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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION CORRELATION BETWEEN THE ANTIOXIDANT, ANTIMICROBIAL, ANTI-FUNGI ACTIVITIES AND CONTENT OF PHENOLIC COMPOUNDS OF RASPBERRY LEAF (*RUBUS IDAEUS* L.) EXTRACTS

AHUDUDU YAPRAĞI (RUBUS IDAEUS L.) SIVI EKSTRELERİNİN ANTİOKSİDAN, ANTİMİKROBİYAL, ANTİ-FUNGAL AKTİVİTELERİ İLE FENOLİK BİLEŞİKLERİNİN İÇERİĞİ ARASINDAKİ KORELASYONUN İNCELENMESİ

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ABSTRACT

Objective: *The purpose of work was study the total content of some biologically active substances (BAS), determine antimicrobial, anti-fungi and antioxidant activities of obtained raspberry leaf extracts, and study a correlation analysis between the content of natural compounds and antimicrobial/antifungal and antioxidant activities.*
Material and Method: *The quantity of phenolic compounds, catechins, flavonoids and hydroxycinnamic acids was determined by spectrophotometric method of analysis, whereas organic acids by alkalimetric method; antioxidant activity of obtained extracts was evaluated by potentiometric method, antimicrobial and anti-fungi wad determined by method of "well".*
Result and Discussion: *Results demonstrates the highest amount of polyphenols, flavonoids, catechins and hydroxycinnamic acids were 1.85±0.02, 1.01±0.02, 0.33±0.01 and 0.24±0.005% in 60% ethanolic extract, respectively. The organic acids were dominated in aqueous extract (1.02±0.02%). The most potent antioxidant property possessed 60% extract of raspberry leaf. There is a high correlation between the content of polyphenols, catechins and antioxidant activity, in the case of inhibition of S. aureus, P. aeruginosa, B. subtillis depends on polyphenols, catechins and antioxidant activity, whereas fungi C. albicans significantly depends only on the content of hydroxycinnamic acids as well as E. coli is not depend on any BAS. These findings show the great potential in the development and creation of new medicines with antimicrobial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.*
Keywords: *Antibacterial activity, anti-fungi, antioxidant activity, correlation analysis, polyphenols, raspberry leaf*

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ÖZ

Amaç: *Çalışmanın amacı, bazı biyolojik olarak aktif maddelerin (BAS) toplam içeriğini incelemek, elde edilen ahududu yaprağı ekstrelerinin antimikrobiyal, anti-fungal ve antioksidan aktivitelerini belirlemek ve doğal bileşiklerin içeriği ile antimikrobiyal/antifungal ve antioksidan aktivitleri arasındaki korelasyon analizini incelemektir.*

Gereç ve Yöntem: *Fenolik bileşikler, kateşinler, flavonoitler ve hidroksisinamik asitlerin miktarı spektrofotometrik analiz yöntemiyle, organik asitlerin miktarı ise alkalimetrik yöntemle belirlenmiştir. Elde edilen ekstrelerin antioksidan aktiviteleri potansiyometrik yöntemle değerlendirilmiş, antimikrobiyal ve anti-fungal aktivite ise "kuyu" yöntemiyle belirlenmiştir.*

Sonuç ve Tartışma: *Sonuçlar; en yüksek polifenol, flavonoit, kateşin ve hidroksisinnamik asit miktarının sırasıyla %1.85±0.02, 1.01±0.02, 0.33±0.01 ve %0.24±0.005 olarak %60 etanolik ekstrede olduğunu göstermiştir. Sulu ekstrede organik asitler baskın bulunmuştur (%1.02±0.02). En güçlü antioksidan özelliği %60'lık ahududu yaprağı ekstresi göstermiştir. Polifenol ve kateşin içeriği ile antioksidan etki arasında yüksek korelasyon tespit edilmiştir; S. aureus, P. aeruginosa, B. subtillis'in inhibisyonunun polifenoller ile kateşinlerin içeriğine ve ve antioksidan aktiviteye bağlı olduğu görülürken; C. albicans mantarlarına karşı etkinin yalnızca hidroksisinnamik asit içeriği ile önemli ölçüde ilişkili olduğu tespit edilmiştir. E. coli üzerine etkinin ise herhangi bir BAS'a bağlı olmadığı gözlenmiştir. Bu bulgular; sentetik analogların etkilerinden daha düşük olmayan ve hatta onlardan daha üstün etkinlik göstermesi dolayısıyla, antimikrobiyal, antioksidan ve anti-fungal etkilere sahip yeni ilaçların geliştirilmesi ve yaratılmasındaki büyük potansiyeli göstermektedir.*

Anahtar Kelimeler: *Ahududu yaprağı, antibakteriyel aktivite, anti-fungal, antioksidan aktivite, korelasyon analizi, polifenoller*

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 per year die from bacterial infections in the world. The mortality rate for all ages was 99.6 deaths per 100.000 population. Of the pathogens studied, *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas aeruginosa* accounted for 54.9% of the 7.7 million deaths, with *S aureus* being associated with more than 1.1 million deaths. *S. aureus* was the leading bacterial cause of death in 135 countries and was associated with the largest number of deaths among people over 15 years of age (940.000) [1]. In addition, this problem is compounded by the emergence of resistance in bacteria to widely used antibiotics, which makes treatment more complex, time-consuming and expensive [2]. 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 [3]. Thus, the search for new antimicrobial natural compounds is perspective for today.

Scientific community has paid a high attention to research of pharmacological activity of natural compounds such as derivatives of flavon-3-ols and flavonols. According to literature sources natural compounds have certain advantages over synthetic compounds. Above all, natural are safe as they possess limited number of side effects, secondly, natural compounds are more effective, and thirdly, it is a cheap production of compounds [4,5].

In the world exist 700 species of raspberry (*Rubus idaeus* L.), most of them occurring on the territory of East and West Europe, Russia and the North America. The chemical composition of *R. idaeus* is represented by derivatives of flavon-3-ols (catechins), flavonols, ellagic and organic acids. The main constituent of leaf is ellagic acid and its derivatives [6]. There are exist of three forms: Free ellagic acid, ellagic acid glycosides, and ellagotannins. In folk medicine *R. idaeus* leaf applied tin the treatment of influenza, diabetes mellitus, respiratory and renal disorders [7].

There are a lot of scientific researches about determination a level antioxidant activity of *R. idaeus* leaf extracts [8-10]. However, there is no date about assessing antioxidant\antimicrobial\anti-fungi activities and its correlation with content of BAS by potentiometric method. So, the aim of the study was to determine the total content of polyphenols, flavonoids, hydroxycinnamic and organic acids, catechins, moreover study antimicrobial activities against *Staphylococcus aureus, Proteus vulgaris,*

Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and fungi *Candida albicans*. In addition, it was aimed to study a correlation analysis between the content of BAS in *R. idaeus* leaf extracts and antimicrobial and antioxidant activities.

MATERIAL AND METHOD

Plant Material

Rubus idaeus leaf were the object of the study, which were collected in the places of its cultivation. The material was collected in 2021 during the fruiting period in the vicinity of the village of Ternova, Kharkiv region.

Equipment

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

Extraction Procedure

A six samples of 10.0 g (exact mass) of *R. idaeus* leaf had the size of particles 1-2 mm. The extraction was conducted with distilled water, 20%, 40%, 60%, 96% ethanol at 80º C within 1 hour with a condenser, ratio raw material/solvent – 1/20. The extraction technique was completed twice to provide totally extract all BAS, then the filtrates were joint and evaporated by vacuum rotary to ratio of extract to raw material 1:2. The five extracts of 96, 60, 40, 20% ethanol and aqueous were obtained. The extraction was carried by a little correction [11]. The green tea (*Camellia sinensis* L.) extract was obtained by the mentioned above method with 60% ethanol.

Quantitative Analysis

The total content of phenolic compounds was measured by the Folin-Ciocaltau assay, the absorbance was measured at 760 nm [11].

The vanillin reagent assay was applied to find out the total catechins [12], the absorbance was measured at 505 nm.

The total flavonoids were determined using assay of complex formation with $AlCl_3$, the absorbance was measured at 415 nm [13].

The total hydroxycinnamic acids derivatives content was measured by assay of complex formation with $NaNO_2$-Na_2MoO_4, the absorbance was measured at 505 nm [14].

The total organic acids content was determined by acid-base titration with the fixation end-point by potentiometric method [14,16].

Antioxidant Activity Assay

Antioxidant activity of extract was evaluated by potentiometric method [17,18]. The standardized green tea leaf 60% extract was used as the reference drug.

Test Organisms

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 used in accordance with the recommendations for the assessment of antimicrobial activity of drugs**.**

Antimicrobial Activity Assay

The method of diffusion of the drug into agar carried out using the method of "wells" [19,20]. Gentamycin, and fluconazole were used as reference drugs for assessing antimicrobial activity.

Correlation Analysis

Pearson's (r) correlation coefficient was used to analyze the correlation between antioxidant activity (AOA) and the amount of phenolic, catechins-, flavonoid, hydroxycinnamic acids derivatives and organic acids. The correlation coefficient to takes a value in the range of -1 to +1. Correlation is very high if it is within the range from 0.90 to 1.00; from 0.70 to 0.90 is a high correlation; from 0.50 to 0.70 is a moderate correlation; from 0.30 to 0.50 is a low correlation; from 0.00 to 0.30 negligible correlation [21].

RESULT AND DICUSSION

According to obtained results shown in Table 1, the 60% extract (1.85±0.02%) had the most significant amount of polyphenols, followed by 40% extract (1.53±0.02%), whereas the lowest one – ethanolic extract (0.38±0.01%).

The content of catechins increasing in the following order 96% extract (0.20±0.01%) > aqueous extract (0.73±0.01%) > 20% extract (0.74±0.01%) > 40% extract (0.96±0.02%) > 60% extract (1.01±0.02%). The percentage of catechins out of total of polyphenols was 53.1, 54.2, 63.0, 50.1 and 54.3% for 96%, 60%, 40%, 20% and aqueous extracts, respectively. The highest percentage of catechins was in 40% extract, whereas the lowest in 20% extract (Table 1).

Table 1 demonstrates that the most significant content of flavonoids was found in 60% extract (0.33±0.01%), whereas in the aqueous extract (0.20±0.002%) was the lowest one. The percentage of flavonoids out of total of polyphenols was 63.2, 18.1, 43.2, 15.3 and 15.0% for 96%, 60%, 40%, 20% and aqueous extracts, respectively. The highest percentage of flavonoids was in 96% extract, whereas the lowest in aqueous and 20% extract.

The content of hydroxycinnamic acids increasing in the following order aqueous and 20% extract (0.10±0.005%) > 40% extract (0.15±0.005%) > 96% extract (0.11±0.005%) > 60% extract (0.24±0.005%). The percentage of hydroxycinnamic acids out of total of polyphenols was 29.0, 13.1, 10.2, 7.1 and 6.2% for 96%, 60%, 40%, 20% and aqueous extracts, respectively. The highest percentage of hydroxycinnamic acids was in 96% extract, whereas the lowest in 6% extract. (Table 1)

The highest amount of organic acids was determined in aqueous extract (1.02±0.02%), followed by 20% extract (1.00±0.02%), whereas the lowest one in 96% extract (0.42±0.01%). The total content of organic acids was lower 153.3%, 110.2%, 48.1%, 32.1% than content polyphenols in 96%, 60%, 40%, 20% extracts, respectively (Table 1).

Table 1. The sum of phenolic compounds, flavonoids, catechins, hydroxycinnamic acids and organic acids in *R. idaeus* leaf liquid extracts

Sample	Amount of polyphenols, %±SD[a]	Amount of catechins	Amount of flavonoid		Amount of hydroxycinnamic acids		Amount of organic acids, %± SD[a]
		% ±SD[a]	% ±SD[a]	Part out polyphenols	% ±SD[a]	Part out polyphenols	% ±SD[a]
96% EtOH extract	0.38±0.01	0.20±0.01	0.24±0.01	63.2	0.11±0.005	29.0	0.42±0.01
60% EtOH extract	1.85±0.02	1.01±0.02	0.33±0.01	18.1	0.24±0.005	13.1	0.73±0.01
40% EtOH extract	1.53±0.02	0.96±0.02	0.25±0.01	43.2	0.15±0.005	10.2	0.73±0.01
20% EtOH extract	1.48±0.02	0.74±0.01	0.22±0.01	15.3	0.10±0.005	7.1	1.00±0.02
Aqueous extract	1.35±0.02	0.73±0.01	0.20±0.01	15.0	0.10±0.005	6.2	1.02±0.02

[a]Standard deviation, n=4

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts of *R. idaeus* leaf. Table 2 shows that the level of antioxidant activity increases in the following order: 96% extract (28.9±0.3 mmol-eqv./$m_{dry\ res.)}$ > aqueous extract (54.3±0.5 mmol-eqv./$m_{dry\ res.}$) > 20% extract (60.8±0.6 mmol-eqv./$m_{dry\ res.}$) > 40% extract (69.9±0.7 mmol-eqv./$m_{dry\ res.}$) > 60% extract (76.1±0.8 mmol-eqv./$m_{dry\ res.}$). In light of the data obtained, it can be established that the 60% extract has the highest level of antioxidant activity. According to the modern classification of antioxidant activity, which was previously developed in our previous research [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 60% extract of *C. sinensis* leaf. The *C. sinensis* leaf extract was obtained by the same technological method as *R. idaeus* 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 96% extract had the highest antioxidant effect, and the least - 20% extract (Table 3).

Table 2. The level of antioxidant activity of *R. idaeus* leaf liquid extracts

Sample	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD[a]	Conditional term of antioxidant level
96% EtOH extract	28.9±0.3	High level
60% EtOH extract	76.1±0.8	High level
40% EtOH extract	69.9±0.7	High level
20% EtOH extract	60.8±0.6	High level
Aqueous extract	54.3±0.5	High level
Green tea leaf 60% EtOH extract	548.8±5.5	Very high level

[a]Standard deviation, n=4

The potentimetric assay was chosen for evaluation antioxidant activity for several reasons: above all, the potentiometric assay is expressive secondly, cheap and moreover, this assay accurate and precise. To compare the antioxidant effect, we used the obtained green tea leaf extract, the results showed that green tea extract inactivates free radicals significantly better than *R. idaeus* leaf extracts. The 60% extract was found to have the highest level of antioxidant activity than other *R. idaeus* extracts. After, we decided to compare the antioxidant effect of extracts at the same concentration of phenolic compounds, as a result, it was shown that green tea extract works lower than in 96%, 60%, 40% extracts of *R. idaeus* leaf. In addition, it was found that the order of levels of antioxidant activity of the extracts changed dramatically. At different concentrations of phenolic compounds, the 60% extract had the highest level of antioxidant activity, and when compared at the same concentration, the ethanolic extract was the best.

Table 3. Comparing the value of antioxidant activity of *R. idaeus* leaf liquid extracts with *C. sinensis* leaf 60% extract at the concentration 0.03 mol/l expressed in the total phenolic compounds as gallic acid

Sample	Concentration of polyphenols, mol/l	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ±SD[a]
96% EtOH extract	0.03	43.3±0.4
60% EtOH extract		20.7±0.2
40% EtOH extract		23.3±0.2
20% EtOH extract		20.3±0.2
Aqueous extract		20.3±0.2
Green tea leaf 60% EtOH extract		27.9±0.3

[a]Standard deviation, n=4

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

S. aureus was most sensitive to the 60% extract (25.0 ± 0.20 mm) and least sensitive to the 96% extract (20.0 ± 0.4 mm). When comparing the results of the gentamicin standard and the 60% extract, it was found that the 60% extract was 12.1% 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 highly sensitive to the 60% extract (25.0 ± 0.3 mm), followed by 40% extract (22.0 ± 0.4 mm), and the aqueous and ethanolic extracts inhibited the growth of the bacterial strain the least. (21.0+0.5 mm). The most resistant strains of bacteria to the action of *R. idaeus* leaf extracts was *P. aeruginosa*. *E. coli* were most sensitive to the action of 20% extract, in second place – 60% extract, whereas *P. vulgaris* were most sensitive to the action of 20% extract (Table 4).

When studying antifungal activity against *C. albicans*, the results showed that 60% extract of *R. idaeus* leaf was the most actively inhibited the growth of the fungus, whereas the ethanolic, 20% and aqueous extracts were least active inhibited the growth of fungi. When compared with the fluconazole standard, it was found that the 60% extracts inhibited fungal growth 10.2% better than fluconazole (Table 4).

The studied *R. idaeus* leaf extract showed 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. idaeus* leaf extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was significantly 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 [22]. Comparing the antifungal effects of fluconazole and *R. idaeus* leaf extract, 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-fungi medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but to *R. idaeus* leaf extracts both strains of bacteria and fungus are sensitive. Thus, *R. idaeus* leaf extracts is a combined 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.

Table 4. The value of antimicrobial activity of *R. idaeus* leaf liquid extracts

Sample	**Concentration mmol/l, (expressed in total polyphenols as gallic acid)**	**Diameter of the growth retardation zone, mm±SD[a]**					
		Gram-positive		**Gram-negative**			**Fungi**
		S. aureus	*B. subtilis*	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*
96% EtOH extract	0.006	20.0±0.4	21.0±0.4	20.0±0.4	20.0±0.4	20.0±0.4	20.0±0.4
60% EtOH extract	0.033	25.0±0.3	25.0±0.3	21.0±0.4	20.0±0.4	18.0±0.6	23.0±0.4
40% EtOH extract	0.027	22.0±0.4	23.0±0.4	20.0±0.5	20.0±0.4	18.0±0.6	21.0±0.4
20% EtOH extract	0.027	25.0±0.3	22.0±0.5	22.0±0.4	23.0±0.3	18.0±0.6	20.0±0.4
Aqueous H_2O extract	0.024	23.0±0.4	21.0±0.5	20.0±0.5	21.0±0.4	18.0±0.6	20.0±0.4
Gentamycin	0.003	22.0±0.5	24.0±0.3	25.3±0.3	25.0±0.3	25.7±0.2	12.0±0.8
Fluconazole	0.003	18.0±0.5	12.0±0.6	14.3±0.8	12.3±0.8	10.0±0.8	20.0± 0.4

[a]Standard deviation, n=4

The dependence of antioxidant, antimicrobial activity on the content of different groups of BAS was studied using the method of linear regression. In Figure 1 shows that the correlation between the antioxidant effect and the content of polyphenols was very high (R=0.9789), in the case of catechins was very high (R=0.9809), flavonoids was moderate (R=0.5168), in the case of hydroxycinnamic acids was moderate (R=0.6512), and the lowest correlation value was observed for organic acids. The results

obtained are similar from those obtained by other Ispiryan et al. [23], they reported about the correlation between antioxidant and the polyphenols content in extracts of raspberry shoots, leaves, seeds and fruits. In their research correlation coefficient was 0.845, 0.854, and 0.700 beetwen antioxidant and polyphenols, flavonoids, and hydroxycinnamic acids content.

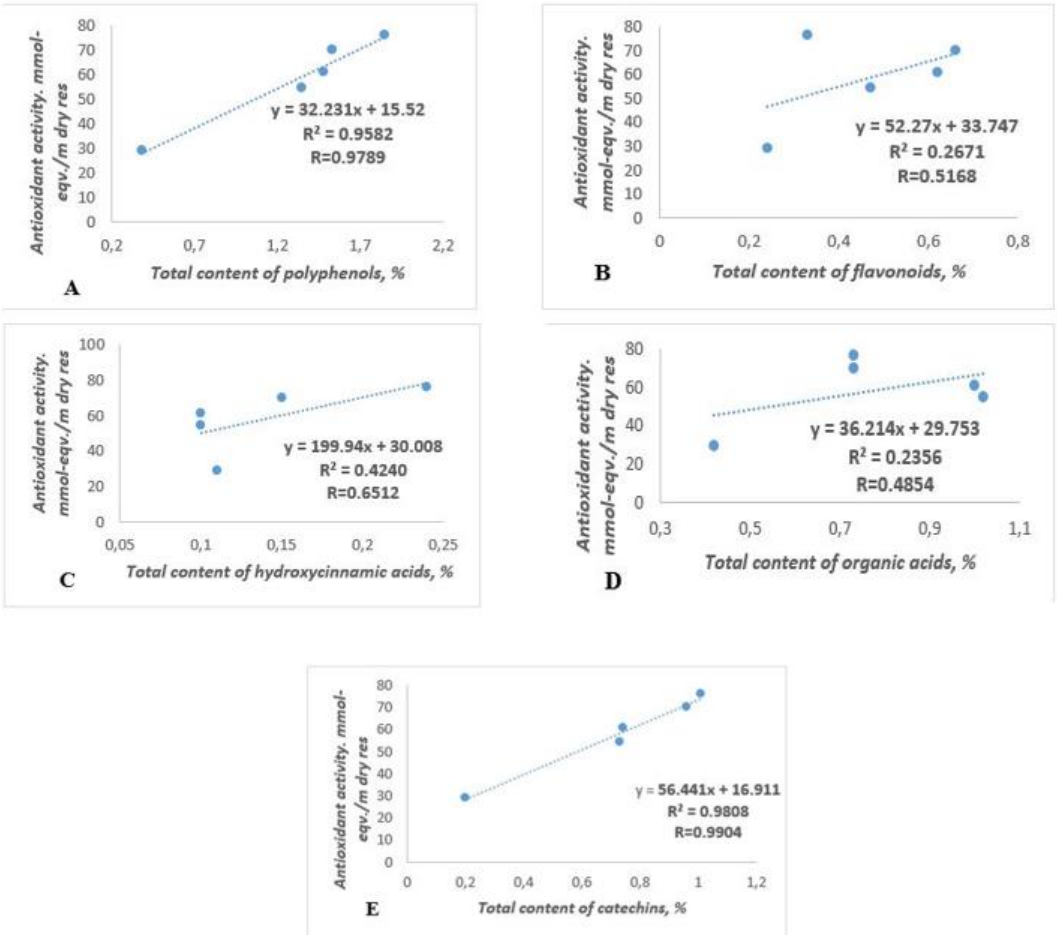

Figure 1. Correlation relationship between value of antioxidant activity and total content of polyphenols (A), flavonoids (B), hydroxycinnamic acids (C), organic acids (D) and catechins (E)

According to the research results presented in Figure 2 it was found that there is a high correlation between phenolic compounds (R=0.8462), catechins (R=0.7122), organic acids (R=0.7064), antioxidant activity (R=0.7554) and inhibition of the growth of *S. aureus*, in the case of flavonoids (R=0.3387) and hydroxycinnamic acids (R=0.3936) – low correlation. Comparing with results obtained of research of Ispiryan *et al.* [23], they are different as in the research of Ispiryan et al. correlation coefficient was 0.300, 0.315, 0.380 and 0.370 between antimicrobial activity against *S. aerous* and polyphenols, flavonoids, hydroxycinnamic acids and antioxidant effect.

In Figure 3 shows that the antimicrobial effect against *B. subtilis* is very highly dependent on the content of hydroxycinnamic acids (R=0.9528), high dependent on polyphenols (R=0.7297), flavonoids (R=0.7465) and antioxidant activity (R=0.8088), in turn, the content of organic acids and flavonoids there is no dependence. Stagos et al. [24] investigated antibacterial activity of *Mentha*, *Salvia* and *Sideritis* leaf extracts, results were shown that there was observed a low correlation between antibacterial and polyphenols contenr.

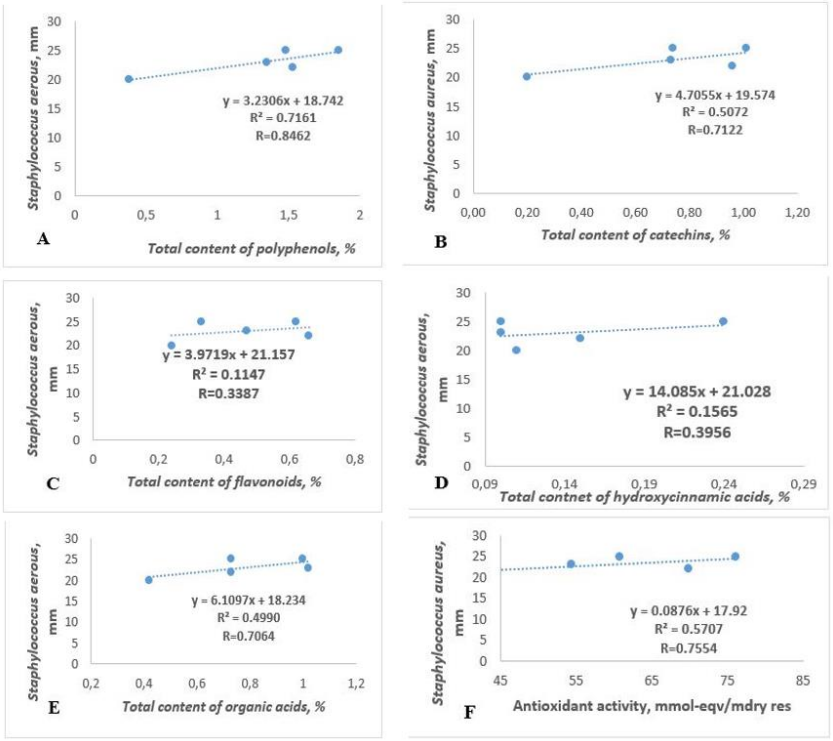

Figure 2. Correlation relationship between value of antimicrobial activity against *Staphylococcus aureus* and total content of polyphenols (A), catechins (B) flavonoids (C), hydroxycinnamic acids (D), organic acids (E) and antioxidant activity (F)

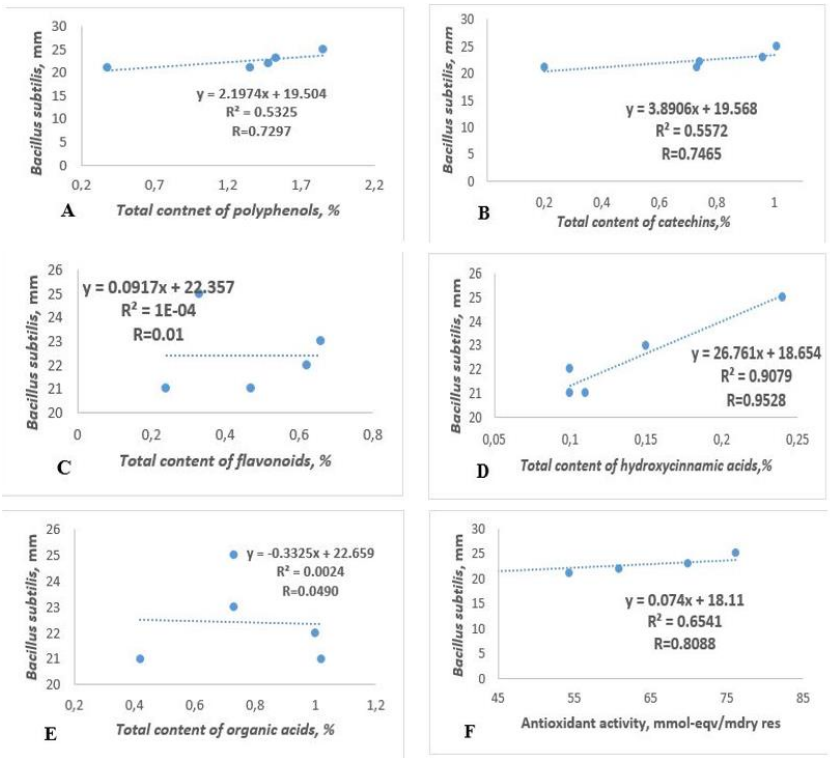

Figure 3. Correlation relationship between value of antimicrobial activity against *Bacillus subtilis* and total content of polyphenols (A), catechins (B), flavonoids (C), hydroxycinnamic acids (D), organic acids (E), and antioxidant activity (F)

The study showed that there is a low correlation between phenolic compounds (R=0.4306), organic acid (R=0.4445), antioxidant effect (R=0.3622) and inhibition of *E. coli* growth, while flavonoids, hydroxycinnamic acids and catechins are not effect on the growth inhibition of *E. coli*. (Figure 4). Cirovic *et al.* [25] investigated antibacterial activity of *Sanguisorba minor* root extracts against *E. coli*, results were shown that there was observed a low correlation between antibacterial and polyphenols, flavonoids content. Comparing results with our, there are similar.

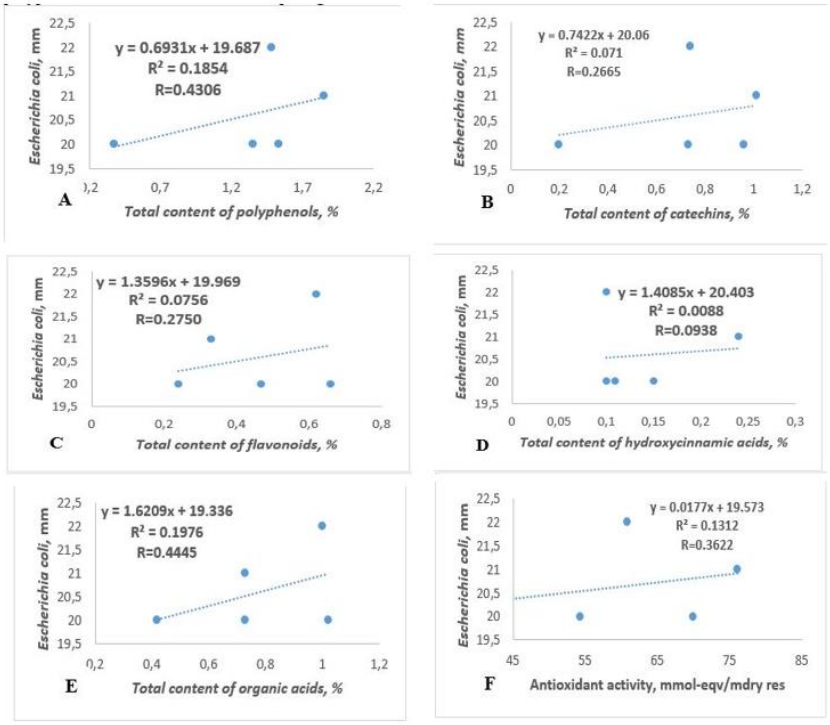

Figure 4. Correlation relationship between value of antimicrobial activity against *Escherichia coli* and total content of polyphenols (A), catechins (B) flavonoids (C), hydroxycinnamic acids (D), organic acids (E), and antioxidant activity (F)

When studying the relationship between inhibition of growth of *P. vulgaris* and the content of different groups of BAS, it was found that there is a very high dependence of antimicrobial activity on the amount of polyphenols (R=0.8838), flavonoids (R=0.7262) and hydroxycinnamic acid (R=0.7347), in turn, the antioxidant effect had a moderate correlation, while catechins, organic acids had not correlation at all. (Figure 5) Cirovic *et al.* [25] investigated antibacterial activity of *Sanguisorba minor* root extracts against *P. vulgaris*, results were shown that there was observed a high correlation between antibacterial and polyphenols, flavonoids and hydroxycinamic acid content. Comparing results with our, there are similiar.

Figure 6 shows that the correlation between the growth inhibition of *P. aeruginosa* and the sum of polyphenols (R=0.9437), catechins (R=0.9194) is very high, with the sum of organic acids and antioxidant activity was high, in the case of flavonoids it was found moderate correlation. Whereas, the total content of hydroxycinnamic acids was not effect on the inhibition of growth *P. aeruginosa*. The results obtained are similar from those obtained by other Katalinic *et al.* [26], they reported about the correlation between antioxidant and the polyphenols content in *Vitis vinifera* leaf extracts against *P. aeruginosa*. In their research correlation coefficient was 0.745, 0.600 beetwen antibacterial effect and polyphenols content, antioxidant effect, respectively.

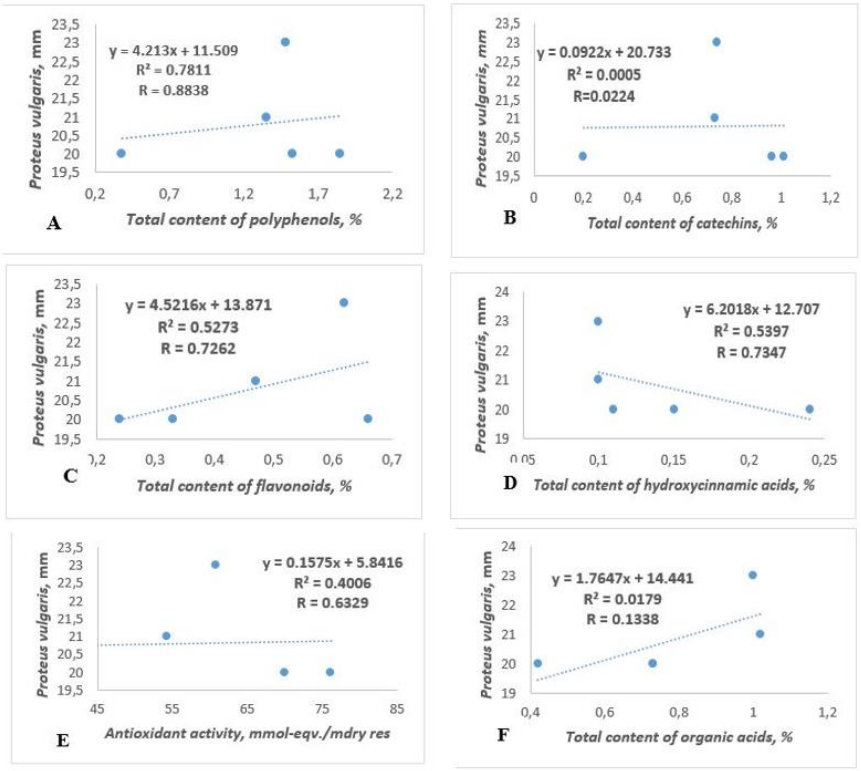

Figure 5. Correlation relationship between value of antimicrobial activity against *Proteus vulgaris* and total content of polyphenols (A), catechins (B), flavonoids (C), hydroxycinnamic acids (D), organic acids (F), and antioxidant activity (E)

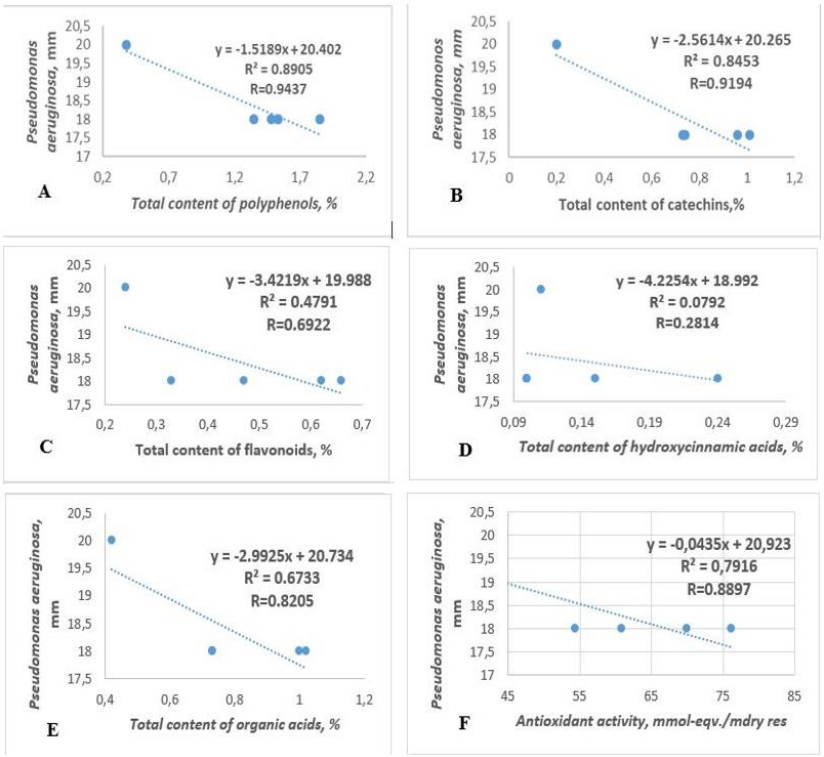

Figure 6. Correlation relationship between value of antimicrobial activity against *Pseudomonas aeruginosa* and total content of polyphenols (A), catechins (B), flavonoids (C), hydroxycinnamic acids (D), organic acids (F), and antioxidant activity (E)

In Fig. 7 shows a significant high correlation between inhibition of the growth of *C. albicans* and the content of hydroxycinnamic acids (R=0.9976), in turn, with the sum of polyphenols (R=0.6239), catechins (R=0.6438) and antioxidant activity (R=0.6940) was found a moderate dependence, and in the case of organic acids and flavonoids there was no correlation at all. The results obtained are similar from those obtained by other Chen *et al.* [27], they reported about the correlation between antifungi and the polyphenols content in *Curcuma longa* extract against *Fusarium graminearum*.

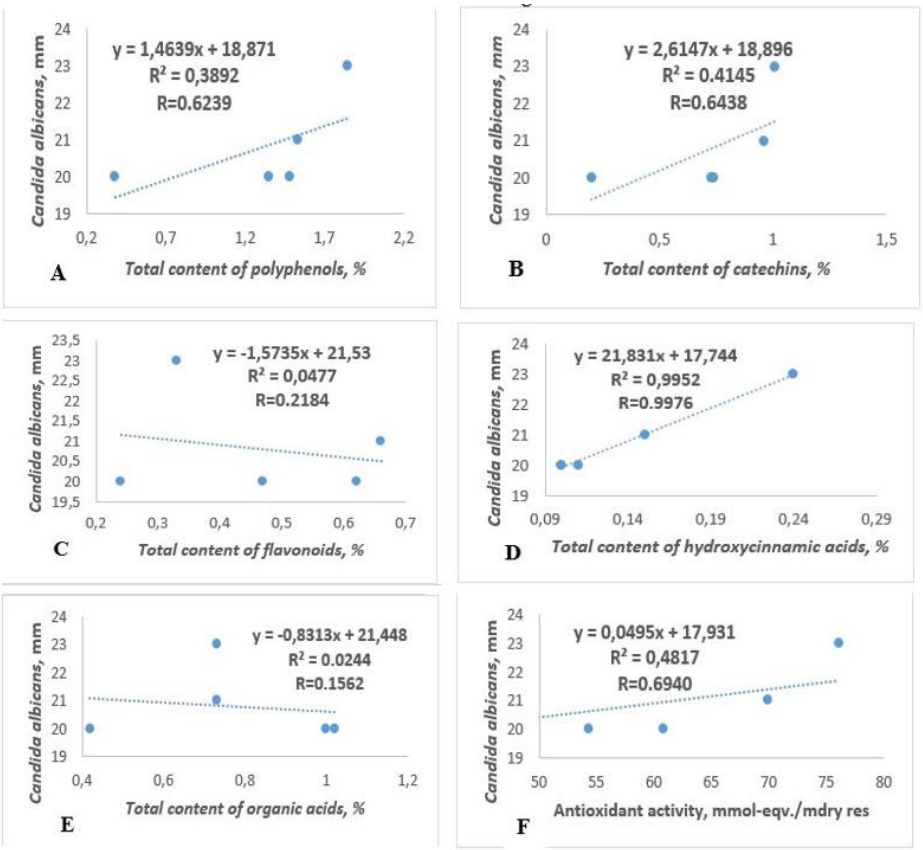

Figure 7. Correlation relationship between value of antimicrobial activity against *Candida albicans* and total content of polyphenols (A), catechins (B), flavonoids (C), hydroxycinnamic acids (D), organic acids (E), and antioxidant activity (F)

When studying the dependence of antioxidant action on the content of different groups of BAS, it was found that phenolic compounds and catechins most strongly influence on the level of antioxidant action. The investigation of correlation between inhibition of the growth of bacteria strains *S. aureus*, *B. subtillis*, *P. aeruginosa*. and the content of BAS, it was shown that the growth of microorganisms is most strongly influenced by phenolic compounds, catechins and antioxidant effects, in addition, the inhibition of the growth of *S. aureus* and *P. aeruginosa* depends on the presence of organic acids, in turn, the growth of the strain of *B. subtillis* is not dependent, but, on the contrary, is highly correlated with content of hydroxycinnamic acids. The growth of *E. coli* does not depend on the content of BAS or on the antioxidant effect. In our opinion, this may be due to the fact that bacterial growth depends on another group of compounds, for example, ellagitannins, which were not represented in our research. The growth of fungi *C. albicans* significantly depends only on the content of hydroxycinnamic acids.

Conclusion

In the research, it has been determined the content of BAS, antioxidant, antimicrobial activity of the obtained extracts of *R. idaeus* 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% extract has a high level of antioxidant activity, all obtained extracts actively inhibits the growth of all studied Gram-positive, Gram-negative strains of bacteria and the fungus *C. albicans* in the range from 18 to 25 mm (diameter of growth inhibition). We have shown that there is a high correlation between the content of polyphenols, catechins and antioxidant activity, in the case of inhibition of *S. aureus*, *P. aeruginosa*, *B. subtillis* depends on polyphenols, catechins and antioxidant activity, whereas fungi *C. albicans* significantly depends only on the content of hydroxycinnamic acids as well as E. coli is not depend on any BAS. 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.

AUTHOR CONTRIBUTIONS

Concept: O.M., M.K., S.P., T.O., S.K.; Design: O.M., M.K., S.P., T.O., S.K.; Control: T.O., S.K.; Sources: O.M., M.K., S.P.; Materials: O.M., M.K., S.P.; Data Collection and/or Processing: O.M., M.K., S.P.; Analysis and/or Interpretation: O.M., M.K., S.P.; Literature Review: O.M., M.K., S.P.; Manuscript Writing: O.M., M.K., S.P.; Critical Review: T.O., S.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Ikuta, K.S., Swetschinski, L.R., Robles Aguilar, G., Sharara, F., Mestrovic, T., Gray, A.P., Davis Weaver, N., Wool, E.E., Han, C., Gershberg Hayoon, A., Aali, A., Abate, S.M., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abd-Elsalam, S., Abebe, G., Abedi, A., Abhari, A.P., Abidi, H., Naghavi, M. (2022). Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. The Lancet, 400, 2221-2248 [CrossRef]
2. Bongomin, F., Gago, S., Oladele, R., Denning, D. (2017). Global and multi-national prevalence of fungal diseases-estimate precision. Journal of Fungi, 3(4), 57. [CrossRef]
3. Denning, D. (2022). Global incidence and mortality of severe fungal disease. Lancet, 400, 1-21. [CrossRef]
4. Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Supuran, C.T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20(3), 200-216. [CrossRef]
5. Zaid, H., Raiyn, J., Nasser, A., Saad, B., Rayan, A. (2010). Physicochemical properties of natural based products versus synthetic chemicals. The Open Nutraceuticals Journal, 3(1), 194-202. [CrossRef]
6. Derymedvid, L.V., Horopashna, D.O., Kalko, K.O., Mishchenko, O.Y., Okipniak, I.V., Komissarenko, A.M., Komisarenko, M.A., Sevastianova, T.V. (2021). Anti-inflammatory properties of raspberry shoot extract. Pharmacologyonline, (2), 657-662.
7. Komisarenko, M.A., Polischuk, I.M., Upyr, T.V., Saidov, N.B. (2021). Study of amino acid composition and immunomodulatory activity of *Rubus idaeus* alcoholic extract. Research Journal of Pharmacy and Technology, 14(3), 1329-1332. [CrossRef]
8. Krzepiłko, A., Prażak, R., Święciło, A. (2021). Chemical composition, antioxidant and antimicrobial activity of raspberry, blackberry and raspberry-blackberry hybrid leaf buds. Molecules, 26(2), 327. [CrossRef]
9. Asnaashari, M., Tajik, R., Khodaparast, M.H.H. (2014). Antioxidant activity of raspberry (*Rubus fruticosus*) leaves extract and its effect on oxidative stability of sunflower oil. Journal of Food Science and Technology, 52(8), 5180-5187. [CrossRef]
10. Veljkovic, B., Djordjevic, N., Dolicanin, Z., Licina, B., Topuzovıc, M., Stankovıc, M., Zlatıc, N., Dajıc-Stevanovıc, Z. (2018). Antioxidant and anticancer properties of leaf and fruit extracts of the wild raspberry (*Rubus idaeus* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(2), 359-367. [CrossRef]
11. Maslov, O.Y., Komisarenko, M.A., Kolisnyk, O.V., Ponomarenko, S.V. (2020). Antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts. Pharmacologyonline, (3), 291-298.

12. Maslov, O., Kolisnyk, S., Komisarenko, M., Golik, M. (2022). Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). Herba Polonica, 68(1), 1-9. [CrossRef]
13. Maslov, O.Y., Komisarenko, M.A., Golik, M.Y., Kolisnyk, S.V., Altukhov, A.A., Baiurka, S.V., Karpushina, S.A., Tkachenko, O., Iuliia, K. (2023). Study of total antioxidant capacity of red raspberry (*Rubus idaeous* L.) shoots. Vitae, 30(1), 1-9. [CrossRef]
14. Maslov, O., Komisarenko, M., Kolisnyk, S., Kostina, T., Golik, M., Moroz, V., Tarasenko, D., Akhmedov, E. (2023). Investigation of the extraction dynamic of the biologically active substances of the raspberry (*Rubus idaeus* L.) shoots. Current Issues in Pharmacy and Medical Sciences, 36(4), 194-198. [CrossRef]
15. Maslov, O.Y., Kolesnik, S.V., Komisarenko, M.A., Altukhov, A.A., Dynnyk, K., Kostina, T. (2021). Development and validation of a titrimetric method for quantitative determination of free organic acids in green tea leaves. Pharmakeftiki, 33(4), 304-311.
16. Maslov, O. Y., Kolisnyk, S. V., Kostina, T.A., Shovkova, Z.V., Ahmedov, E.Y., Komisarenko, M.A. (2021). Validation of the alkalimetry method for the quantitative determination of free organic acids in raspberry leaves. Journal of Organic and Pharmaceutical Chemistry, 19(1(73)), 53-58. [CrossRef]
17. Maslov, O.Y., Kolisnyk S.V., Komissarenko N.A., Kostina T.A. (2021) Development and validation potentiometric method for determination of antioxidant activity of epigallocatechin-3-O-gallate. Pharmacologyonline, (2), 35-42.
18. Maslov, O., Komisarenko, M., Kolisnyk, S., Derymedvid, L. (2024b). Evaluation of anti-Inflammatory, antioxidant activities and molecular docking analysis of *Rubus idaeus* leaf extract. Jordan Journal of Pharmaceutical Sciences, 17(1), 105-122. [CrossRef]
19. Maslov, O., Komisarenko, M., Ponomarenko, S., Horopashna, D., Osolodchenko, T., Kolisnyk, S., Derymedvid, L., Shovkova, Z., Akhmedov, E. (2022). Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeous* l.) leaf extract. Current Issues in Pharmacy and Medical Sciences, 35(4), 229-235. [CrossRef]
20. Maslov O., Kolisnyk S., Komisarenko M., Komisarenko A., Osolodchenko T., Ponomarenko S. (2022). *In vitro* antioxidant and antibacterial activities of green tea leaves (*Camella sinensis* l.) liquid extracts. AMI, (2), 64-67.
21. Maslov, O., Komisarenko, M., Horopashna, D., Tkachenko, O., Derymedvid, L., Kolisnyk, S., Kolisnyk, O. (2023). Antioxidant activity of red raspberry shoots (*Rubus idaeus* L.) liquid extracts. Herba Pol, 69(4), 45-53.
22. Maslov, O.Y., Kolisnyk, S.V., Komisarenko, M.A., Altukhov, A.A., Dynnyk, K.V., Stepanenko, V.I. (2021). Study and evaluation antioxidant activity of dietary supplements with green tea extract. Current Issues in Pharmacy and Medicine: Science and Practice, 14(2), 215-219. [CrossRef]
23. Ispiryan, A., Atkociuniene, V., Makstutiene, N., Sarkinas, A., Salaseviciene, A., Urbonaviciene, D., Viskelis, J., Pakeltiene, R., Raudone, L. (2024). Correlation between antimicrobial activity values and total phenolic content/antioxidant activity in *Rubus idaeus* L. Plants, 13(4), 504. [CrossRef]
24. Stagos, D., Portesis, N., Spanou, C., Mossialos, D., Aligiannis, N., Chaita, E., Panagoulis, C., Reri, E., Skaltsounis, L., Tsatsakis, A.M., Kouretas, D. (2012). Correlation of total polyphenolic content with antioxidant and antibacterial activity of 24 extracts from Greek domestic Lamiaceae species. Food and Chemical Toxicology, 50(11), 4115-4124. [CrossRef]
25. Cirovic, T., Barjaktarevic, A., Cupara, S., Mitic, V., Nikolic, J., Jovanovic, V.S. (2019). Antioxidant and antimicrobial activity of sanguisorba minor L. Extracts. Serbian Journal of Experimental and Clinical Research. [CrossRef]
26. Katalinic, V., Mozina, S.S., Generalic, I., Skroza, D., Ljubenkov, I., Klancnik, A. (2012). Phenolic profile, antioxidant capacity, and antimicrobial activity of leaf extracts from six *Vitis vinifera* L. varieties. International Journal of Food Properties, 16(1), 45-60. [CrossRef]
27. Chen, C., Long, L., Zhang, F., Chen, Q., Chen, C., Yu, X., Liu, Q., Bao, J., Long, Z. (2018). Antifungal activity, main active components and mechanism of *Curcuma longa* extract against *Fusarium graminearum*. Plos One, 13(3), Article e0194284. [CrossRef]