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Eur. Pharm. J. 2024, 71(1), 26-31.
ISSN 1338-6786 (online) and ISSN 2453-6725 (print version),
DOI: 10.2478/afpuc-2024-0006

EUROPEAN PHARMACEUTICAL JOURNAL

Antimicrobial, Antifungal, Antioxidant Activity and Phytochemical Investigation of Phenolcarboxylic Acids by GC–MS of Raspberry (*Rubus idaeus* L.) Shoot Lipophilic Extract

Research paper

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Received 2 January, 2024, accepted 9 April, 2024

Abstract **Aim:** To determine the content of phenolcarboxylic acids using gas chromatography–mass spectrometer (GC–MS) in the obtained extract, conduct a study of the antimicrobial, antifungal, and antioxidant activities of *Rubus idaeus* shoot lipophilic extract.
Material/methods: The quantification of phenolcarboxylic was accomplished through GC–MS, antioxidant activity was assessed by potentiometric method, antimicrobial and antifungal activities were determined by well method.
Results: The 8 compounds were identified by the GC–MS method. The vanillic acid (2.59 ± 0.08 mg/100 g), benzoic acid (1.51±0.08 mg/100 g), and ferulic acid (0.79±0.04 mg/100 g) dominated in the obtained lipophilic *R. idaeus* shoot extract. *Bacillus subtilis* (17.00 ± 0.50 mm) was the most sensitive to lipophilic extract, whereas *Proteus vulgaris* was the most resistant to the lipophilic extract. Moreover, *Candida albicans* was medium sensitive to lipophilic extract (13.50 ± 0.50 mm). The antioxidant activity was 1.00 mmol-equiv./$m_{dry\ res}$; according to Maslov`s antioxidant level classification it has low level.
Conclusions: The lipophilic extract from *R. idaeus* shoots contains various phenolcarboxylic acids, including vanillic acid, benzoic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid, gentisic acid, salicylic acid, and phenylacetic acid, with the highest concentrations observed for vanillic, benzoic, and ferulic acids. This study highlights the antimicrobial and antifungal properties of the *R. idaeus* shoot lipophilic extract. However, the obtained lipophilic extract showed a relatively low level of antioxidant activity. Consequently, the derivatives of phenolcarboxylic acids play a substantial role in the antimicrobial and antifungal effects, whereas their contribution to antioxidant activity appears to be limited.

Keywords *raspberry – phenolcarboxylic acids – shoot – lipophilic extract – antimicrobial activity – antioxidant activity*

INTRODUCTION

Group of international researches have estimated that global mortality is associated with bacterial infections. They have analyzed 343 million individual patient records and pathogen isolates; overall, the researches have estimated 13.7 million infection-related deaths in 2019, with 7.7 million associated with the 33 bacterial pathogens and 11 infectious syndromes studied. These deaths made up 13.6% of all global deaths and 56.2% of all sepsis-related deaths in 2019. The all-age mortality rate was 99.6 deaths per 100,000 population. Of the investigated pathogens, five—*Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*—accounted for 54.9% of the 7.7 million deaths, with *S. aureus* associated with more than 1.1 million deaths. *S. aureus* was the leading bacterial cause of death in 135 countries and was associated with most deaths in people over 15 years (940,000). *S pneumoniae* was associated with most deaths in children under 5 years (225,000), while *K. pneumoniae* was associated with most newborn deaths (124,000). *Salmonella enterica* serovar Typhi was linked to most deaths in children ages 5 to 14 years (49,000). The study notes that these estimates would put these infections ahead of HIV, cancer, and self-harm as leading causes of death globally in 2019 (Ikuta et al., 2022). Meanwhile, nearly a billion people suffer from fungal infections of the skin, nails, and hair, and more than 150 million people suffer from serious fungal diseases that can later be fatal (Bongomin et al., 2017). Therefore, the search and elaboration of new antimicrobial and antifungal medicines against Gram-positive and Gram-negative strains is topical for medicine and pharmacy.

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The genus *Rubus* consists of around 700 species that usually occur in the temperate climate (Maslov et al., 2023). Raspberries are an aggregate fruit commonly grown and consumed throughout Asia, Europe, and America as members of the rose family, and are closely related to blackberries and other brambles or caneberries. Although many species and types of raspberries exist, red and black are the most common (Maslov et al., 2023).

The raspberry leaves and fruits are a rich source of flavonoid derivatives, which are represented by quercetin derivatives as well as phenolcarboxylic acids, organic acids, vitamin C (Komisarenko et al., 2021). Phenolcarboxylic acids are represented by ferulic acid, gentisic acid, syringic acid, vanillic acid, and ellagic acid in *Rubus idaeus* leaf and shoot. The recent study showed that the main compound in *R. idaeus* leaf and shoot is ellagotannins (Derymedvid et al., 2021).

The recent literature search showed that many researches estimated antimicrobial and antifungal activities against Gram-positive and Gram-negative of ethanolic-aqueous and aqueous-ethanolic *R. idaeus* shoot and leaf extracts [Maslov et al., 2022]. The results showed that all obtained extracts had high level of inhibition growth of bacteria and fungi strains. Moreover, the researches have concluded that exactly the derivatives of ellagotannins and flavan-3-ols are responsible for antimicrobial and antifungal actions. There are no doubts that ellagotannins and flavan-3-ols inhibited the growth of bacteria and fungi strains. However, in our view, the derivatives of phenolcarboxylic acids contribute to antimicrobial and antifungal effects of *R. idaeus* extracts. Therefore, the aim of this study is to determine the content of phenolcarboxylic acids using GC–MS in the obtained extract, conduct a study of the antimicrobial, antifungal, and antioxidant activities of *R. idaeus* shoot lipophilic extract.»

MATERIALS AND METHODS

Plant material

»The *R. idaeus* leafless shoots of second year were the object of the study. The material was collected in 2021 after the fruiting period in the vicinity of the village of Ternova, Kharkiv region (50.193116162220264, 36.66935288403296), in October.»

Reagents

Hexane (≥98%, manufacturer «Reackhim,» c. Kharkiv), chloroform (≥98%, manufacturer «Reackhim,» c. Kharkiv»), vanillic acid (≥97%, manufacturer «Sigma Aldrich Company,» c. Poznan), benzoic acid (≥98%, manufacturer «Reackhim,» c. Kharkiv), ferulic acid (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan), *p*-oxybenzoic acid (≥98%, manufacturer «Reackhim,» c. Kharkiv), syringic acid (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan), gentisic acid (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan), salicylic acid (≥98%, manufacturer «Reackhim,» c. Kharkiv) phenylacetic acid (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan), gentamycin (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan), and fluconazole (≥98%, manufacturer «Sigma Aldrich Company,» c. Poznan) were purchased.

Extraction procedure

»A 250.0 g (exact mass) of *R. idaeus* shoots were ground to the size of 1–2 mm. The extraction was carried out by 60% ethanol at the ratio of raw material/solvent 1/20 (*m/v*) on water bath at 80 °C with a reflux condenser for one hour, the extraction was made two times. Following the cooling process, the solutions were filtered and concentrated to a final volume of 250 mL using a rotary evaporator at 40 °C under vacuum conditions and the obtained extract was extracted by chloroform with a volume of 125 mL for 15 min two times.»

GC–MS method of analysis

The chromatographic separation of phenolcarboxylic acids was carried out on gas chromatography–mass spectrometer (GC–MS) 5973N/6890N MSD/DS «Agilent Technologies» (USA). The MS detector is a quadrupole, the ionization method is electron impact (EI), and the ionization energy is 70 eV. The full ion current recording mode was used for the analysis. A capillary column was used for distribution HP–INNOWAX (30 m × 250 μm). Stationary phase—INNOWAX; mobile phase—helium, gas flow rate—1 ml/min; the temperature of the sample introduction heater is 250 °C; the temperature of the thermostat is programmable from 50 to 250 °C. The introduction of a sample of 2 μL into the chromatographic column was performed in the splitless mode (without flow distribution), which allows you to do this without loss of separation and significantly (up to 20 times) increase the sensitivity of the chromatography method. Sample injection speed = 1 mL/min, time = 0.2 min.

The research was carried out as follows: To 0.50 mg of the dried extract in a 2 mL vial was added an internal standard (50 μg of tridecane in hexane) and 1.0 mL of a methylating agent—14% BCl_3 in methanol, Supelco No. 3–3033. The mixture was kept in a hermetically sealed vial for 8 hours at a temperature of 65 °C. During this time, phenolcarboxylic acids are completely extracted from the extract and transesterification of acids occurs. The reaction mixture was drained from the sediment and diluted with 1 ml of distilled water. To obtain methyl esters of fatty acids, 0.2 mL of methylene chloride was added, shaken for 1 hour and subjected to chromatography (Lowenthal et al., 2013).

Identification of the methyl esters of the acids was based on the calculation of the equivalent aliphatic chain length using data from the NIST 05 and Willey 2007 mass spectra library with a total number of spectra of more than 470,000 combined with the AMDIS and NIST identification programs. The retention time was also compared with the retention

time of standard compounds ("Sigma"). To calculate the quantitative determination of the components, the formula was used:

$$C(mg/kg) = K_1 \times K_2 \times 1000,$$

where, $K_1 = S_1/S_2$ (S_1 = square peak of analyzed substance, S_2 = square peak of standard substance); $K_2 = 50/M$ (50 = mass of internal standard, that injected with analyzed substance, μg); M = sample mass, mg.

Antioxidant activity

Antioxidant activity of extract was evaluated by potentiometric method (Maslov et al., 2021, 2022, 2022). Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:»

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

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

Test organisms

Collection strains of *Staphylococcus aureus ATCC 25923*, *Staphylococcus aureus ATCC 6538*, *Escherichia coli ATCC 25922*, *Proteus vulgaris NTCS 4636*, *Pseudomonas aeruginosa ATCC* 27853, and *Candida albicans ATCC 885/653* were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs**.**

Antimicrobial and antifungal activities

In our study, we used 1% solution of extract, the solvent of which were 60% ethanol. The method of diffusion of the drug into agar carried out using the method of "wells" (Maslov et al., 2021). Studies of antibacterial activity were performed using the method of wells. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using the equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to equipment and information list. Colony forming unit was 107 microorganisms at 1 ml of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 ml of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15–20 minutes Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples were incubated at 37 °C for 16–24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper. «Reference standards: fluconazole and gentamycin at concentrations 0.005 and 0.30%, respectively.

Statistical analysis

For all the experiments, two samples were analyzed and all the assays were carried out 5 times. The results were expressed as mean values with confident intervals. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.»

RESULTS

GC–MS analysis

The GC–MS method was used to carry out a qualitative and quantitative analysis of phenolcarboxylic acids in the obtained lipophilic extract of *R. idaeus* shoot. According to the results of the study, 8 compounds were identified: vanillic acid, benzoic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid, gentisic acid, salicylic acid, and phenylacetic acid (Fig. 1). The sum of phenolcarboxylic acids in the obtained extract was 6.75 mg/100 g (Table 1).
As shown in Table 1, vanillic acid dominates among all phenolcarboxylic acids (38.37% out of the total phenolcarboxylic acids), benzoic acid is in second place (22.37% out of the total phenolcarboxylic acids), and ferulic acid is in the third place, and the lowest content was phenylacetic acid (1.63% out of the total phenolcarboxylic acids). As can be seen from the above results, the content of vanillic, benzoic, and ferulic acids is 72.44% out of total phenolcarboxylic acids (Table 1).»

Antimicrobial and antifungal activities

Analyzed extract showed the antimicrobial and antifungal activities against *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 4636, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633,* and *Candida albicans ATCC 653/885* strains. According to the conducted research, it was found that extract strongly inhibited the growth of *B. subtilis* (17.00 ± 0.50 mm). In the case of Gram-negative bacteria, it was found that it strongly inhibited the growth of *E. coli* (15.00 ± 0.50 mm) and *P. aeruginosa* (17.00 ± 0.50 mm). The most resistant strains

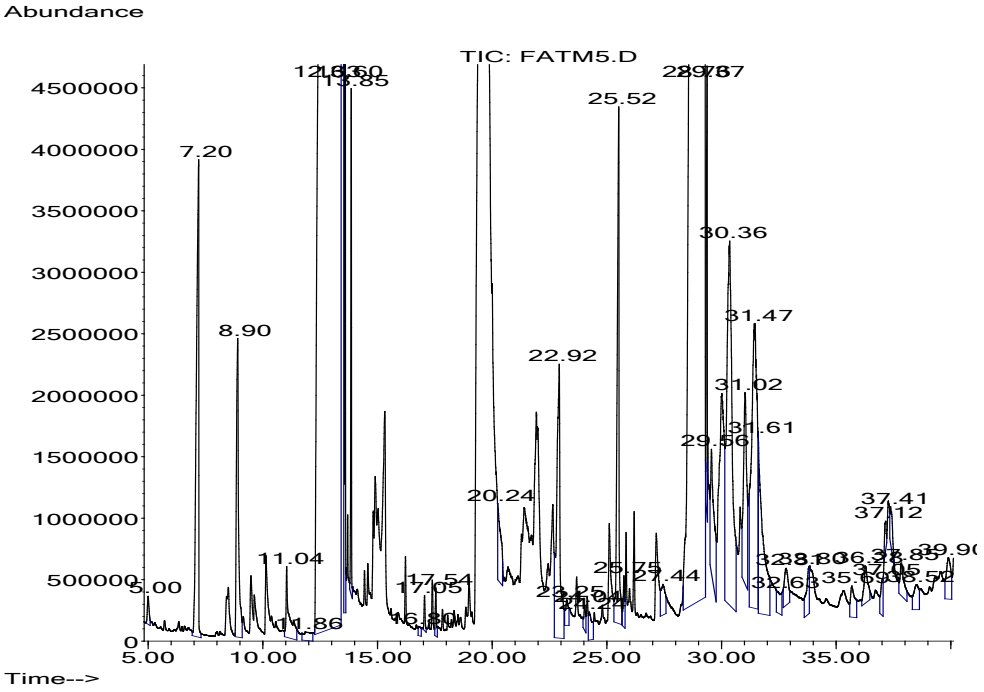


Figure 1. GC–MS fingerprint of Rubus idaeus shoot lipophilic extract.

Table 1. Qualitative composition and quantitative content of phenolcarboxylic acids in R. idaeus shoot lipophilic extract.

	Compound	Rt, min	Quantitative content in extract, mg/100 g	% out of sum phenolcarboxylic acids
1	Vanillic acid	31.664	2.59±0.08	38.37
2	Benzoic acid	14.076	1.51±0.08	22.37
3	Ferulic acid	39.815	0.79±0.04	11.70
4	*p*-hydroxybenzoic acid	36.935	0.61±0.04	9.04
5	Syringic acid	37.428	0.42±0.04	6.22
6	Gentisic acid	37.803	0.32±0.04	4.74
7	Salicylic acid	17.154	0.17±0.01	2.52
8	Phenylacetic acid	16.806	0.11±0.01	1.63
	Total content of identified compounds		**6.75**	**100**

among bacteria turned out to be *P. vulgaris. C. albicans* was medium sensitive for the obtained extract (13.50 ± 0.50 mm). Moreover, the obtained results showed that the obtained extract possessed antimicrobial effect against Gram-positive bacteria than Gram-negative (Table 2).

Antioxidant activity

The antioxidant activity was estimated by the potentiometric method. Table 3 shows that the antioxidant activity of lipophilic extract was 1.00 ± 0.01 mmol-equiv./$m_{dry\ res.}$. Comparing with standard «Ascorutin,» the level of antioxidant activity of the analyzed extract was lower at 98%. According to the developed new conditional classification of antioxidant activity by Maslov (Maslov et al., 2021), the lipophilic extract had a low level of antioxidant activity, whereas the standard «Ascorutin» was medium level (Table 3).

DISCUSSION

GC–MS analysis

The phenolcarboxylic acids describe the phenolic compounds having one carboxylic acid group. They are divided in two subgroups: hydroxybenzoic and hydroxycinnamic acids. In plant, typically, they are present in bound as esters, amides, and glycosides (Kumar & Goel, 2019). In a recent research by

Table 2. Antimicrobial and antifungal activities of R. idaeus shoot lipophilic extract.

Sample	Concentration, %	Diameter of the growth retardation zone, mm					
		Staphylococcus aureus ATCC 25923	*Escherichia coli ATCC 25922*	*Proteus vulgaris ATCC 4636*	*Pseudomonas aeruginosa ATCC 27853*	*Bacillus subtilis ATCC 6633*	*Candida albicans ATCC 653/885*
Lipophilic extract	**1.00**	16.50 ± 0.50	15.00 ± 0.50	14.00 ± 0.50	15.00 ± 0.50	17.00 ± 0.50	13.50 ± 0.50
Gentamycin	**0.005**	22.00 ± 0.20	24.00 ± 0.20	25.33 ± 0.33	25.00 ± 0.20	25.67 ± 0.67	12.00 ± 0.20
Fluconazole	**0.30**	18.00 ± 0.20	12.00 ± 0.20	14.33 ± 0.33	12.33 ± 0.33	10.00 ± 0.20	20.00 ± 0.50
60% ethanol	**—**	11.00 ± 0.20	10.00 ± 0.20	Growth	Growth	Growth	Growth

Table 3. Result of antioxidant activity of R. idaeus shoot lipophilic extract.

Sample	AOA, mmol-equiv./$m_{dry\ res.}$	The conditional level of antioxidant activity
Lipophilic extract	1.00 ± 0.01	Low level
«Ascorutin»	51.10 ± 1.05	Medium level

Pavlovic *et al.* (Pavlović et al., 2016), it has been evaluated that the extract was obtained with 70% methanol from *R. idaeus* shoot of cultivar "Willmatte." It was found that total phenolcarboxylic acids content was 24.19 mg/100 g in extract, whereas the content of gallic acid was 1.49 mg/100 g, *p*-hydroxybenzoic acid was 2.24 mg/100 g, gentisic acid was 0.10 mg/100 g, protocatechuic acid was 1.49 mg/100 g, 5-O-caffeoylquinic acid was 8.15 mg/100 g, *p*-coumaric acid was 1.05 mg/100 g, caffeic acid was 0.52 mg/100 g, and ferulic acid was 9.5 mg/100 g. Comparing with our research, the sum of phenolcarboxylic acids was lower at 72%, the content of *p*-hydroxybenzoic acid was lower at 73%, and the content of ferulic acid was lower at 92%. But the content of gentisic acid was higher at 69%. Moreover, in the *R. idaeus* leaf extract, the following compounds were not identified: benzoic, syringic, salicylic, and phenylacetic acid. In the case of *R. idaeus* lipophilic shoot extract, the following phenolcarboxylic acids were not determined: gallic, caffeic, *p*-coumaric, protocatechuic, and 5-O-caffeoylquinic acid. According to the compared results, the sum content of phenolcarboxylic acids in *R. idaeus* leaf extract is higher than in the obtained lipophilic extract. In our view, it relates with the biometabolism of flavonols in plant. According to the shikimate pathway, derivatives of phenolcarboxylic acids serve as precursors for flavonols. In the leaf extract, the content of flavonols was much higher than in the shoot, and it follows that the amount of phenolcarboxylic acids should be also higher in *R. idaeus* leaf extract.»

Antimicrobial, antifungal and antioxidant activities

In our recent studies, it has been demonstrated that the primary groups of biologically active compounds in *R. idaeus* shoots are derivatives of catechins and ellagitannins. Owing to the presence of these compound groups, the extracted *R. idaeus* shoot extract exhibits powerful antioxidant, antimicrobial, and antifungal effects. However, other phenolic compounds, such as phenolcarboxylic acids (vanillic, ferulic, salicylic acid, etc.), are also present in *R. idaeus* shoots but in minor quantities. We were interested in understanding whether these phenolcarboxylic acids possess antioxidant, antimicrobial, and antifungal properties, or if they are merely inert compounds with no pharmacological value.

To investigate this hypothesis, we conducted the following extraction method: Initially, a dual extraction of the raw material was performed using 60% ethanol, followed by ethanol evaporation and liquid–liquid extraction with chloroform. This is because phenolcarboxylic acid derivatives have high solubility in nonpolar solvents. The obtained extract was evaporated to a 1:2 mass ratio relatively to the raw material, and subsequent analysis was conducted to evaluate antimicrobial, antifungal, and antioxidant activities.

Our study revealed that the obtained lipophilic extract demonstrates antimicrobial activity against both Gram-positive and Gram-negative bacterial strains and exhibits antifungal activity against *C. albicans*. However, it shows low level of antioxidant activity. Consequently, derivatives of phenolcarboxylic acids contribute significantly to the antimicrobial and antifungal actions, while catechin and ellagitannins derivatives are responsible for the antioxidant activity. Based on these findings, it can be concluded that in the development of a pharmaceutical product with antioxidant properties, phenolcarboxylic acid derivatives should be eliminated.

According to the obtained data, at first glance, it can be considered that the antimicrobial and antifungal activities

of the lipophilic extract are significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was significantly lower than the content of phenolcarboxylic acids in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys, and liver, which can lead to serious complications of the disease. Comparing the antifungal effects of fluconazole and lipophilic extract, it was found that fluconazole inhibited the growth of the fungal strain at the higher level. We can declare that fluconazole is a leader as antifungal medicine, but at the same time it weakly inhibits the growth of Gram-negative and Gram-positive bacteria.

CONCLUSION

The lipophilic extract from *R. idaeus* shoots contains various phenolcarboxylic acids, including vanillic acid, benzoic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid, gentisic acid, salicylic acid, and phenylacetic acid, with the highest concentrations observed for vanillic, benzoic, and ferulic acids. This study highlights the antimicrobial and antifungal properties of the *R. idaeus* shoot lipophilic extract. The extract exhibited antimicrobial activity against all tested strains, with the most significant impact observed against *B. subtilis*. However, the obtained lipophilic extract showed a relatively low level of antioxidant activity. Consequently, the derivatives of phenolcarboxylic acids play a substantial role in the antimicrobial and antifungal effects, whereas their contribution to antioxidant activity appears to be limited.»

ACKNOWLEDGMENTS

This work was not supported by any program.

CONFLICTS OF INTEREST

The authors declare that there is no actual or potential conflict of interest, including any financial, personal, or other conflicts.

References

[1] Ikuta KS, Swetschinski LR, Robles AG et al. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*.2022;400:2221-48. doi: https://doi.org/10.1016/s0140-6736(22)02185-7

[2] Bongomin F, Gago S, Oladele R, Denning D. Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision. *J Fungi*. 2017;3(4):57. doi: https://doi.org/10.3390/jof3040057

[3] Maslov O 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: https://doi.org/10.2478/cipms-2023-0034

[4] Maslov OY et al. Study of total antioxidant capacity of red raspberry (Rubus idaeus L.) shoots. *Vitae*;30(1):1-8. doi: https://doi.org/10.17533/udea.vitae.v30n1a351486

[5] Komisarenko MA, Polischuk IM, Upyr TV, Saidov NB. Study of Amino acid composition and immunomodulatory activity of Rubus idaeus alcoholic extract. *Res J Pharm Technol*. 2021;14(3):1329-32. doi: https://doi.org/10.5958/0974-360x.2021.00236.5

[6] Derymedvid LV et al. Anti-inflammatory properties of raspberry shoot extract. Pharmacologyonline. 2021;(2):657-62.

[7] Maslov O et al. Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeus* l.) leaf extract. *Curr Issues Pharm Med Sci*. 2022;35(4):229-35. doi: https://doi.org/10.2478/cipms-2022-0040

[8] Lowenthal MS, Andriamaharavo NR, Stein SE, Phinney KW. Characterizing Vaccinium berry Standard Reference Materials by GC-MS using NIST spectral libraries. *Anal Bioanal Chem*. 2013;405(13):4467-76. doi: https://doi.org/10.1007/s00216-012-6610-6

[9] Maslov O, Kolisnyk S, Komisarenko M, Komisarenko A, Osolodchenko T, Ponomarenko S In vitro antioxidant and antibacterial activities of green tea leaves (*Camella sinensis* L.) liquid extracts. *An Mechnikov's Inst*. 2022;(2):64–7. doi: https://doi.org/10.5281/zenodo.6634846

[10] Maslov O, Kolisnyk S, Komisarenko M, Golik M. Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). *Herba Pol*. 2022;68(1):1-9. doi: https://doi.org/10.2478/hepo-2022-0003

[11] Maslov OY, Kolisnyk SV, Komisarenko NA, Kostina TA. Development and validation potentiometric method for determination of antioxidant activity of epigallocatechin-3-o-gallate. *Pharmacologyonline*. 2021;(2):35-42. doi: https://doi.org/10.5281/zenodo.7813097

[12] Maslov OY, Kolisnyk SV, Komisarenko MA, Kolisnyk OV, Ponomarenko SV. Antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts. *Pharmacologyonline*. 2021;(3):291-298. doi: https://doi.org/10.5281/zenodo.7813114.

[13] Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI. Study and evaluationantioxidant activity of dietary supplements with green tea extract. *Curr Issues Pharm Med*. 2021;14(2):215-9. doi: https://doi.org/10.14739/2409-2932.2021.2.233306

[14] Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Rep*. 2019;24:e00370. doi: https://doi.org/10.1016/j.btre.2019.e00370

[15] Pavlović AV et al. Phenolics composition of leaf extracts of raspberry and blackberry cultivars grown in Serbia. *Ind Crop Prod*. 2016;87:304-14. doi: https://doi.org/10.1016/j.indcrop.2016.04.052