



Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeus* L.) leaf extract

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ABSTRACT

The aim of the study. To determine phenolic and organic acids compound profiles, and the antioxidant, antibacterial and anti-inflammatory activities of raspberry leaf extract.

Materials and methods. The object of the study was red raspberry leaf extract. The quantity of phenolic compounds was determined by applying a spectrophotometric method of analysis, whereas organic acids content was assessed by means of the alkalimetric method, while the antioxidant activity of the obtained extract was evaluated by employing the potentiometric method, and antibacterial and antifungal activity was ascertained through the wells method, and anti-inflammatory activity was found via carrageenan – induced paw edema assay.

Results. The content of phenolic compounds was 18.45 ± 0.37 mg/ml, catechins was 10.12 ± 0.20 mg/ml, flavonoids was 3.32 ± 0.07 mg/ml, hydroxycinnamic acids derivatives was 2.39 ± 0.05 mg/ml and organic acids was 7.25 ± 0.15 mg/ml. Moreover, the antioxidant activity was 76.11 ± 1.48 mmol-equiv./m_{res dry}, which was higher by 32.80% than the reference drug „Ascorutin”. Staphylococcus aureus bacteria was the most sensitive to the extract (25.00 ± 0.00 mm), whereas Pseudomonas aeruginosa was the most resistant (21.67 ± 0.66 mm). Treatment with red raspberry leaf extract at 1 ml/kg showed a significant edema reduction at 1, 2 and 3 h at 38.8, 41.8 and 48.8%, compared with the saline group.

Conclusion. The present study indicated that red raspberry leaf extract possesses antioxidant, antibacterial and anti-inflammatory activities. Thus, red raspberry leaves are a promising source of bioactive substances that can be used for further developing medicines in the treatment and prevention of lifestyle diseases.

INTRODUCTION

The genus *Rubus* consists of around 700 species that usually occur in the temperate climate [1]. Raspberries are an aggregate fruit of the rose family that is commonly grown and consumed throughout Asia, Europe and America. They 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 [2].

Raspberry fruits, leaves and blossoms are commonly used for medicinal purposes. Raspberry leaves have been applied to treat gastrointestinal disorders, respiratory disorders, heart problems, the flu, fever and diabetes. The fruits traditionally have been consumed as cardioprotective, antitumor, anti-inflammatory and antipyretic agents. Moreover, raspberry blossoms are sourced for eye ointments or to treat stomach ailments [3].

Raspberry leaves and fruits are a rich source of flavonoid derivatives, including quercetin derivatives, as well as phenolic acids, organic acids and vitamin C [4]. Durgo *et al.*

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[5] have concluded that the main component among phenolic compounds is ellagic acid in three different forms: ellagitannins (in which ellagic acid forms esters with a sugar); free ellagic acid and ellagic acid glycosides.

A search of recent publications in Science Direct and PubMed has revealed that oxidative stress plays a main role in neuro- and cardiogenerative diseases, diabetes mellitus and arthritis. Oxidative stress is caused by decrease in endogenous antioxidant defense or by increase of free radicals. Free radicals are highly reactive compounds due to presence of an unpaired electron in an outer orbital [6]. It is known that the inflammation process is a protective mechanism against different damaging factors such as viruses, microorganisms, mechanic influences and others. Most studies have revealed that activated neutrophils, eosinophils, monocytes and macrophages produce free radicals at lysosomal enzymes where inflammation is occurring [7].

A search of ScienceDirect and PubMed has showed there is great problem with microbial mutation that is caused by uncontrolled prescription, mismanagement and maladministration of antibiotics [8]. This process has led to the development of drug-resistant strains. As result, antibiotics that have applied to cure infectious diseases have lost their effectiveness. Therefore, the search for new antimicrobial drugs from natural sources is warranted.

We can conclude that oxidative stress and inflammation process are underlying issues in disease development. Hence, it is important to create a medicine with potent antioxidant and anti-inflammatory potential. There are various non-steroidal anti-inflammatory drug (NSAIDs) which are used in clinical practice for treating intestinal diseases, atherosclerosis, reperfusion injuries, cardiac diseases, neurodegeneration, respiratory disorders, diabetes and cancer. Their major disadvantage is low level of antioxidant and antibacterial activities. This outcome decreases the effectiveness of treating inflammation. That is why it is important to pay attention to herbal medicines containing various natural compounds. Literature sources have shown that many time-tested herbal medicines have potent antioxidant activity and low toxicity – and rarely cause serious adverse reactions [9].

In the view of the above, the aim of the study was to determine phenolic and organic acids compound profiles, antioxidant, antibacterial and anti-inflammatory activities of raspberry leaf extracts.

MATERIALS AND METHODS

Plant material

Red raspberry leaves were the object of the study. These were collected in the places of its cultivation in 2021, during the fruiting period, in the vicinity of the village of Ternova, Kharkiv region. All solvent and other chemical used in the study were of analytical grade.

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 using an UV-spectrophotometer UV – 1000 (China) with matched 1 cm

quarts cells. Weighing was carried out employing a digital analytical balance AN100 (AXIS, Poland) with $d=0.0001$ g.

Extraction procedure

A 10.0 g (exact mass) of fresh red raspberry leaves were ground down using a mortar and pestle until it could pass through a mesh of size 1-2 mm. The material was then placed in a water bath with a condenser. The extraction was carried by 60% ethanol at 80°C within 1 hour, raw material/solvent ratio being 1/20. The procedure was performed twice to provide completely extraction of biological active substances (BAS), then the filtrates were united and concentrated by vacuum evaporator to a ratio of extract to raw material of 1:2.

Qualitative analysis

The total content of phenolic compounds was measured via Folin-Ciocalteu assay, the optical density was measured at 760 nm [10]. The calibration curve was plotted with interval concentrations 1.0-5.0 $\mu\text{g/ml}$, the calibration equation being $Y = 0.1055X + 0.1745$ ($R^2=0.9951$) expressed as gallic acid and calculated according to the following equation:

$$X (\text{mg/ml}) = \frac{C_x \times K_{\text{dil}} \times 1000}{V} \quad (\text{Eq.1})$$

where: C_x – concentration of gallic acid according to the calibration curve, $C \times 10^{-6}$, g/ml; V – volume of extract, ml; K_{dil} – coefficient of dilution

Vanillin reagent assay was then applied to determine total catechins [11]. The absorbance was measured at 505 nm. The calibration curve was plotted with interval concentrations 100 – 400 $\times 10^{-6}$ g/ml, the calibration equation being $Y = 0.0025X - 0.0851$ ($R^2=0.9951$). The total catechins content in the extract, expressed as epigallocatechin-3-O-gallate, was calculated according to the following equation:

$$X (\text{mg/ml}) = \frac{C_x \times K_{\text{dil}} \times 1000}{V} \quad (\text{Eq.2})$$

where: C_x – concentration of epigallocatechin-3-O-gallate according to calibration curve, $C \times 10^{-6}$ g/ml; V – volume of extract, ml; K_{dil} – coefficient of dilution

The total flavonoids were determined using an assay of complex formation with AlCl_3 , the absorbance was measured at 417 nm [12]. The total flavonoids content in the extract, expressed as rutin, was calculated according to the following equation:

$$X (\text{mg/ml}) = \frac{A \times K_{\text{dil}} \times 1000}{A_{\text{st}} \times V} \quad (\text{Eq.3})$$

where: A – absorbance of analyzed solution; A_{st} – absorbance of standard solution of rutin; V – volume of extract, ml; K_{dil} – coefficient of dilution

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 [13]. The total content of hydroxycinnamic acids derivatives in the extract, expressed as chlorogenic acid was calculated according to the following equation:

$$X (\text{mg/ml}) = \frac{C_x \times K_{\text{dil}} \times 1000}{188 \times V} \quad (\text{Eq.4})$$

where: A – absorbance of analyzed solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, ml; K_{dil} – coefficient of dilution

The total organic acids content was determined by acid-base titration via the fixation of end-point by applying the potentiometric method [14]. The total content of organic acids in the extract, expressed as citric acid, was calculated according to the following equation:

$$X(\text{mg/ml}) = \frac{(V_{\text{equiv}} - V_x) \times 0,0032 \times K_{\text{dil}} \times K \times 1000}{V} \quad (\text{Eq. 5})$$

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

Antioxidant activity assay

Antioxidant activity (AOA) of the extract was evaluated by utilizing the potentiometric method [15,16], and was calculated according to the following equation and expressed as mmol-equiv./m_{dry res.}:

$$AOA = \frac{C_{\text{ox}} \times \alpha \times C_{\text{red}}}{1 + \alpha} \times K_{\text{dil}} \times 10^3 \times \frac{m_1}{m_2} \quad (\text{Eq. 6})$$

where: $\alpha = C_{\text{ox}}/C_{\text{red}} \times 10^{(\Delta E - E_{\text{ethanol}})/nF/2.3RT}$; C_{ox} – concentration of $K_3[\text{Fe}(\text{CN})_6]$, mol/l; C_{red} – concentration of $K_4[\text{Fe}(\text{CN})_6]$, mol/l; $E_{\text{ethanol}} = 0.0546 \cdot C_{\text{ethanol}} - 0.0091$; C_{ethanol} – concentration of ethanol; ΔE – change of potential; $F = 96485.33 \text{ C/mol}$ – Faraday constant; $n = 1$ – number of electrons in electrode reaction; $R = 8.314 \text{ J/molK}$ – universal gas constant; $T = 298 \text{ K}$; 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

Anti-inflammatory activity assay

The antiexudative activity of the extract was studied on 56 white outbred male rats weighing 180-220 g, in which a model of acute inflammation induced by subplantar injection of 0.1 ml of 1% carrageenan (Fluka, Switzerland) into the right hind paw was reproduced. Measurement of paw edema in rats was carried out after 1, 2, 3, 8, 24 hours, taking into account that after the administration of carrageenan, maximum edema is observed by the third hour and the edema gradually decreases during the day. For this, the volume of the paws in cm³ was measured using a digital plethysmometer Panlab (Spain) model LE 7500 version V29/10/2014. The amount of edema in each case was determined by the difference in volume between the edematous and healthy paws and was expressed in %, indicating how much the study drug inhibits the development of edema compared to the control, where the amount of edema was taken as 100%. The activity of extract and reference drug was calculated using the formula [17]:

$$A = \frac{(M_s - M_h) \times 100}{M_{\text{sh}} - M_{\text{hc}}} \quad (\text{Eq. 7})$$

where: A – antiexudative activity, %; M_s is the volume of the swollen paw in the experiment; M_h is the volume of a healthy paw in the experiment; M_{sc} – the volume of the swollen paw in the control; M_{hc} is the volume of a healthy paw in the control

All animals were divided into 6 groups. The first group was control pathology (animals that were subplantar administered a solution of carrageenan and intragastrically administered with 0.5 ml/kg of distilled water). The second

and third group were animals that were administered carrageenan solution subplantar, while the studied extract was administered intragastrically at a dose of 0.5 ml/kg and 1 ml/kg, respectively. Animals of groups 4 and 5 were administered intragastrically, drugs of comparison against the background of the introduction of carrageenan: diclofenac sodium at a dose of 8 mg/kg; group 6 consisted of intact animals, which were administered 0.1 ml of saline subplantar.

Experimental animals

The experiment was performed on 56 white outbred male rats weighing 180-220 g. The animals were obtained from the vivarium of the National University of Pharmacy (NUPH) (Ukraine, Kharkiv). At the time of the study, the rats were kept in macrolon boxes (5 animals each) with free access to water and food. Water and feed were supplied daily; bedding was changed once every three days. Containment conditions were as follows: temperature 22±2°C, relative humidity 60±5%, daily cycle – 12 hours a day, 12 hours a night. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee.

Test organisms

Museum 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 activity assay

Studies of antibacterial activity were performed by applying the wells method. In our work, we used 1% solution of extract, the solvent of which was 60% ethanol. Diffusion of the drug into agar came about via the wells method as per [18]. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) using a Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to the equipment and information list of [19]. The colony forming unit was 107 microorganisms at 1 ml of growth medium and was assessed by applying the McFarland standards. In doing so, 1 ml of the microorganism suspension was placed on solidified agar in sterile Petri dishes, using a pipette under sterile conditions. After uniform distribution of these 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 subsequently 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 was measured using a caliper. Chlorophyll spray manufactured by the State Scientific

Center of Drugs (DNCLZ) with concentration 1% in 96% ethanol was used as the reference drug.

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 interval. Programs MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.

RESULTS

Determination the content of phenolic compounds and organic acids in extract

Total phenolic compounds were determined by application of the Folin-Ciocalteu method, and expressed as gallic acid equivalent. As shown in Table 1, the content of phenolic compounds in the extract was 18.45 ± 0.37 mg/mL, whereas the amount of catechins was 10.12 ± 0.20 mg/mL. The percentage of catechins out of total phenolic compounds was 54.85%. Total amount of flavonoids was measured via AlCl_3 complex formation assay. The flavonoids content was 3.32 ± 0.07 mg/ml. The percentage of flavonoids out of total phenolic compounds was 18%. Total hydroxycinnamic acids derivatives content was determined by NaNO_2 – NaMoO_4 and expressed as chlorogenic acid. The amount of hydroxycinnamic acids derivatives was 2.39 ± 0.05 mg/ml. The percentage of hydroxycinnamic acids derivatives out of total phenolic compounds was 12.95%. The content of organic acids observed in 60% extract was 7.25 ± 0.15 mg/ml, the amount of free organic acids was 61% less than total phenolic compounds in extract.

Table 1. The total content of phenolic, flavonoid, hydroxycinnamic acids derivatives compounds and organic acids in red raspberry leaves liquid extract

Sample	Mass of dry residue of extract (%)	Total phenolic content (mg/ml)	Total catechins content (mg/ml)	Total flavonoid content (mg/ml)	Total hydroxycinnamic acids derivatives content, (mg/ml)	Total organic acids, (mg/ml)
60% extract	16.70 ± 0.17	18.45 ± 0.37	10.12 ± 0.20	3.32 ± 0.07	2.39 ± 0.05	7.25 ± 0.15

Determination of antioxidant property of red raspberry leaves extract

The AOA values of investigated extract was estimated by applying the potentiometric method. Based on the conducted research, the antioxidant activity of investigated extract was $76.11 \text{ mmol-equiv./m}_{\text{dry res}}$ – which is 32.80% higher than the reference drug „Ascorutin” (Table 2).

Table 2. Result of antioxidant activity of red raspberry leaves liquid extract

Sample	AOA (mmol-equiv./m _{dry res.})
60% extract	76.11 ± 1.52
„Ascorutin”	51.10 ± 1.05

Determination of antibacterial and antifungal properties of red raspberry leaves extract

The analysed extract showed antimicrobial and antifungal properties 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. We found that the extract strongly inhibited the growth of *Staphylococcus aureus* (25.00 ± 0.00 mm). In the case of Gram-negative bacteria, the extract strongly inhibited the growth of *Escherichia coli* (24.67 ± 0.66 mm). The most resistant strain among the tested bacteria turned out to be *Pseudomonas aeruginosa*. In contrast, *Candida albicans* was highly sensitive to the obtained extract (24.67 ± 0.66 mm). Our results showed that the obtained extract possessed higher antimicrobial effect against Gram-positive bacteria than Gram-negative. Moreover, on comparing the results of the investigated extracts and the reference drug – „Chlorophyllipt” (DNCLZ), the reference drug was found to be inferior to the antibacterial activity of the analysed extracts. Results are shown in Table 3.

Table 3. Antimicrobial and antifungal activity of red raspberry leaves extract and reference drug

Sample	Diameter of the growth retardation zone, mm					
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Proteus vulgaris</i> ATCC 4636	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 653/885
60% extract	25.00 ± 0.00	24.67 ± 0.66	22.67 ± 0.66	21.67 ± 0.66	24.33 ± 0.33	24.67 ± 0.66
Chlorophyllipt (DNCLZ)	19.33 ± 0.33	19.33 ± 0.33	17.67 ± 0.66	17.67 ± 0.66	19.33 ± 0.33	19.00 ± 0.00

Determination of anti-inflammatory properties of red raspberry leaves extract

Treatment with red raspberry leaves extract brought about significantly reduced mice paw edema (38.8%) when compared with the saline group effect from the first hour of test ($p < 0.05$). Thereafter, these treatments reduced edema by 41.8%, 48.8%, 20.2% and 17.8% by 2, 3, 8 and 24 hrs, respectively ($p < 0.05$ at all times), compared to saline. Treatment with the 0.5 ml/kg dose of red raspberry leaves extract showed lower results to that of treatment with the 1 ml/kg dose. As of the first hour, mice paw edema was reduced by 25.6%, thereafter, edema was mitigated by 27.2%, 36.1%, 14.1% and 5.1% by 2, 3, 8 and 24 hrs, respectively ($p < 0.05$ at all times), compared to saline. Treatment with red raspberry leaves extract at 1 ml/kg showed significant edema reduction by 1, 2 and 3 hrs after induction, compared to the effect generated by diclofenac sodium ($p < 0.05$), but by 8 and 24 hrs, lower reduction of edema than that of diclofenac sodium was evident. The effect of treatment with 0.5 ml/kg of red raspberry leaves extract was significant worse than that of diclofenac sodium. All results are shown in Table 4.

Table 4. Effect of extract on the exudation process in carrageenan inflammation in rats ($M \pm m$, $n=8$)

Studied sample	Dose	% of edema inhibition compared to control				
		1 hour	2 hours	3 hours	8 hours	24 hours
60% extract	1 ml/kg	38.8 ± 2.6	41.8 ± 7.2	48.8 ± 4.4	20.2 ± 3.8	17.8 ± 7.2
60% extract	0.5 ml/kg	25.6 ± 6.1	27.2 ± 4.1	36.1 ± 2.8	14.1 ± 6.1	5.1 ± 1.1
Diclofenac sodium	8 mg/kg	32.4 ± 3.7	40.5 ± 5.1	54.6 ± 4.8	36.7 ± 5.9	24.7 ± 3.3

DISCUSSION

Phenolic compounds are the most important type of plant phytochemical with regard to potential medical use. They possess a variety of pharmacological effects, among others, as antioxidants, anti-inflammatories, antidiabetics, antivirals and antimicrobials [20]. Our results are similar to those of Bobinatie R. *et al.* [21], who investigated the red raspberry leaf medicinal properties of 41 cultivars. In their work, they found that the phenolic compounds content varied in different samples from 1.0 to 6.0 mg/mL in ethanolic extract. However, compared to our results, it can be seen that in our study, the amount of contained phenolic compounds is higher from 3 to 18 times.

Catechins are polyphenolic compounds that are flavanols of the flavonoid family that are found in a variety of plants [22]. They play a significant role in scavenging free radicals as metal ion chelators and are known to inhibit oxidative enzymes and activate antioxidants defense systems. Due to such properties, they are beneficial in preventing and protecting against diseases caused by oxidative stress (among others, ischemia, diabetes mellitus, Alzheimer's disease, cancer and atherosclerosis) [23]. In the recent research of Durgo K. *et al.* [24], the total catechins content was 0.17 mg/mL in an aqueous extract of red raspberry leaves. In our research, the amount of catechins in aqueous extract was higher by 60 times.

Flavonoids are polyphenolic compounds which are abundant in fruits, vegetables and teas, and have well-documented cardio- and neuroprotective actions through multiple mechanistic routes [25]. Moreover, they improve platelet and endothelial function, inhibit oxidative enzymes and strengthen antioxidant defense [26]. In the research of Costa T. *et al.* [27], the amount of flavonoids was 12.92 mg/mL in a 20% ethanolic extract of red raspberry leaves. Compared to our result, the amount of flavonoids in the obtained 60% extract was lower by 74%.

Phenolic acids are secondary plant metabolites belonging to the polyphenols that are found throughout the Plant Kingdom. The high interest in phenolic acids shown by researchers is associated with their high potential antioxidant, anti-collagenase, anti-inflammatory, antimicrobial and anti-tyrosinase activities, as well as their ultraviolet (UV) protective effects [28,29]. Costa T. *et al.* [27] has established the amount of hydroxycinnamic acids derivatives in 20% ethanolic extract of red raspberry leaves to be 9.28 mg/mL in 20% ethanolic extract, whereas in our case, our outcome was 74.25% higher.

Organic acids are a group of biologically active compounds that hold vitamin properties and a chlorotic effect and enhance the secretion of bile and pancreatic juices [30]. According to recent studies, organic acids also show potent antibacterial activity against Gram-positive and Gram-negative strains [31]. Mikulic-Petkovsek M. *et al.* [32] investigated the quantity of organic acids in aqueous extract of red raspberry fruits by applying high performance liquid chromatography. This study revealed that the total organic acids content was 2.2 mg/mL. When compared with our results with regard to 60% extract, the amount of organic

acids is greater by 69.66% than in that in the study reported by the authors.

With regard to obtained results of quantitative analysis of biological active compounds in the extracts, the main phenolic compounds were catechins, followed by flavonoids and hydroxycinnamic acids derivatives. We also observed a high content of organic acids. Hence, organic acids have an important role in the pharmacological effects of red raspberry leaves.

In this study, we chose to apply the potentiometric method, as it is characterized by high sensitivity, rapid analysis procedure and relatively low equipment and reagents cost, and, hence, cost of analysis as a whole [33]. Based on our recent study, in determining antioxidant activity of natural antioxidants by the potentiometric method, the ethanol that was used as solvent contributed to the level of antioxidant activity of the analyzed sample. As result, an analytical method has been put forward that takes the influence of ethanol into account [34].

Different extract antioxidant activity with regard to plant part was indicated in the work of Teleszko M. *et al.* [35]. They studied and compared the antioxidant activity of red raspberry fruits and leaves extracts using ABTA and FRAP assays. According to the research, the strongest antioxidant potential was demonstrated by leaf extract rather than by fruits. This effect is related to the higher content of polyphenolic compounds within leaf tissues.

Staszowska-Karkut M. *et al.* [36], in determining the main biologically active compounds of red raspberry leaf, revealed the order of activity to be, primarily, ellagic acid derivatives, secondarily – catechins. In our conducted study, we observed that the percentage of catechins among the phenolic compounds was 54.85% in the analyzed 60% extract. Our work indicates that the catechins and ellagic acid derivatives are the main contributors to the antibacterial, antioxidant and anti-inflammatory activities of red raspberry leaf extract. According to the literature sources, the mechanism of antibacterial activity of catechins and ellagic acid derivatives is based in inhibiting biofilm formation, as well as dehydrofolate reductase, and DNA gyrase activity [36,37], which in turn leads to changes in cell wall permeability, denaturation of proteins present in microbial cells and ultimately the death of bacteria [38]. Many studies have revealed that catechins and ellagic acid derivatives are able to modulate cellular components that participate in the mechanism of inflammation, such as the procytokines, TNF- α and IL-1, and to inhibit the activity of enzymes involved in the arachidonic acid pathway, such as cyclooxygenase and lipoxygenase [39].

CONCLUSION

The red raspberry leaves extract shows a very high content of phenolic compounds, catechins and organic acids. The present work shows that a 60% extract of red raspberry leaves possesses remarkable antioxidant, antibacterial and anti-inflammatory activities. Moreover, the red raspberry leaves extract has quite high antimicrobial activity in relation to all strains, while the greatest effect is upon *Staphylococcus aureus*. Over all, red raspberry leaves are

a promising source of bioactive substances that can be used as replacements for synthetic antioxidant, antibacterial and anti-inflammatory medicines in the treatment and prevention of lifestyle diseases. They may also be used as valuable food additives and cosmetological products, thus increasing the functional qualities of food.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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