

Anti-inflammatory and antioxidant activity of *Rubus idaeus* shoot extract: An *in silico* molecular docking study

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Abstract

Oxidative stress and inflammation are key contributors to cardiovascular, oncological, and neurodegenerative diseases, making natural compounds with dual antioxidant and anti-inflammatory effects of particular interest. This study investigated raspberry (*Rubus idaeus*) shoot extract by identifying its main biologically active substances (BAS) and evaluating its activity through *in silico*, *in vitro*, and *in vivo* approaches, as well as exploring the relationship between its antioxidant and anti-inflammatory potential. High-performance liquid chromatography (HPLC) revealed eleven compounds, dominated by epicatechin, (+)-catechin, and ellagic acid derivatives. Molecular docking demonstrated a strong affinity of catechins for the active sites of cyclooxygenase-2 (COX-2) and NADPH oxidase, indicating their role in modulating both oxidative and inflammatory pathways. The extract exhibited clear dose-dependent antioxidant activity, while *in vivo* testing using a carrageenan-induced edema model confirmed a significant reduction in inflammation. Importantly, correlation analysis showed a very high positive association between antioxidant and anti-inflammatory activities, suggesting that the latter is largely mediated through the extract's redox-modulating capacity. The consistency between theoretical predictions and experimental outcomes strengthens the evidence for the extract's biological efficacy. Taken together, these findings highlight raspberry shoot extract as a valuable source of polyphenolic compounds, particularly catechins, with significant antioxidant and anti-inflammatory activity, supporting its potential as a natural therapeutic candidate for conditions associated with oxidative stress and inflammation.

Key words: antioxidant activity; anti-inflammatory activity; correlation; HPLC; molecular docking

Introduction

Inflammation and oxidative stress are always found in many chronic diseases: diabetes mellitus, hypertension, atherosclerosis, Alzheimer's disease, cancer and aging (Biswas, 2016). During the inflammatory process, neutrophils and macrophages produce large amounts of free radicals to kill the invading foreign agent. In the course of research, it was found that in addition to phagocytic cells, also non-phagocytic cells are capable of producing free radicals. For example, recent studies have shown that interleukin-6 (IL-6) produces free radicals through expression of NADPH oxidase (Drummond *et al.*, 2011). In addition to the fact that the inflammatory process can cause oxidative stress, oxidative stress can also cause inflammation. In studies, hydrogen peroxide free radicals have been found to induce inflammation by activating the transcriptional enzyme NF- κ B, p38 MAP kinase, and N-terminal c-Jun kinase (Fan *et al.*, 2013; Keshari *et al.*, 2013; Yarza *et al.*, 2016). All of the above discussions indicate that inflammation and oxidative stress are closely related pathophysiological processes, one of which can easily induce the other. Thus, new drugs should have antioxidant and anti-inflammatory activity.

In the countries of Eastern Europe, red raspberry (*Rubus idaeus* L.) is a fairly well-known plant, not only because of the edible fruits, but because of the use of leaves and fruits in traditional medicine as a diaphoretic, antiviral and anti-inflammatory agent (Maslov *et al.*, 2024). In addition, in folk medicine, the use of common *R. idaeus* shoots was noted in the treatment of influenza (Polishchuk *et al.*, 2018).

R. idaeus shoots are a rich source of phenolic compounds (Komisarenko *et al.*, 2021). The chemical composition is dominated by derivatives of ellagitannins and flavan-3-ols. Ellagotannins are a group of hydrolysable tannins, the monomer of ellagitannins is an ester of glucose and ellagic acid. Sanguin H-6, H-10 and lambertianin C are the main compounds of ellagitannins (Kula *et al.*, 2016). The main representatives of flavan-3-ol derivatives are epicatechin and (+)-catechin (Krauze-Baranowska *et al.*, 2014; Garjonyte *et al.*, 2022).

In recent scientific studies indexed in PubMed and Scopus, ellagic acid and epicatechin have been found to have powerful anti-inflammatory and antioxidant effects. In recent research (Ghorbanzadeh *et al.*, 2024) studied anti-inflammatory and antioxidant activity in a carrageenan-induced mouse paw edema model. The results of the study showed that systemic administration of ellagic acid (1-30 mg/kg) reduces edema in a dose-dependent manner in the inflammatory paws of rats, reduces the serum concentration of nitric oxide (NO), prostaglandin E2 (PGE2), suppresses the expression of endothelial NOS (eNOS) enzymes, COX-2 and attenuates the production of tumor necrosis factor (TNF- α), interleukin-1 β (IL-1 β) in inflammatory paw tissue.

In recent research *in vitro*, Yang *et al.* (2015) studied the effect of epicatechin on lipopolysaccharide-induced production of pro-inflammatory mediators in RAW264.7 cells. During the analysis, it was found that epicatechin suppresses the expression of eNOS, COX-2, production of NO, PGE2, pro-inflammatory cytokines IL-6, IL-1 β and TNF- α in RAW264.7 cells.

In our recent studies, we have shown that *R. idaeus* leaves extract has anti-inflammatory, antioxidant and antimicrobial effects (Maslov *et al.*, 2022). But, there is no data on the antioxidant and anti-inflammatory activity of *R. idaeus* shoots, as well as their correlation with these pharmacological effects. Taking into account the pharmacological action of biologically active substances (BAS) contained in red *R. idaeus* shoots, we support the hypothesis that the resulting extracts of red *R. idaeus* shoots have anti-inflammatory and antioxidant activity.

Therefore, the aim of our study was to determine the main BAS by HPLC method, to conduct theoretical and practical studies to determine the antioxidant and anti-inflammatory activity *in silico*, *in vitro*, *in vivo* of *R. idaeus* shoot extract, and to study the correlation of pharmacological actions.

Materials and Methods

Raw material

The leafless second year red *Rubus idaeus* shoots were the object of the study, which were collected in the places of its cultivation. The material was collected in 2021 after the fruiting period in the vicinity of the village of Ternova, Kharkiv region (50°19'31" N, 36°66'93" E). Green tea leaves of cultivars Chun Myn were collected in Anhui province

Equipment

The pH meter HANNA 2550 (FRG) with a combined platinum electrode EZDO 50 PO (Taiwan) was applied for potentiometric measurements. Weighing was carried out using digital analytical balance AN100 (AXIS, Poland) with $d = 0.0001$ g.

Technique of Extraction

A 10.0 (exact mass) g of red *R. idaeus* shoots were grinded in the size 1-2 mm. The extraction was carried out one by 60% ethanol at the ratio raw material/solvent 1/20 (m/v) on water bath at 80 °C with a reflux condenser during 1 hour, the extraction was made two times. After cooling, the solutions were filtrated and concentrated to 20 mL by a rotary evaporator at 40 °C under a vacuum. The green tea extract was obtained by 60% ethanol according to procedure mentioned in our previous research (Maslov *et al.*, 2021).

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.

HPLC method of analysis

The chromatographic separation was carried out by Agilent Technology model 1100 chromatograph with 150 mm × 2.1 mm ZORBAX-SB C-18 column with granularity at 3.5 μm. Elution flow rate was 0.25 ml/min. All determinations were undertaken at 45 °C. The mobile phase binary solvent system consisted of solvent A (0.6% trifluoroacetic acid) and solvent B (70% methanol). All solvents passed through ultrasonic degassing and 0.22 μm pore size membrane filtering. The sample injection volume was 2 μml and the detection was carried out at 254, 280, 350, 525 nm. The linear mobile phase gradient was as follows (Table 1).

Table 1. Linear mobile phase gradient

Time (min)	0.6% trifluoroacetic acid	70% methanol
0	92	8
8	62	38
24	0	100
24.1	0	100
29	0	100

Molecular docking

Molecular docking study was with the tool known as AutoDockTools 1.5.6 (Morris *et al.*, 2008). The preparation of protein was involved optimization process of it, removing water and other atoms, then adding a polar hydrogen group. Autogrid was used to arrange the grid coordinates (X, Y and Z) on the binding site. The genetic algorithm parameters were applied for ligand interaction with using 10 runs of this one criteria.

COX-2 (PDB ID: 1ddx) and NADPH oxidase (PDB ID: 5o0X) structures were obtained from PDB database (Burley *et al.*, 2025). The ligand structures of (+)-catechin (CID_9064), (-)-epicatechin (CID_72276), and ellagic acid (CID_5281855) were obtained from PubChem database (Kim *et al.*, 2025). Active site of docking protein was determined using the Computed Atlas for Surface Topography of Proteins (Tian *et al.*, 2018).

Antioxidant activity

Antioxidant activity of extract was evaluated by potentiometric method (Maslov *et al.*, 2023). Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./m_{dry res.}:

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

where, $\alpha = C_{ox}/C_{red} \times 10(\Delta E - E_{ethanol})nF/2.3RT$; 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.

Anti-inflammatory activity

The anti-exudative activity of 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 of rats 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, then 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 (Stefanov, 2001):

$$A = \frac{(M_s - M_h) \times 100}{M_{sh} - M_{hc}} \quad (Eq.2)$$

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 subplantarily administered solution of carrageenan and intragastrically administered with 0.5 ml/kg of distilled water). The second, third and fourth group - animals that were administered carrageenan solution subplantarily and the studied extract was administered intragastrically at a dose of 0.65 mg/kg and 6.0 mg/kg, 13.0 mg/kg, respectively. Animals of group 5 were administered intragastrically drugs of comparison against the background of the introduction of carrageenan: diclofenac sodium at a dose of 8 mg/kg; The 6 group was consisted of intact animals, which were administered 0.1 ml of saline subplantarily.

Correlation analysis

Pearson's (r) correlation coefficient was used to analyze the correlation between antioxidant activity (AOA) and the amount of phenolic, catechin, flavonoid, hydroxycinnamic acids derivatives and organic acids. The correlation coefficient 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 (Mukaka, 2012).

Statistical analysis

For all the experiments, two samples were analyzed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis. Comparisons between study groups were performed using parametric and nonparametric methods (Newman-Keuls and Mann-Whitney test). Differences between study groups were considered statistically significant at $p < 0.05$.

Results

HPLC analysis

The HPLC method was used to carry out a qualitative and quantitative analysis of phenolic compounds in the obtained extract of *R. idaeus* shoots. According to the results of the study, 11 compounds were identified (Figures 1 and 2). The total content of phenolic compounds in the obtained extract was 1.906% of which flavan-3-ols (catechins) - 1.362% (71.46% out of the total polyphenols), ellagitannins - 0.085% (4.46% out of the total polyphenols), ellagic acid derivatives - 0.459% (24.08% out of the total polyphenols) (Table 2).

Among flavan-3-ols, epicatechin dominates – $0.882 \pm 0.018\%$ (46.28% out of the total polyphenols), and (+)-catechin – $0.480 \pm 0.005\%$ (25.19% out of the total polyphenols). Among ellagitannins, 3 compounds were identified: sanguine H-10 isomer 1 - $0.003 \pm 0.0005\%$ (0.16% out of the total polyphenols), sanguine H-10 isomer 2 - $0.032 \pm 0.001\%$ (1.69% out of the total polyphenols), lambertianin C - $0.010 \pm 0.001\%$ (0.53% out of the total polyphenols) and sanguine H-6 - $0.040 \pm 0.001\%$ (2.10% out of the total polyphenols) (Table 2).

As shown in Table 2, sanguine H-6 dominates among all ellagitannins, sanguine H-10 isomer 2 is in second place, and sanguine H-10 isomer 1 is in third place, and the lowest content was lambertianin C. The content of ellagic acid was $0.181 \pm 0.004\%$ (0.50% of the total phenolic compounds). The content of ellagic acid and its derivatives is 81% higher than that of ellagitannins.

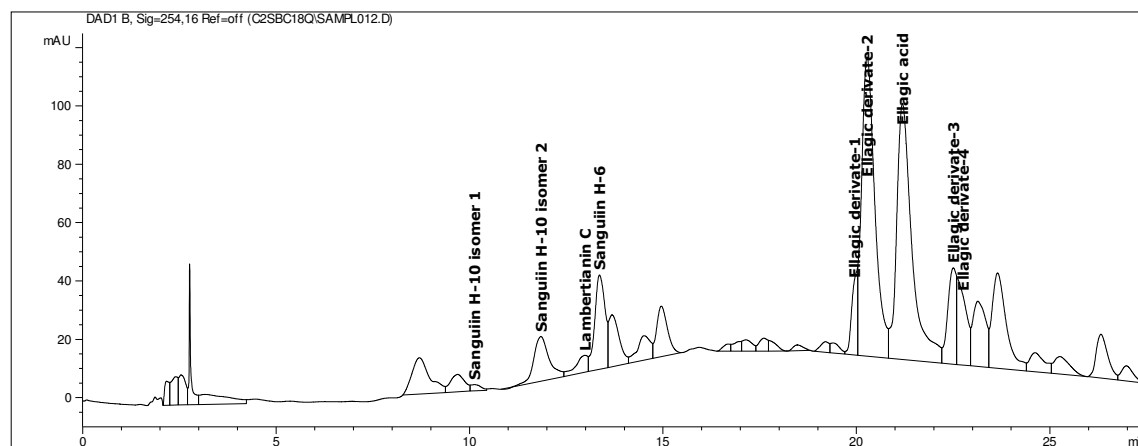


Figure 1. HPLC fingerprint (254 nm) of the *R. idaeus* shoots extract

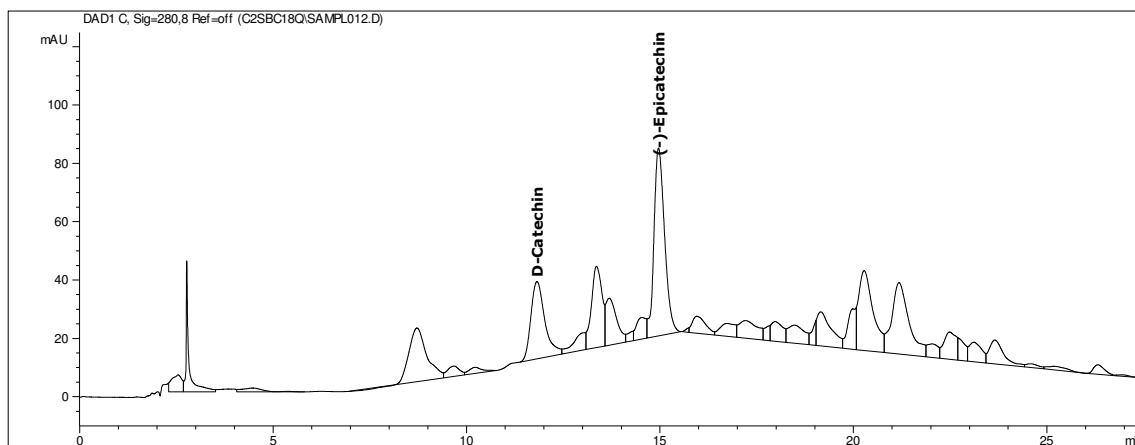


Figure 2. HPLC fingerprint (280 nm) of the *R. idaeus* shoots extract

Table 2. Qualitative composition and quantitative content of polyphenols in *R. idaeus* shoots extract

No	Compound	Rt (min)	Quantitative content (%)	% out of sum polyphenols
1	Sanguiin H-10 isomer 1	10.08	0.003±0.0005	0.16
2	(+)-Catechin	11.89	0.480±0.005	25.19
3	Sanguiin H-10 isomer 2	11.91	0.032±0.001	1.69
4	Lambertianin C	12.91	0.010±0.001	0.53
5	Sanguiin H-6	13.38	0.040±0.001	2.10
6	(-)-Epicatechin	14.96	0.882±0.018	46.28
7	Ellagic acid derivatives 1	19.96	0.021±0.001	1.10
8	Ellagic acid derivatives 2	20.26	0.184±0.004	9.66
9	Ellagic acid	21.20	0.181±0.004	9.50
10	Ellagic acid derivatives 3	22.48	0.037±0.001	1.92
11	Ellagic acid derivatives 4	22.75	0.036±0.001	1.87
	Total content of identified compounds		1.906	100

*Note: SD - standard deviation, n=3, p<0.05

Molecular docking

For molecular modelling of the theoretical antioxidant and anti-inflammatory activity, we took (+)-catechin, epicatechin and ellagic acid. Epicatechin and (+)-catechin were selected because their content was 71.46% of all phenolic compounds in the obtained extract.

All studied compounds have a high level of affinity for the structure of the COX-2 enzyme (+)-catechin had the highest free energy value (-8.40 kcal/mol), followed by epicatechin (-7.94 kcal/mol). When comparing the obtained results with the diclofenac sodium standard, the affinity of (+)-catechin with the COX-2 active site was 45%, and in the case of epicatechin, 25% more than that of diclofenac sodium, respectively. Also, we calculated the theoretical dose in terms of kg of rat weight of the studied compounds for 50% inhibition of the enzyme. Thus, the dose of (+)-catechin was 0.10 mg/kg, and for epicatechin it was 0.55 mg/kg. The dose of (+)-catechin was significantly lower than the dose of diclofenac sodium by 84 times, and in the case of epicatechin, 12 times less. (Table 3).

The interaction of (+)-catechin with the active center of COX-2 is represented by hydrogen bonds with Ala199, Ala202, Thr206, and hydrophobic bonds - by Tyr385, Glu203, His 388, Leu 391, Leu390, Trp 387.

The interaction of epicatechin with the active center is represented by hydrogen bonds: Ala202, Tyr348, Thr206, Tyr385, Trp387, and by hydrophobic bonds: Glu203, Ala199, Leu390 (Figure 3).

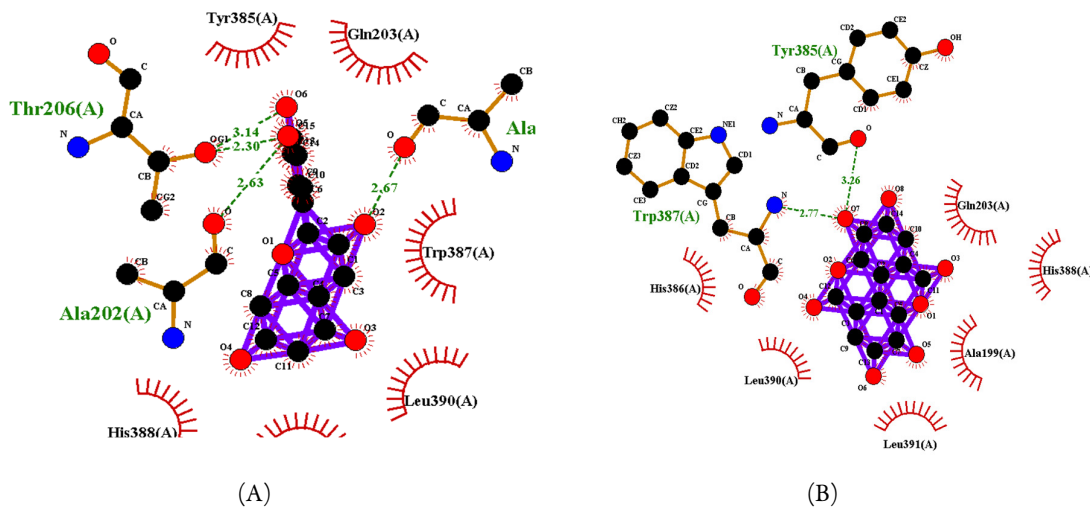
All studied compounds exhibit high affinity for the active site of NADPH oxidase. Epicatechin (-7.11 kcal/mol) had the highest level of free energy, and epicatechin (-6.60 kcal/mol) was in second place. When comparing the obtained results with the epigallocatechin-3-O-gallate standard, the affinity of (+)-catechin with the active site of NADPH oxidase was 10.55%, and in the case of epicatechin it was 19.00% higher than that of epigallocatechin-3-O-gallate, respectively. Also, we calculated the theoretical dose per kg of rat weight of the studied compounds for 50% inhibition of the enzyme. Thus, the dose of (+)-catechin was 1.40 mg/kg, for epicatechin it was 0.59 mg/kg. The dose of epicatechin was significantly lower than the dose of epigallocatechin-3-O-gallate, namely 11 times, and in cases of (+)-catechin 4.60 times less (Table 3).

The interaction of (+)-catechin with the active center of NADPH oxidase is represented by hydrogen bonds with Glu691, Ser522, Glu443, Thr462, Cys668, Phe667, and hydrophobic bonds - Pro521, Thr520, Tyr445, Pro542, Asp444, Phe 693. Epicatechin interacts with the active center enzyme by hydrogen bonds: Asn692, Thr462, and by hydrophobic bonds: Glu691, Trp695, Thr520, Cys668, Tyr445, Pro542, Phe693. Also, all tested compounds interact with flavin adenine dinucleotide (Figure 4).

Table 3. Results of molecular docking of the compounds identified by the HPLC in the *R. idaeus* shoots extract with the COX-2 and NADPH oxidase structures

COX-2				NADPH oxidase			
Ligand	ΔG_{bind}^a (kcal/mol)	K_i^b (mmol)	K^c (mg/kg)	Ligand	ΔG_{bind}^a (kcal/mol)	K_i^b (mmol)	K^c (mg/kg)
Epicatechin	-7.20	0.00526	0.55	Epicatechin	-7.11	0.00616	0.59
(+)-Catechin	-8.40	0.00070	0.10	(+)-Catechin	-6.60	0.00	1.41
Diclofenac sodium	-5.76	0.05977	5.85	Epigallocatechin-3-O-gallate	-5.97	0.04237	6.42

Notes: a – free-binding energy; b – inhibition constant, IC50; c – dose per kg rat weight, for 50% inhibition of the enzyme structure



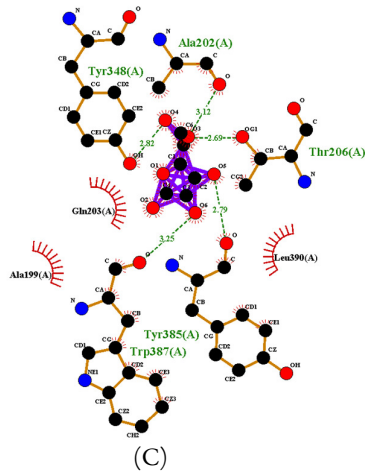


Figure 3. 2D representation of the interactions of COX-2 residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines – represent hydrogen bonds; red lines – hydrophobic bonds

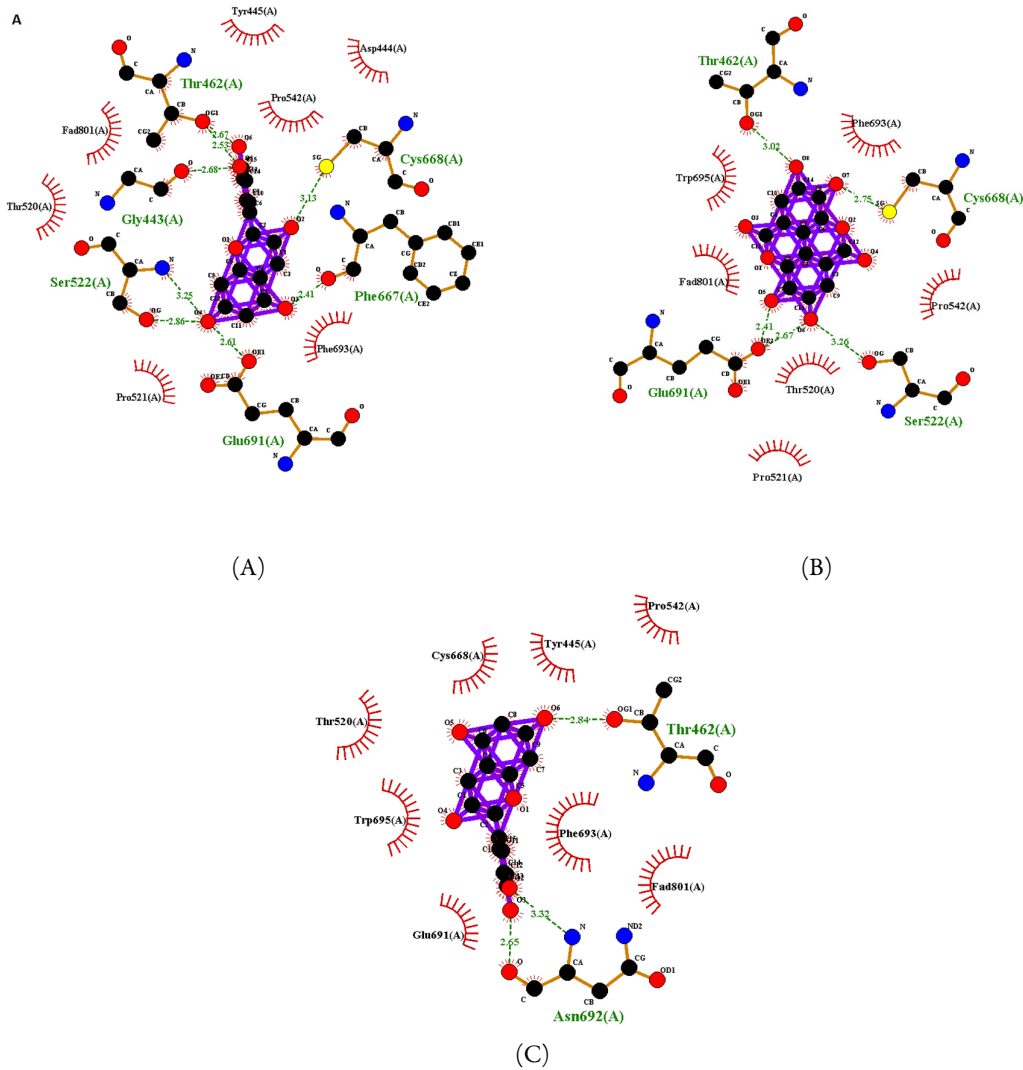


Figure 4. 2D representation of the interactions of NADPH oxidase residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines – represent hydrogen bonds; red lines – hydrophobic bonds

Antioxidant power

To compare the theoretical and practical results of the study of the antioxidant activity of the obtained extract of *R. idaeus* shoots, we studied the antioxidant activity by the potentiometric method at three levels of theoretical concentrations in terms of the amount of catechins in the extract: 4.00 mg/mL (double the sum of the theoretical dose of (+)-catechin and epicatechin), 2.00 mg/mL (the sum of the theoretical dose of (+)-catechin and epicatechin) and 0.20 mg/mL (half the sum of the theoretical dose of (+)-catechin and epicatechin). 60% green tea leaf extract was taken as the reference standard, as our study showed that epigallocatechin-3-O-gallate was the dominant compound. Green tea leaf extract was taken in three concentrations in terms of the amount of catechins: 4.00, 2.00 and 0.20 mg/mL.

As result of study, it was found that at a dose of 4.00 mg/mL, the antioxidant activity of 60% *R. idaeus* shoot extract was 29.79% higher, at a dose of 2.00 mg/mL - by 51.98%, and at 0.20 mg/mL - 39.15%. According to the developed conditional classification of antioxidant activity according to Maslov (Maslov *et al.*, 2021), we determined that the extracts at a dose of 4.00 mg/mL had an average level of antioxidant activity, and at a dose of 2.00 and 0.20 mg/mL they were below average (Table 4).

Table 4. Results of determination of antioxidant activity of the obtained *R. idaeus* shoots extracts

Sample	Concentration of catechins (mg/mL)	AOA (mmol-equiv./ m _{dry res.})	Conditional terms of AOA
60% raspberry extract	4.00	32.11±3.00	Middle
	2.00	18.80±2.00	Lower middle
	0.2	2.60±0.03	Lower middle
60% green tea leaves extract	4.00	24.74±2.30	Middle
	2.00	12.37±1.30	Lower medium
	0.20	1.43±0.03	Lower medium

Anti-inflammatory activity

To compare the theoretical and practical results of the study of the anti-inflammatory activity of the obtained extract of *R. idaeus* shoots, we studied the anti-inflammatory activity at three levels of theoretical concentrations in terms of the amount of catechins in the extract: 13.0 mg/kg (twenty-fold sum of the theoretical dose of (+)-catechin and epicatechin), 6.5 mg/kg (ten times the theoretical dose of (+)-catechin and epicatechin) and 0.65 mg/kg (the sum of the theoretical dose of (+)-catechin and epicatechin).

R. idaeus shoot extract at a dose of 13.0 mg/kg in rats significantly reduced paw edema by 43.1% compared to the saline group from the first hour of the test. Thereafter, edema decreased by 46.4%, 49.8%, 24.2%, and 19.6% at 2, 3, 8, and 24 hours, respectively, compared with saline. Treatment with *R. idaeus* shoot extract at a dose of 6.5 mg/kg showed lower results compared to treatment at a dose of 13.0 mg/kg; in the first hour, the paw edema of mice decreased by 28.6%, and later it was possible to reduce edema by 30.2%, 38.1%, 16.1% and 7.1% after 2, 3, 8 and 24 hours, respectively, compared with saline. Treatment with *R. idaeus* shoot extract at a dose of 13.0 mg/kg showed a significant reduction in edema at 1, 2 and 3 hours after induction compared with diclofenac sodium, but after 8 and 24 hours it provided less reduction in edema than diclofenac sodium. Treatment with *R. idaeus* shoot extract at 6.5 and 0.65 mg/kg was significantly worse than treatment with diclofenac sodium (Table 5).

Table 5. Results of determination of antioxidant activity of the obtained *R. idaeus* shoots extracts

Sample	Dose	% of edema inhibition compared to control during				
		1 hour	2 hours	3 hours	8 hours	24 hours
60% raspberry extract	13.0 mg/kg	43.1±2.6	46.4±7.2	49.8±4.4	24.2±3.8	19.6±7.2
	6.5 mg/kg	28.6±6.1	30.2±4.1	38.1±2.8	16.1±6.1	7.1±1.1
	0.65 mg/kg	13.6±1.5	26.2±1.3	22.2±1.5	11.6±1.4	0
Diclofenac sodium	8 mg	32.4±3.7	40.5±5.1	54.6±4.8	36.7±5.9	24.7±3.3

Correlation analysis

To confirm the hypothesis about the dependence of anti-inflammatory activity on antioxidant activity, we carried out a correlation analysis. The Pearson correlation coefficient (r) between antioxidant activity and 1, 2, 3, 8 and 24 h anti-inflammatory activity of the *R. idaeus* shoot extract was 0.9963, 0.9666, 0.9866, 0.9962 and 0.9967 (Table 6).

Table 6. Pearson's (r) correlation coefficient between antioxidant and anti-inflammatory actions

Correlation	1 hour	2 hour	3 hour	8 hour	24 hour
r	0.9963	0.9666	0.9866	0.9962	0.9967

From the results of the correlation analysis, it can be seen that in all cases there is a significant positive correlation. Thus, the hypothesis that anti-inflammatory activity directly depends on antioxidant activity is confirmed.

*Discussions**HPLC analysis*

Ellagotannins and catechins are believed to participate in the defense of plants against insects such as moth, viruses, bacteria and herbivores by rendering their tissues unpalatable and non-nutritious, making unacceptable as food sources (Salminen, 2014). A recent study of Krauze-Baranowska M. *et al.* (2014), they have studied the methanolic extract of *R. idaeus* shoots of cultivar "Willamette". They found that sum of polyphenols content was 2.39%, sanguin H6 – 1.36%, ellagic acid – 0.29%, (+)-catechin – 0.03% and epicatechin – 0.02% in *R. idaeus* shoots extract. Compared to our results, in our research, the sum of polyphenols was in 20.25% lower, the content of sanguin H6 was lower in 97% and ellagic acid was in 38% less. But, the content of (+)-catechin and epicatechin were higher in 94% and 98%, respectively. We can see that the content of catechins derivatives were dominated in our examined extract, whereas in case of compared extract the content of ellagotannins and ellagic acid were higher. The difference in the chemical composition may be related with the different cultivars and vegetative phase of plant. A big role for accumulation of BAS plays a growing season. Salminen *et al.* (2004) studied a seasonal variation of ellagotannins and catechins in the leaves of oak from April to October. They showed that accumulation of ellagotannins was higher than catechins in young leaves whereas in the mature leaves the content of catechins was dominated. Thus, the compared extract may be prepared from *R. idaeus* shoots collected in April or May, whereas we obtained extract from *R. idaeus* shoots collected in July.

Molecular docking

One of the main links in the development of inflammation is COX-2. The main role of COX-2 in the inflammatory process is the conversion of arachidonic acid into prostaglandins, prostocyclins and thromboxane A2. Thus, COX-2 is an important target for studying the anti-inflammatory activity of drugs (Lopes *et al.*, 2022).

Oxidation stress is condition in which observes excess of reactive oxygen species as superoxide, hydroxyl radical, hypochlorite and singlet oxygen. They cause cellular damages through oxidative stress. The NADPH oxidase is the only enzyme that are devoted exclusively to the production of reactive oxygen species (Drummond *et al.*, 2011). The NADPH oxidase are membrane proteins and consist of six transmembrane domains (TM) and a cytosolic C-terminal dehydrogenase (DH) domain. DH includes the flavin adenine dinucleotide (FAD) and NADPH binding sites, while TM binds with two hemes. For the development of

superoxide and reactive oxygen molecules, DH transfers the electron from NADPH to oxygen bound to the moieties of the heme (Chikhale *et al.*, 2021).

To obtain a theoretical dose of COX-2 and NADPH oxidase inhibition, we performed molecular docking of (\pm)-catechin and epicatechin to the active centers of the above enzymes.

Antioxidant power

To assess the antioxidant activity of the obtained extracts, we used a potentiometric method. The resulting green tea leaf extract was taken as a reference standard. Since green tea leaves contain in their gold standard - epigallocatechin-3-gallate. This compound, according to a number of studies, has a powerful antioxidant effect (Srivastava *et al.*, 2010).

When comparing the obtained theoretical and experimental results of antioxidant activity, it was found that in the study of antioxidant activity *in silico* (+)-catechin and epicatechin showed a better result than the comparison standard epigallocatechin-3-O-gallate, and in the case of determining the antioxidant The *in vitro* activity of *R. idaeus* shoot extract also had a better result than the reference standard green tea leaf extract, which indicates a matching of experimental and theoretical research results.

Anti-inflammatory activity

To study the anti-inflammatory activity, we used the carrageenan edema model. This model of inflammation consists of two stages: the first stage begins in 1 hour after administration, when edema is formed, which is associated with the release of vasoactive amines (histamine and serotonin) and kinins; the second stage begins in 3 hours after the formation of edema, which is associated with an increase in the activity of COX-2, which produces a large amount of prostaglandins and the release of nitrogen monoxide (Lopes *et al.*, 2022).

When comparing the obtained theoretical and experimental results of antioxidant activity, it was found that in the study of antioxidant activity *in silico* (+)-catechin and epicatechin showed a better result than the reference standard diclofenac sodium, and in the case of determining antioxidant activity *in vivo*, *R. idaeus* shoot extract at a dose of 13.0 mg/kg in the first 2 hours had a better result than the reference standard diclofenac sodium, which indicates a matching of experimental and theoretical results of studies. In our opinion, the *R. idaeus* shoot extract at a dose of 13.0 mg/kg showed a better result than at a dose of 6.5 and 0.65 mg/kg, since at this dose 100% of COX-2 activity is inhibited, and at a dose of 6.5 mg/kg only 50%.

Correlation analysis

Srivastava *et al.* (2010), studied the correlation between antioxidant (ferric reducing antioxidant power assay) and anti-inflammatory (percent inhibition of edema in model of ear thickness) activities of blackberry extracts. They reported about high positive correlation, where Pearson's coefficient of 0.8520. Compared to our research, in our study, in all cases we had a high positive correlation between antioxidant and anti-inflammatory actions ($r = 0.9963$, $r = 0.9666$, $r = 0.9866$, $r = 0.9962$, $r = 0.9967$). Thus, the hypothesis that anti-inflammatory activity directly depends on antioxidant activity is confirmed.

Conclusions

The (+)-catechin and epicatechin dominated in the *Rubus idaeus* shoots extract. It was established that (+)-catechin and epicatechin have a high level of affinity for the active sites of COX-2 and NADPH oxidase. The extract showed a significant level of antioxidant and anti-inflammatory activity in *in vitro* and *in vivo* studies, respectively. Moreover, there was a matching of experimental and theoretical results of the study of antioxidant and anti-inflammatory activities. In addition, correlation analysis confirmed the dependence of the anti-inflammatory action on the antioxidant one.

Authors' Contributions

Conceptualization: OM, MK, DH; Data curation: OM, MK, DH; Formal analysis: OM, MK; Funding acquisition: DH; Investigation: OM, DH, LD; Methodology: LD, MK; Project administration: SK, LD; Resources; Software; Supervision: SK, LD; Writing - original draft: OM; Writing - review and editing: SK, LD.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article

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