

Evaluation of Anti-Inflammatory, Antioxidant Activities and Molecular Docking Analysis of *Rubus idaeus* Leaf Extract

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ABSTRACT

The study aimed to identify the most abundant compounds in raspberry leaf extract via HPLC analysis, conduct theoretical and practical assessments of antioxidant and anti-inflammatory activities both in silico, in vitro, and in vivo, and evaluate the correlation between antioxidant and anti-inflammatory activities. Polyphenols were quantified using HPLC; molecular docking was carried out using AutoDockTools 1.5.6; antioxidant activity was ascertained via the potentiometric method; and anti-inflammatory activity was examined based on the carrageenan edema method. The extract was found to be rich in epicatechin (0.417%), (+)-catechin (0.501%), and ellagitannins (0.401%). The free energy of (+)-catechin and epicatechin was -8.40 and -7.20 respectively for the active sites of cyclooxygenase-2 (COX-2), and -6.60 and -7.11 for nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). Notably, the antioxidant activity of the raspberry leaf extract was 1.43%, 1.04%, and 10.62% higher than that of green tea leaf extract for doses of 4.00, 2.00, 0.20 mg/mL, respectively. Treatment with the raspberry leaf extract at a dose of 13.0 mg/kg resulted in a significant decrease in edema after 1, 2, and 3 hours by 38.8%, 41.8%, and 48.8%, respectively, compared to the control group. The study demonstrated a correspondence between experimental and theoretical results in evaluating antioxidant and anti-inflammatory activities. Correlation analysis further substantiated that the anti-inflammatory action is dependent on antioxidant activity.

Keywords: *Rubus idaeus* L., Leaf, HPLC, Molecular docking, Antioxidant activity, Anti-inflammatory activity, Correlation.

1. INTRODUCTION

In many chronic diseases such as diabetes mellitus, hypertension, atherosclerosis, Alzheimer's disease, and cancer [1], inflammation and oxidative stress invariably play pivotal roles. During the inflammatory response, neutrophils and macrophages generate substantial quantities of free radicals to combat and eliminate foreign invaders [2,3]. Furthermore, recent research has revealed

that non-phagocytic cells, like interleukin-6 (IL-6), can also produce free radicals by expressing NADPH oxidase [4]. Importantly, oxidative stress does not only arise from inflammation, but can also provoke it. Studies have shown that hydrogen peroxide free radicals can initiate inflammation by activating transcriptional enzymes like nuclear factor of kB (Nf-kB), p38 mitogen activated protein kinase, and N-terminal c-Jun kinase [5, 6, 7]. These findings underscore the interconnected nature of inflammation and oxidative stress, as each of these enzymes has the potential to trigger the other. Consequently, it is crucial for new medications to possess both antioxidant and anti-inflammatory properties.

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The genus *Rubus* consists of around 700 species that usually occur in the temperate climate [8]. Raspberries, members of the rose family, are aggregate fruits 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 berries are the most common [9]. Recent research indicates that raspberry leaves and fruits are a rich source of flavonoid derivatives, represented by quercetin derivatives as well as phenolic acids, organic acids, and vitamin C [10]. Durgo et al. [11] have declared that ellagic acid is the main component among phenolic compounds. It is represented in three different forms: ellagitannins, where ellagic acid forms esters with a sugar; free ellagic acid; and ellagic acid as glycosides. Raspberry fruits, leaves, and blossoms have been used for medicinal purposes. Raspberry leaves are typically applied to treat gastrointestinal disorders, respiratory issues, heart problems, the flu, fever, and diabetes. The fruits have traditionally been used as cardioprotective, antitumor, anti-inflammatory, and antipyretic agents [12, 13]. Raspberry blossoms were used to create eye ointments or to treat stomach ailments [14].

Prominent scientific studies indexed in PubMed and Scopus have unveiled the robust anti-inflammatory and antioxidant effects of ellagic acid and epicatechin. Mansury et al. [15] studied the anti-inflammatory and antioxidant activity in a carrageenan-induced mouse paw edema model. The study's findings revealed that ellagic acid, administered systemically at doses ranging from 1 to 30 mg/kg, displayed a dose-dependent reduction in edema in the inflamed paws of rats. Moreover, ellagic acid treatment led to decreased serum levels of nitric oxide (NO) and prostaglandin E2 (PGE2). Additionally, the expression of endothelial NOS (eNOS) and cyclooxygenase-2 (COX-2) enzymes were suppressed, while the production of tumor necrosis factor-alpha (TNF- α) and interleukin-1 β (IL-1 β) in the inflammatory

paw tissue was attenuated.

In a recent *in vitro* study by Yang et al. [16], the objective was to explore the impact of epicatechin on the production of pro-inflammatory mediators in RAW264.7 cells induced by lipopolysaccharide. The results of the analysis revealed that epicatechin effectively suppressed the expression of eNOS and COX-2, along with reducing the production of NO, PGE2, and pro-inflammatory cytokines IL-6, IL-1 β , and TNF- α in RAW264.7 cells.

In our recent studies, we found that *Rubus idaeus* leaf extract has anti-inflammatory, antioxidant, and antimicrobial effects [17]. Currently, limited data is available concerning the correlation between antioxidant and anti-inflammatory activities of *Rubus idaeus* leaves. Taking into account the pharmacological action of biologically active substances (BAS) contained in red *Rubus idaeus* leaves, we hypothesized a correlation between the anti-inflammatory and antioxidant activities of the obtained *Rubus idaeus* leaf extract. Therefore, the aim of this investigation was to determine the main BAS using the HPLC method, conduct theoretical and practical studies to ascertain the antioxidant and anti-inflammatory activity *in silico*, *in vitro*, and *in vivo* of *Rubus idaeus* leaf extract, and to study the correlation of pharmacological actions.

2. MATERIALS AND METHODS

2.1 Plant material

The object of the study was the leaves of *Rubus idaeus*, which were collected from places of its native cultivation. The material was gathered in 2021, after the fruiting period, in the vicinity of the village of Ternova, Kharkiv region (50.193116162220264, 36.66935288403296). Green tea leaves from the Chun Mee cultivar were collected in Anhui province, China, during the months of March to May.

2.2 Reagents

Methanol and trifluoroacetic acid were purchased from Allchem. Sanguinin H-10 isomer 1, Lambertianin C, Sanguinin H-6, (+)-catechin, (-)-epicatechin, ellagic acid, cyanidin-3-O-glucoside, and quercetin-3-O-glucuronide were procured from the Sigma-Aldrich Company.

2.3 Extraction procedure

An exact mass of 10.0g of *Rubus idaeus* leaves were ground to a size of 1-2 mm. The extraction was carried out in 60% ethanol, at a raw material to solvent ratio of 1/20 (m/v), on a water bath at 80°C with a reflux condenser for one hour. This process was performed twice. After cooling, the solutions were filtered and concentrated to a final volume of 20 mL using a rotary evaporator at 40°C under vacuum conditions.

The green tea extract was obtained by 60% ethanol according to the procedure mentioned in our previous research [18].

2.4 Experimental animals

The study involved 56 male rats of the outbred white strain, weighing between 180 and 220 grams. These rats were sourced from the vivarium of the National University of Pharmacy (NUPh). Throughout the experiment, the rats were housed in macrolon boxes, with five animals in each box. Rats had unrestricted access to water and food, which were provided on a daily basis. The bedding was replaced on a three-day cycle. The rats were maintained under specific conditions, including a temperature of 22±2°C, relative humidity of 60±5%, and a daily light cycle consisting of 12 hours of light and 12 hours of darkness.

All procedures carried out during the study adhered to the guidelines set by the National Institute of Health for the care and use of laboratory animals, as well as the European Council Directive on 24 November 1986 for the Care and Use of Laboratory Animals (86/609/EEC). The study protocol was approved by the Local Ethics Committee.

2.5 HPLC method of analysis

The chromatographic separation was carried out on an Agilent Technology model 1100 chromatograph with a 150 mm × 2.1 mm ZORBAX-SB C-18 column with a granularity at a pore size of 3.5 µm. The 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) [19]. All solvents utilized in the experiment underwent ultrasonic degassing and were subjected to 0.22 µm pore size membrane filtering. The sample injection volume was set at 2 µL, and detection occurred at wavelengths of 254, 280, and 350 nm. The mobile phase gradient used was linear and followed the profile given in Table 1. The concentrations of phenolic compounds in the extract were calculated from standard curves using the standard of individual compounds.

Table 1. Linear mobile phase gradient

Time, min	0.6% trifluoroacetic acid	70% methanol
0	92	8
8	62	38
24-29	0	100

Standard calibration

Stock solutions (2 mg/mL) for phenolic compounds were prepared by accurately weighing 50 mg of each substance into 25.0 mL of methanol. Dilution of the above stock solutions yielded a set of standard solutions of 200, 100, 50, and 25 µg/mL for each individual compound, respectively. Calibration curves were obtained for each individual compound by plotting concentrations versus peak areas. Regression equations were obtained from the calibration curves for each individual phenolic compound. Identification of the phenolic compounds was done by comparing the retention time of the unknown compounds with those of authentic phenolic compounds at three wavelengths (254, 280, 350 nm). The identities were then

confirmed by spiking the unknown samples with authentic compounds.

2.7 Molecular docking

A molecular docking study was conducted using a tool known as AutoDockTools 1.5.6 [20]. The preparation of the protein involved an optimization process, which included the removal of water and other atoms, followed by the addition of a polar hydrogen group. Autogrid was used to configure the grid coordinates (X, Y, and Z) on the binding site. Genetic algorithm parameters were applied for ligand interaction, with 10 runs of this criterion.

COX-2 (PDB ID: 1ddx) and NADPH oxidase (PDB ID: 5o0X) structures were obtained from the PDB database [21]. The resolution of 1ddx was 3.00 Å, whereas 5o0X was 2.20 Å. For the docking experiment, protein structure is selected if the resolution is above 2 Å. So, these two proteins can be used for the experiment. The ligand structures of (+)-catechin (CID_9064), (-)-epicatechin (CID_72276), and ellagic acid (CID_5281855) were obtained from the PubChem database [22]. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [23].

2.8 Antioxidant activity

The extract's antioxidant activity was assessed using the potentiometric method [24]. 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, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E_{ethanol})nF/2.3RT}$; 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.

2.9 Anti-inflammatory activity

The extract's anti-exudative activity was investigated using 56 male rats of the outbred white strain, weighing between 180 and 220 grams. The anti-inflammatory activity was carried out according to the carrageenan edema method [25]. The activity of the extract and reference drug were calculated using the following formula:

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

where A represents the anti-exudative 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} is the volume of the swollen paw in the control, and M_{hc} is the volume of a healthy paw in the control.

The animals in the study were divided into six groups for experimental purposes. The first group served as the control group, where the animals were subplantarily administered a carrageenan solution and orally given 0.5 mL/kg of distilled water. The second, third, and fourth groups received the carrageenan solution subplantarily, along with intragastric administration of the studied extract at doses of 0.65 mg/kg, 6.0 mg/kg, and 13.0 mg/kg, respectively. Animals in the fifth group were given a comparison drug, specifically indomethacin at a dose of 2 mg/kg, intragastrically in addition to the carrageenan injection. The sixth group consisted of intact animals who received a subplantar administration of 0.1 mL saline solution.

2.10 Statistical analysis

The measurements were made five times (replicates). The results were expressed as mean values accompanied by standard deviations, reflecting the level of certainty in the measurements. Statistical analysis was performed using MS Excel 7.0 and STATISTIKA 6.0 software, enabling thorough data evaluation and interpretation.

3.RESULTS

3.1 HPLC analysis

The HPLC method was used to carry out a qualitative and quantitative analysis of phenolic compounds in the obtained extract of *Rubus idaeus* leaves. According to the results of the study, 15 compounds were identified (Fig. 1, 2, 3, and 4). The sum of polyphenols in the obtained extract was 1.680%, of which flavan-3-ols (catechins) accounted for 0.918% (54.64% of the total polyphenols), ellagitannins - 0.401% (23.87% of the total polyphenols), flavonols – 0.245% (14.58% of the total polyphenols), and ellagic acid derivatives - 0.113% (6.73% of the total polyphenols) (Table 2).

Among the flavan-3-ols, epicatechin dominates – 0.417±0.004% (24.82% of the total polyphenols), and (+)-catechin – 0.501±0.005% (29.82% of the total polyphenols). Among ellagitannins, 6 compounds were

identified: Sanguiin H-10 isomer 1 - 0.026±0.001% (1.55% of the total polyphenols), Lambertianin C without ellagic acid fragment – 0.007±0.0001% (0.42% of the total polyphenols), Sanguiin H-10 isomer 2 - 0.024±0.001% (1.43% of the total polyphenols), Lambertianin C - 0.141±0.001% (8.39% of the total polyphenols), Sanguiin H-6 - 0.192±0.002% (11.43% of the total polyphenols), and Lambertianin C isomer – 0.011±0.001% (0.65% of the total polyphenols) (Table 2).

As shown in Table 1, Sanguiin H-6 dominates among all ellagitannins, Lambertianin C is in second place, and Sanguiin H-10 isomer 1 is in third place. The compound with the lowest content was Lambertianin C without the ellagic acid fragment. The content of ellagic acid was 0.068±0.004% (4.50% of the total phenolic compounds). As shown in the results, the content of ellagic acid and its derivatives is 72% lower than that of ellagitannins (Table 2).

Only one flavonol – quercetin-3-O-glucuronide – was identified in the *Rubus idaeus* leaves. The content of quercetin-3-O-glucuronide was 0.245±0.002% (14.58% of the total polyphenols). Moreover, one anthocyanin was identified – cyanidin-3-O-glucoside (0.003±0.001% (0.18% of the total polyphenols)), and its content is minor compared with other compounds (Table 2).

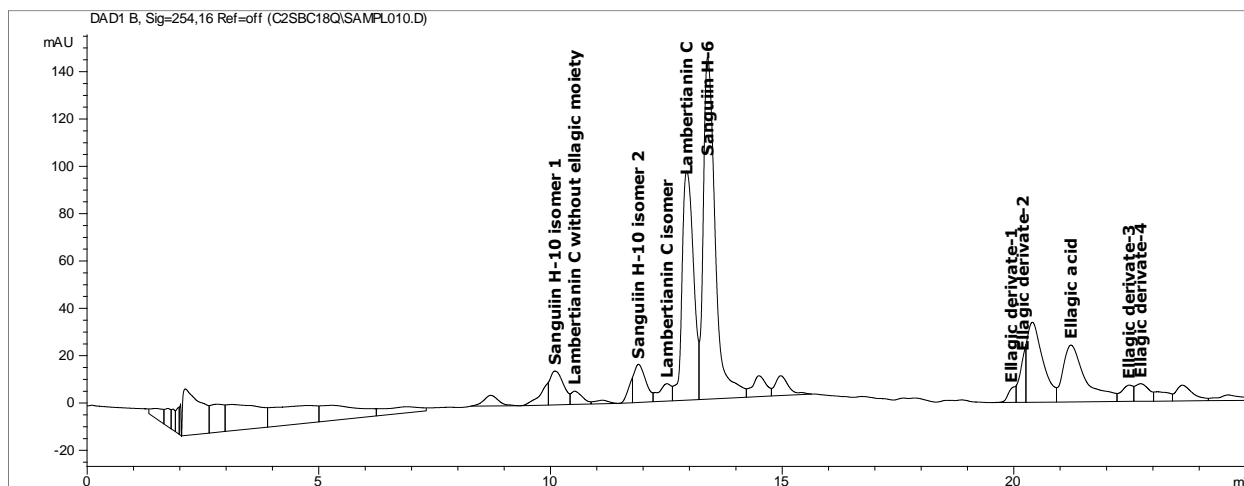


Figure 1. HPLC fingerprint (254 nm) of the *Rubus idaeus* leaves extract

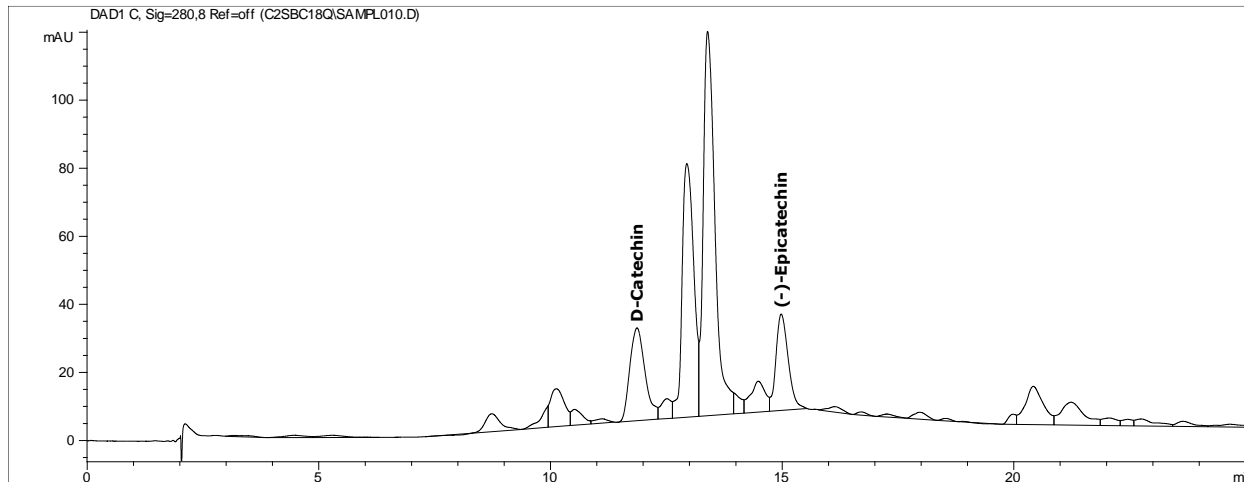


Figure 2. HPLC fingerprint (280 nm) of the *Rubus idaeus* leaves extract

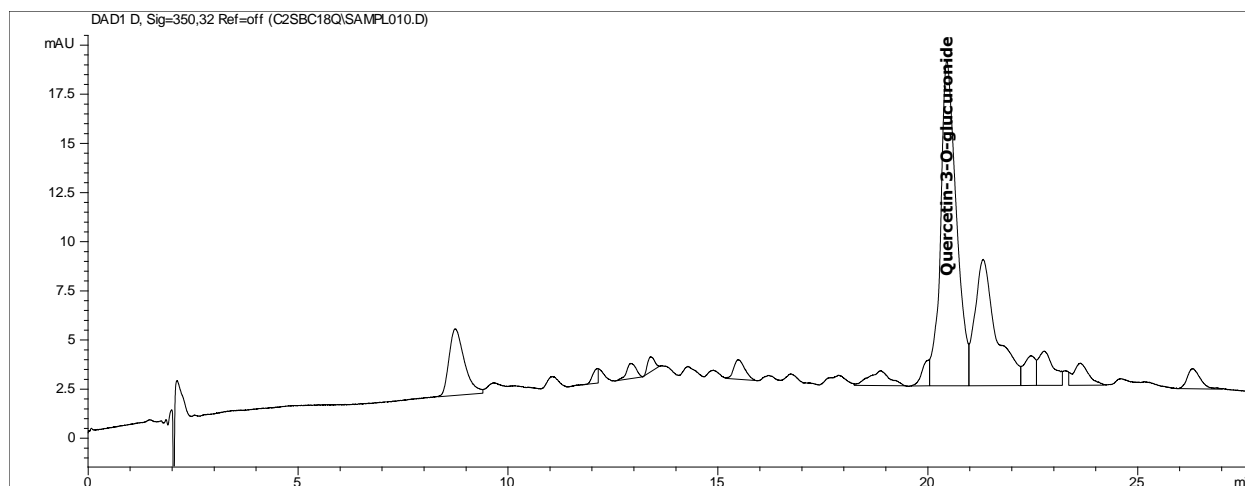


Figure 3. HPLC fingerprint (350 nm) of the *Rubus idaeus* leaves extract

Table 2. Qualitative composition and quantitative content of polyphenols in the extract of *Rubus idaeus* leaves

	Compound	Rt, min	Quantitative content, %	% out of sum polyphenols
1	Sanguin H-10 isomer 1	10.08	0.026±0.001	1.55
2	Lambertianin C without ellagic fragment	10.51	0.007±0.0001	0.42
3	(+)-Catechin	11.89	0.501±0.005	29.82
4	Sanguin H-10 isomer 2	11.91	0.024±0.001	1.43
5	Lambertianin C isomer	12.48	0.011±0.001	0.65
6	Lambertianin C	12.91	0.141±0.001	8.39
7	Sanguin H-6	13.38	0.192±0.002	11.43
8	(-)-Epicatechin	14.96	0.417±0.004	24.82
9	Cyanidin-3-O-glucoside	18.43	0.003±0.001	0.18
10	Ellagic acid derivatives 1	19.96	0.006±0.001	0.36
11	Ellagic acid derivatives 2	20.26	0.016±0.001	0.95
12	Quercetin-3-O-glucuronide	20.44	0.245±0.002	14.58
13	Ellagic acid	21.20	0.068±0.001	4.05
14	Ellagic acid derivatives 3	22.48	0.010±0.001	0.60
15	Ellagic acid derivatives 4	22.75	0.013±0.001	0.77
	Total content of identified compounds		1.680	100

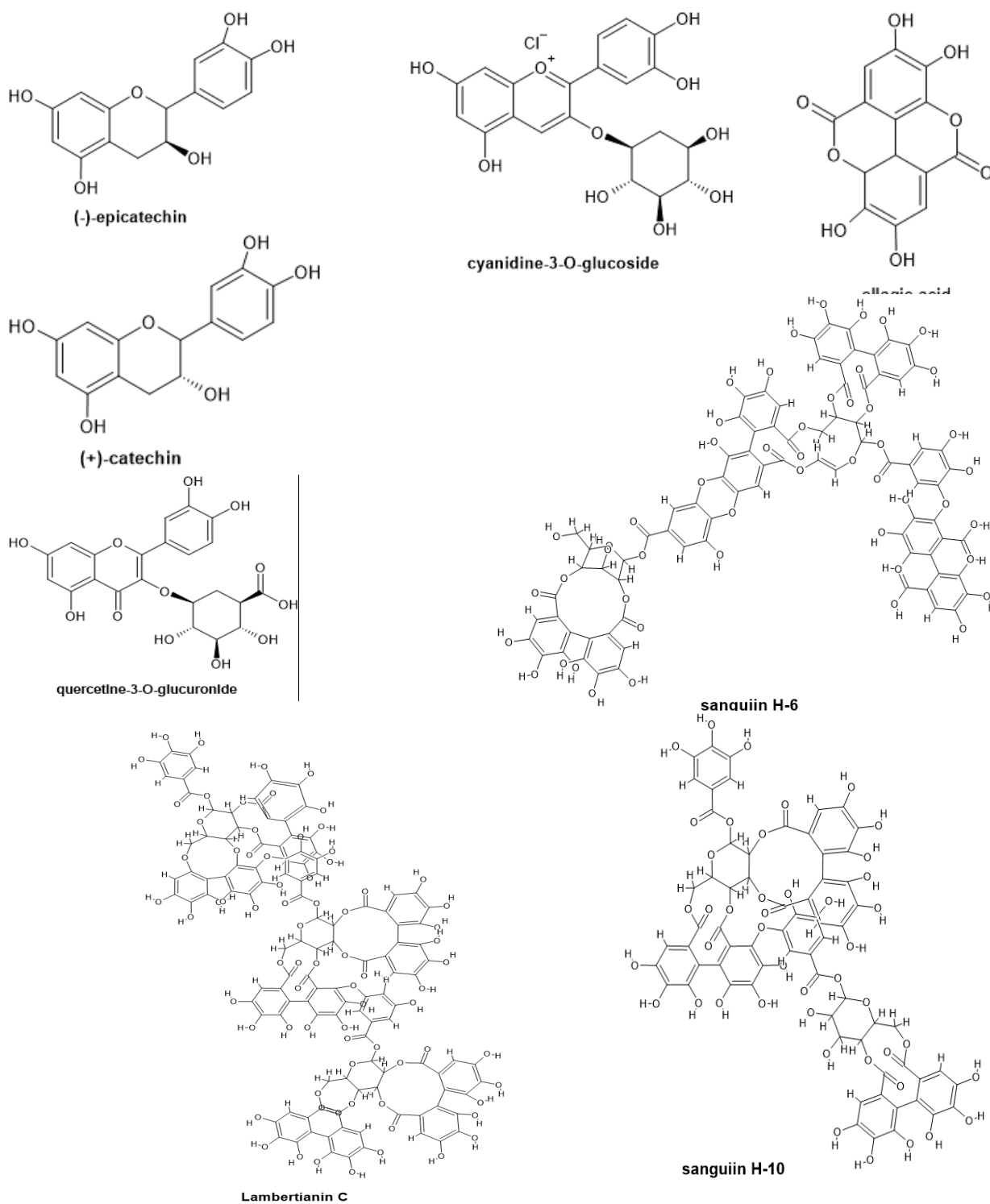


Figure 4. Structures of the identified phenolic compounds in the extract of *Rubus idaeus* leaves

3.2 Molecular docking

For the molecular modeling of theoretical antioxidant and anti-inflammatory activity, we selected (+)-catechin, epicatechin, and ellagic acid. Epicatechin and (+)-catechin were chosen because their content constituted 54.64% of all phenolic compounds in the obtained extract.

All studied compounds demonstrated 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 results with the indometacin standard, the affinity of (+)-catechin with the COX-2 active site was 16% lower, and in the case of epicatechin, it was 21% lower than that of indometacin, respectively. Additionally, the theoretical dose of studied compounds required for 50% inhibition of the enzyme was calculated per kg of rat weight. 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 higher than the dose of indomethacin by 17 times, and in the case of epicatechin, it was 92 times higher (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 with Tyr385, Glu203, His 388, Leu 391, Leu390, Trp 387. The interaction of epicatechin with the active center is represented by hydrogen bonds with Ala202, Tyr348,

Thr206, Tyr385, Trp387, and by hydrophobic bonds with Glu203, Ala199, Leu390 (Fig. 5).

All studied compounds exhibited a high affinity for the active site of NADPH oxidase. Epicatechin (-7.11 kcal/mol) had the highest level of free energy, and (+)-catechin (-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% higher, and in the case of epicatechin, it was 19.00% higher than that of epigallocatechin-3-O-gallate, respectively. Moreover, we calculated the theoretical dose per kg of rat weight of the studied compounds necessary for 50% inhibition of the enzyme. Thus, the dose of (+)-catechin was 1.40 mg/kg, and for epicatechin, it was 0.59 mg/kg. The dose of epicatechin was significantly lower than that of epigallocatechin-3-O-gallate, namely 11 times lower, and in the case of (+)-catechin, it was 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 with Pro521, Thr520, Tyr445, Pro542, Asp444, Phe 693. Epicatechin interacts with the active center of the enzyme through hydrogen bonds with Asn692, Thr462, and hydrophobic bonds with Glu691, Trp695, Thr520, Cys668, Tyr445, Pro542, Phe693. Furthermore, all tested compounds interact with flavin adenine dinucleotide (Fig. 6).

Table 3. Results of molecular docking of the compounds identified by the HPLC in the *Rubus idaeus* leaves 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
Indomethacin	-9.99	0.00005	0.006	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

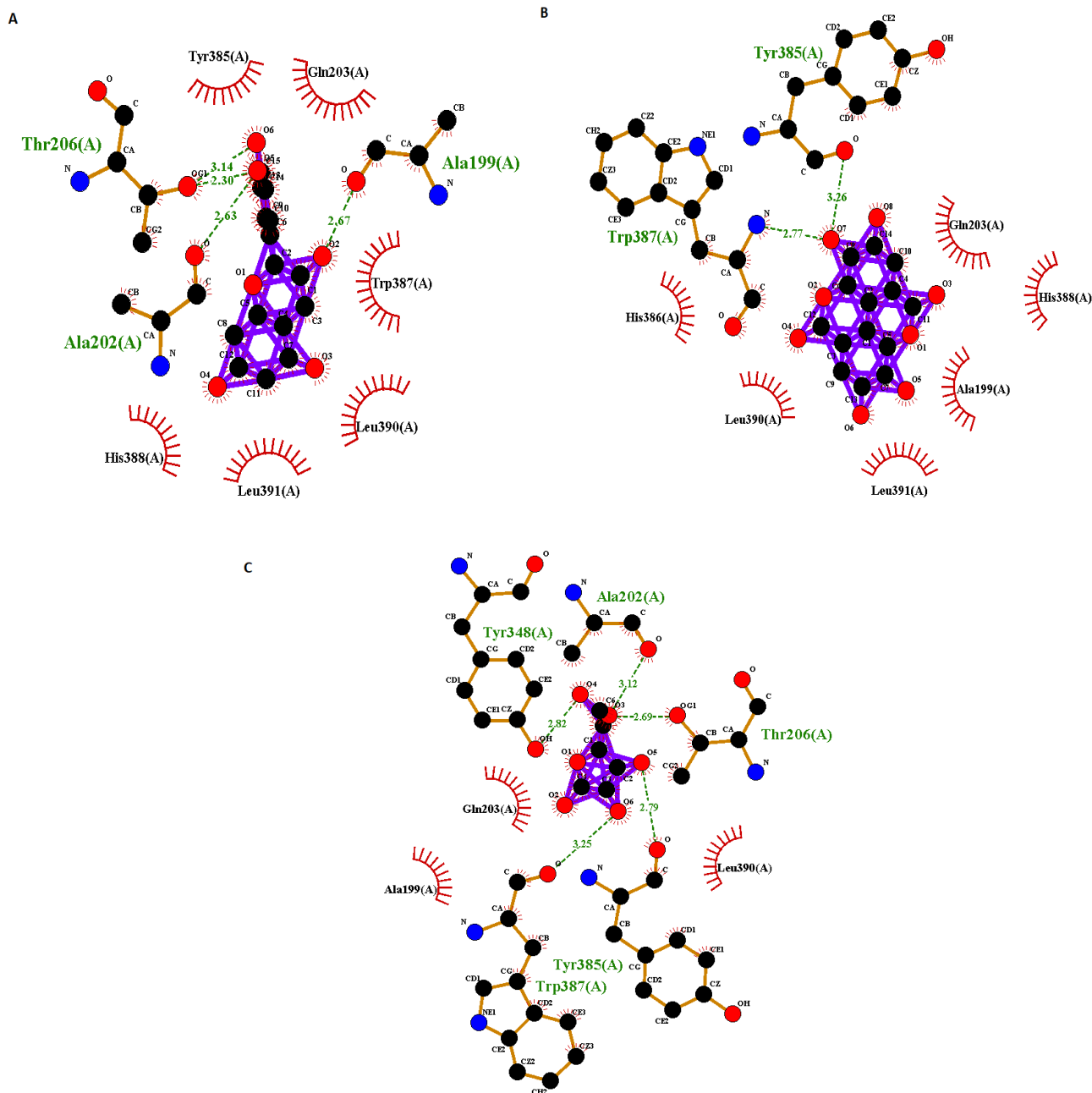


Figure 5. 2D representation of the interactions of COX-2 residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines—represent hydrogen bonds; red lines – hydrophobic bonds.

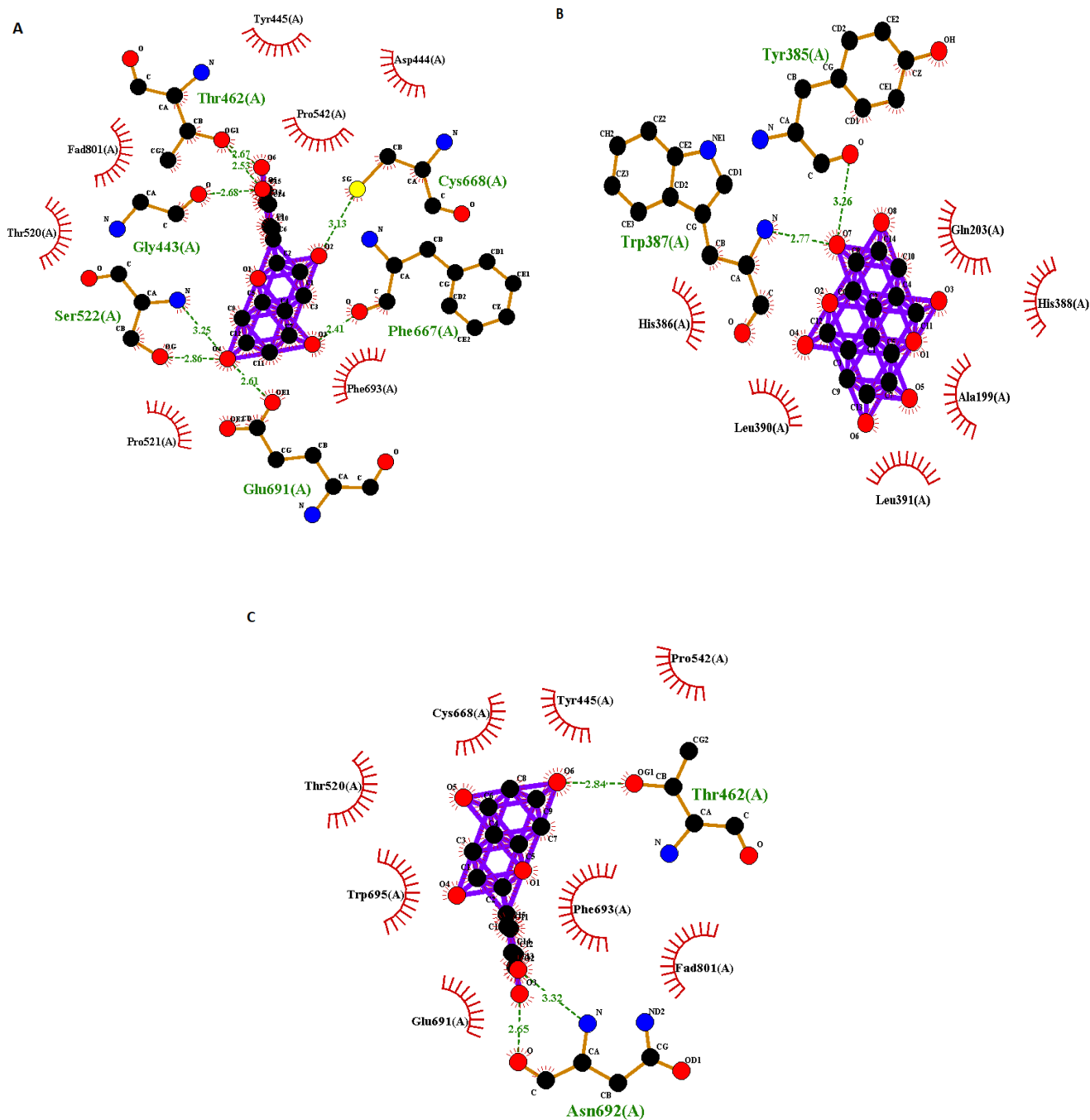


Figure 6. 2D representation of the interactions of NADPH oxidase residues with (+)-catechin (A), ellagic acid (B) and epicatechin (C). Dashed lines—represent hydrogen bonds; red lines – hydrophobic bonds.

3.3 Antioxidant activity

To compare the theoretical and practical results of the study of the antioxidant activity of the obtained extract of *Rubus idaeus* leaves, the antioxidant activity was studied using the potentiometric method at three levels of theoretical concentrations based on 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). A 60% green tea leaf extract was used as the reference standard, as our study showed that epigallocatechin-3-O-gallate was

the dominant compound. Green tea leaf extract was used in three concentrations in terms of the amount of catechins: 4.00, 2.00, and 0.20 mg/mL.

As a result of the study, it was found that at a dose of 4.00 mg/mL, the antioxidant activity of the 60% *Rubus idaeus* leaf extract was 1.43% higher, at a dose of 2.00 mg/mL it was higher by 1.04%, and at 0.20 mg/mL, it was 10.62% higher. According to the developed conditional classification of antioxidant activity according to Maslov [25], it was determined that the extracts at a dose of 4.00 mg/mL had an average level of antioxidant activity, whereas at doses of 2.00 and 0.20 mg/mL, the antioxidant activity level was below average (Table 4).

Table 4. Results of determination of antioxidant activity of the obtained *Rubus idaeus* leaves extracts

Sample	Concentration of catechins, mg/mL	AOA, mmol-equiv./m _{dry} res.	Conditional terms of AOA
60% raspberry extract	4.00	25.10±0.50	Middle
	2.00	12.50±0.25	Lower middle
	0.20	1.60±0.03	Lower middle
60% green tea leaves extract	4.00	24.74±0.50	Middle
	2.00	12.37±0.25	Lower medium
	0.20	1.43±0.03	Lower medium

3.4 Anti-inflammatory activity

To compare the theoretical and practical results of the study of the anti-inflammatory activity of the obtained extract of *Rubus idaeus* leaves, the anti-inflammatory activity was examined at three levels of theoretical concentrations, in terms of the amount of catechins in the extract: 13.0 mg/kg (twenty times the 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).

Rubus idaeus leaf extract administered at a dose of 13.0 mg/kg in rats significantly reduced paw edema by 38.8% compared to the saline group from the first hour of the test. Thereafter, edema decreased by 41.8%, 48.8%, 20.2%, and

17.8% at 2, 3, 8, and 24 hours respectively, compared with saline. Treatment with *Rubus idaeus* leaf 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 25.6%, and subsequently, it was possible to reduce edema by 27.2%, 36.1%, 14.1%, and 5.1% after 2, 3, 8, and 24 hours respectively, compared with saline. Treatment with *Rubus idaeus* leaf extract at a dose of 13.0 mg/kg showed a significant reduction in edema at 1, 2, and 3 hours post-induction compared with indomethacin, but after 8 and 24 hours, it showed less reduction in edema than indomethacin. Treatment with *Rubus idaeus* leaves extract at doses of 6.5 and 0.65 mg/kg was significantly less effective than treatment with indomethacin (Table 5).

Table 5. Results of determination of antioxidant activity of the obtained *Rubus idaeus* leaves 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 ^a	38.8±2.6	41.8±7.2	48.8±4.4	20.2±3.8	17.8±7.2
	6.5 ^a	25.6±6.1	27.2±4.1	36.1±2.8	14.1±6.1	5.1±1.1
	0.65 ^a	12.6±1.5	25.2±1.3	21.1±1.5	10.6±1.4	0
Indomethacin	2 mg	39.1±4.1	42.8±4.4	53.1±5.2	30.3±7.1	21.8±2.4

Note: a – mg/kg.

3.5 Correlation analysis

To confirm the hypothesis about the dependence of anti-inflammatory activity on antioxidant activity, a correlation analysis was carried out. The Pearson correlation coefficient (r) between antioxidant activity and 1, 2, 3, 8 and 24 h anti-inflammatory activity of the *Rubus idaeus* leaves extract was 0.9981, 0.8918, 0.9995, 0.9777 and 0.9559 (Table 6).

From the results of this correlation analysis, it is evident that there is a significant positive correlation in all cases. Therefore, the hypothesis that anti-inflammatory activity directly depends on antioxidant activity is hereby confirmed.

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

	1 hour	2 hour	3 hour	8 hour	24 hour
r	0.9981	0.8918	0.9995	0.9777	0.9559

4. DISCUSSIONS

4.1 HPLC analysis

Ellagitannins and catechins are considered to be involved in plant defense mechanisms against threats such as insects, moths, viruses, bacteria, and herbivores. They contribute to these mechanisms by making the plant tissues unpalatable and non-nutritious, rendering them unsuitable as food sources [27]. In a recent study by Kashchenko N. et. al. [28], the aqueous extract of *Rubus idaeus* leaves from Siberia (Republic of Buryatia) was examined. The study found that the total polyphenols content equated to

2.60%, sanguin H6 amounted to 0.20%, ellagic acid was 0.17%, epicatechin had a 0.05% presence, and both galocatechin and quercetin-3-O-glucuronide each contributed 0.03% to the *Rubus idaeus* leaves extract. In comparison to these results, our research showed that the sum of polyphenols was 35% higher, the content of sanguin H6 was 5% higher, and the presence of ellagic acid was 60% higher. However, the content of epicatechin was 7.4 times lower. In our extract, the content of catechin derivatives was dominant, while in the comparison extract, the content of ellagitannins and ellagic acid was found to be higher. The difference in chemical composition may be attributable to different cultivars and the vegetative phase of the plant. The growing season plays a significant role in the accumulation of bioactive substances. A study by Salminen et al. [29] examined the seasonal variation of ellagitannins and catechins in oak leaves from April to October. The study showed that the accumulation of ellagitannins exceeded that of catechins in young leaves, whereas in mature leaves, catechin content dominated. Therefore, it is possible the comparison extract was prepared from *Rubus idaeus* leaves collected in April or May, while our extract was prepared from *Rubus idaeus* leaves collected in July.

4.2 Molecular docking

One of the main links in the development of inflammation is COX-2. The primary 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 [30].

Oxidative stress is a condition characterized by an excessive presence of reactive oxygen species, such as superoxide, hydroxyl radical, hypochlorite, and singlet oxygen. These reactive oxygen species can lead to cellular damage through oxidative stress [31]. Among the enzymes responsible for generating reactive oxygen species is NADPH oxidase, which stands out as the sole enzyme devoted exclusively to this function [32]. NADPH oxidase is composed of membrane proteins with six transmembrane domains (TM) and a cytosolic C-terminal dehydrogenase (DH) domain. The DH domain contains binding sites for flavin adenine dinucleotide (FAD) and NADPH, while the TM domains bind to two hemes. In the process of generating superoxide and other reactive oxygen molecules, the DH transfers electrons from NADPH to oxygen molecules bound to the heme moieties [33].

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

4.3 Antioxidant activity

To assess the antioxidant activity of the obtained extracts, a potentiometric method was used. The resulting green tea leaf extract was taken as a reference standard since green tea leaves contain their gold standard - epigallocatechin-3-gallate. This compound, according to multiple studies, has a potent antioxidant effect [34].

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. Further, in the case of determining the *in vitro* antioxidant activity, *Rubus idaeus* leaves extract also had a superior result than the reference standard green tea leaf extract. These results indicate a correlation between experimental and theoretical research outcomes.

4.4 Anti-inflammatory activity

To examine anti-inflammatory activity, a carrageenan-induced mouse paw edema model was utilized. This model consists of two distinct stages: the initial stage, occurring an hour after administration, involves edema formation due to the release of vasoactive amines (histamine and serotonin) and kinins. The subsequent stage, beginning three hours after edema formation, is characterized by an increase in COX-2 activity, leading to the production of a significant number of prostaglandins and the release of NO [29].

When comparing the theoretical and experimental results of antioxidant activity, it was found that in the study of antioxidant activity *in silico*, (+)-catechin and epicatechin performed better than the reference standard indomethacin. When determining the antioxidant activity *in vivo*, *Rubus idaeus* leaves extract, at a dose of 13.0 mg/kg within the first two hours, exceeded the reference standard diclofenac sodium. This observation indicates a correlation between experimental and theoretical results. In our study, the *Rubus idaeus* leaves extract, at a dose of 13.0 mg/kg, showed better results than at doses 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% is inhibited.

4.5 Correlation analysis

Srivastova et al. [35] studied the correlation between the antioxidant (ferric reducing antioxidant power assay) and anti-inflammatory activities (percent inhibition of edema in a model of ear thickness) of blackberry extracts. They reported a high positive correlation, with a Pearson's coefficient of 0.8520. In recent research by Vinodhini V. et al. [36], they investigated the phytoconstituents of *Tragia Involucrata* leaf extracts and evaluated their correlation with anti-inflammatory and antioxidant properties. The antioxidant activity was examined using DPPH and H₂O₂ assays while the anti-inflammatory analysis was carried out using a membrane stabilization assay. A strong relationship was observed between the antioxidant and anti-inflammatory

activities ($r = 0.971$).

Compared to these studies, our research consistently observed a very high positive correlation between antioxidant and anti-inflammatory activities ($r = 0.9981$ for 1 hour, $r = 0.9995$ for 3 hours, $r = 0.9777$ for 8 hours, $r = 0.9559$ for 24 hours). The exception was the two-hour duration of anti-inflammatory activity study where a high positive correlation was still evident but somewhat lower ($r = 0.8918$). Consequently, our findings affirm the hypothesis that anti-inflammatory activity directly depends on antioxidant activity.

5. CONCLUSION

The *Rubus idaeus* leaves extract was found to be dominated by (+)-catechin and epicatechin. It was established that (+)-catechin and epicatechin have a high level of affinity for the active sites of COX-2 and NADPH oxidase. In comparison with the antioxidant activity of green tea leaf extracts, the obtained raspberry leaf extract showed higher activity by 1.43% at a dose of 4.00 mg/mL,

1.04% at 2.00 mg/mL, and 10.62% at 0.20 mg/mL. The extract demonstrated a significant level of antioxidant and anti-inflammatory activity in both in vitro and in vivo studies. Furthermore, there was an alignment between the experimental and theoretical results in the study of antioxidant and anti-inflammatory activities. Additionally, correlation analysis confirmed the dependency of the anti-inflammatory action on the antioxidant one.

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تقييم النشاط المضاد للالتهابات ومضادات الأكسدة وتحليل الالتحام الجزيئي لمستخلص أوراق نبات الروبوس إيديوس

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ملخص

هدفت الدراسة إلى تحديد المركبات الأكثر وفرة في مستخلص أوراق التوت عن طريق HPLC، وإجراء دراسات نظرية وعملية لتقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات في السيليكو وفي المختبر وفي الجسم الحي، والتحقق في العلاقة بين الأنشطة المضادة للأكسدة والمضادة للالتهابات. تم تحديد كمية البوليفينول باستخدام HPLC، وتم إجراء الإرساء الجزيئي باستخدام AutoDockTools 1.5.6، وتم تحديد النشاط المضاد للأكسدة باستخدام طريقة قياس الجهد، ودراسة النشاط المضاد للالتهابات باستخدام طريقة الوزمة الكاراجينية. وُجد أن المستخلص غني بمادة الإبيكاتشين (0.417%) و(+)-كاتشين (0.501%) والإيلاجوتانين (0.401%) كانت الطاقة الحرة ل - (+)كاتشين وإبيكاتشين -8.40 و -7.20 على التوالي للمواقع النشطة لأنزيمات الأكسدة الحلقية (COX-2)، و -6.60 و -7.11 لأنزيمات أوكسيداز فوسفات نيكوتيناميد الأدينين أدينين ثنائي النوكليوتيد (NADPH oxidase). كان النشاط المضاد للأكسدة لمستخلص أوراق التوت أعلى بنسبة 1.43% و 1.04% و 10.62% من مستخلص أوراق الشاي الأخضر لجرعات 4.00 و 2.00 و 0.20 ملغم/مل على التوالي. أظهر العلاج بمستخلص أوراق التوت بجرعة 13.0 ملغم/كغم انخفاضاً ملحوظاً في الوزمة بعد 1 و 2 و 3 ساعات بنسبة 38.8% و 41.8% و 48.8% على التوالي مقارنة بالمجموعة الضابطة. كشفت الدراسة عن وجود تطابق بين النتائج التجريبية والنظرية في تقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات. أكد تحليل الارتباط اعتماد النشاط المضاد للالتهابات على النشاط المضاد للأكسدة.

الكلمات الدالة: روبوس إيديوس ل.، ورقة، HPLC، الالتحام الجزيئي، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات، الارتباط.

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