MOLECULAR DOCKING STUDIES OF ANTI-INFLAMMATORY, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL COMPOSITION OF *RUBUS IDAEUS* SHOOT EXTRACT

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Introduction

Nowadays, the main anti-inflammation medium is steroidal (prednisolone) and nonsteroidal (diclofenac, indomethacin) applied to treat acute and chronic inflammatory diseases as rheumatoid arthritis and osteoarthritis [1]. However, the action of anti-inflammatory drugs is related with a high number of side effects. For instance, steroidal medicine causes osteoporosis, adrenal atrophy, suppression of immune system. Non-steroidal drug cause bronchospasm, peptic ulcer that relates with inhibition physiological and inflammatory prostaglandins [2]. Therefore, the search for new anti-inflammatory compounds from herbal sources is topical for today.

There is increasing interest in plant medicines rich in flavan-3-ols due to potential beneficial effects observed in clinical trials against inflammatory-related diseases [3]. Flavan-3-ols or catechins belong to subclass of flavonoids such as epicatechin, (+)-catechin, epigallocatechin-3-O-gallate and so on [4]. In recent study of Sunil *et al.* [1] has been evaluated the effect of (+)-catechin on various inflammatory mediators using lipopolysaccharide-stimulated (LPS) RAW264.7 macrophages. Their study demonstrated that cyclooxygenase-2. 5-lipoxygenase, endothelium nitric oxide synthase (eNOS), and tumor necrosis factor were significantly decreased by (+)-catechin. Moreover, there are a variety of studies that show inhibition potential of flavonoids against NF-kB. It has been showed that epicatechin on acute lung injury induced by LPS in mice inhibit NF-kB, mitogen-activated protein-kinases [5].

Today, the main plant source of flavan-3-ols is a green tea leaf [6]. But, in the East Europe the green tea leaf is not cultivated. That is why, shoots of raspberry have been chosen as a perspective source of flavan-3-ols. Our previous study has been showed 80% out of all content of phenolic compounds presented by flavan-3-ols, where epicatechin and (+)-catechin are the main constituents [7]. Except flavan-3-ols the raspberry shoot is rich with derivatives of ellagotannins. Ellagotannins represent a class of hydrolysable tannins, wherein the monomer consists of a glucose and ellagic acid ester [8]. Sanguiin H-6. H-10 and lambertianin C are the main compounds of ellagitannins [9, 10]

The molecular docking is a bioinformatics instrument that has been applied to determine active sites of protein structures as well as to assess the potential pharmacological activity of researched compounds. The level of interception between ligand and protein active site is estimated by the binding affinity, which is calculated by the measuring values of hydrogen bonding, van der Waals forces, hydrophobic bonding and salt bridges [11].

The aim of work was study on *in silico* antioxidant, anti-inflammatory activity of identified compounds and investigation phytochemical composition of *Rubus idaeus* shoot extract by HPLC and GC.

Materials and methods

The *Rubus idaeus* shoots were the object of the study, which were collected from places of its native cultivation. The material was collected in 2021 after the fruiting period in the vicinity of the village of Ternova, Kharkiv region (50.193116162220264. 36.66935288403296).

Metanol (purchased from "Allchem"), trifluoroacetic acid (purchased from "Allchem"), chloroform (purchased from "Allchem"), sanguiin H-10 isomer 1. lambertianin C, sanguiin H-6. (+)-catechin (purchased from "Sigma-Aldrich"), (-)-epicatechin, ellagic acid, cyanidine-3-O-glucoside, quercetin-3-O-glucurunide from Sigma Aldrich Company.

A 250.0 (exact mass) g of *Rubus idaeus* shoots were grinded in the size 1-2 mm. The extraction was carried out one by 60% ethanol at the ratio of raw material/solvent 1/20 (*m/v*) on water bath at 80º C with a reflux condenser for one hour, the extraction was made two times. Following the cooling process, the solutions were filtered and concentrated to a final volume of 250 mL using a rotary evaporator at 40ºC under vacuum conditions.

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 a pore size 3.5 μm. Elution flow rate was 0.25 mL/min. All determinations were undertaken at 45 ºC. The mobile phase binary solvent system consisted of solvent A (0.6% trifluoroacetic acid) and solvent B (70% methanol) [12]. 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. The mobile phase gradient used was linear and followed the following profile: (Table 1). The concentrations of phenolic compounds in extract were calculated from standard curves using 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

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 gave a set of standard solutions of 200. 100. 50. and 25 μg/mL for each individual compound, respectively. Calibration curves were obtained

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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 with those of authentic phenolic compounds at three wavelengths (254. 280 nm). The identities were then confirmed by spiking the unknown samples with authentic compounds.

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

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

Identification of the methyl esters of the acids was based on the calculation of the equivalent aliphatic chain length using data from the NIST 05 and Willey 2007 mass spectra library with a total number of spectra of more than 470.000 combined with the AMDIS and NIST identification programs. The retention time was also compared with the retention time of standard compounds ("Sigma"). To calculate the quantitative determination of the components, the formula was used:

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

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

A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6 [13]. 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), phospholipase A2 (PDB ID: 3hsw), 5-LOX (PDB: 2q7m), NF-kB (1svc), myeloperoxidase (PDB: 3f9p), xanthine oxidase (PDB: 1fiq), NADPH oxidase (PDB ID: 5o0X) structures were obtained from PDB database [14]. The resolution of 1ddx was 3.00 Å whereas 5o0X – 2.20 Å, 2q7m – 4.25 Å, 1svc – 2.60 Å, 3f9p – 2.93 Å, 1fiq – 2.50 Å. For docking experiment protein structure is selected if resolution above 2 Å. So, all proteins can be used for the experiment. The ligand structures of (+)-catechin (CID_9064), (-)-epicatechin (CID_72276), were obtained from PubChem database [15]. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [16].

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.

Results and discussion

To develop optimal technologies for obtaining an extract with a high level of anti-inflammatory activity, first of all, it is necessary to conduct a qualitative and quantitative analysis of the chemical composition of the native extract of *Rubus idaeus* shoots. HPLC and GC methods were used for analysis of obtained extract. As a result of our research, we found that the extract of *Rubus idaeus* shoots contains the following groups of compounds: catechins, ellagitannins, organic (derivatives of mono-, di-, tricarboxylic and fatty acids) and phenolcarboxylic acids.

The HPLC method was used to carry out a qualitative and quantitative analysis of phenolic compounds in the obtained extract of *Rubus idaeus* shoots. According to the results of the study, 11 compounds were identified. The total content of phenolic compounds in the obtained extract was 1906.00 mg/100 g of which flavan-3-ols (catechins) – 1362.00 mg/100 g (71.46% out of the total polyphenols), ellagitannins – 85.00 mg/100 g (4.46% out of the total polyphenols), ellagic acid derivatives – 459.00 mg/100 g (24.08% out of the total polyphenols) (Table 2).

Among flavan-3-ols, epicatechin dominates – 882.00±2.00 mg/100 g (46.28% out of the total polyphenols), and (+)-catechin – 480.00±5.00 mg/100 g (25.19% out of the total polyphenols). Among ellagitannins, 3 compounds were identified: sanguine H-10 isomer 1 – 3.00±0.50 mg/100 g (0.16% out of the total polyphenols), sanguine H-10 isomer 2 – 32.00±1.00 mg/100 g (1.69% out of the total polyphenols), lambertianin C – 10.00±1.00 mg/100 g (0.53% out of the total polyphenols) and sanguin H-6 – 40.00±1.00 mg/100 g (2.10% out of the total polyphenols) (Table 2).

As shown in Table 1. 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 181.00±4.00 mg/100 g (0.50% of the total phenolic compounds). As can be seen from the above results, the content of ellagic acid and its derivatives is 81% higher than that of ellagitannins (Table 2).

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

	Compound	Rt, min	Quantitative content, mg/100 g	% out of sum polyphenols
1	Sanguiin H-10 isomer 1	10.08	3.00±0.50	0.16
2	(+)-Catechin	11.89	480.00±5.0	25.19
3	Sanguiin H-10 isomer 2	11.91	32.00±1.0	1.69
4	Lambertianin C	12.91	10.00±1.0	0.53
5	Sanguiin H-6	13.38	40.00±1.0	2.10
6	(-)-Epicatechin	14.96	882.00±2.0	46.28
7	Ellagic acid derivatives 1	19.96	21.00±1.0	1.10
8	Ellagic acid derivatives 2	20.26	184.00±4.0	9.66
9	Ellagic acid	21.20	181.00±4.0	9.50
10	Ellagic acid derivatives 3	22.48	37.00±1.0	1.92
11	Ellagic acid derivatives 4	22.75	36.00±1.0	1.87
	Total content of identified compounds		1906.00	100

Qualitative and quantitative analysis of organic, fatty and phenolcarboxylic acids was carried out using the GC method. Based on the results of the study, 36 compounds were identified. The total content of all acids was 163.61 mg/100 g, of which organic acids - 122.96 mg/100 g (39.62% of the total acids), phenolcarboxylic acids - 13.49 mg/100 g (4.10% of total acids), and fatty acids – 101.93 mg/100 g (56.26% of the total acids).

A total of 10 organic acids were identified, including 2 tricarboxylic acids (citric and iso-citric acid), 7 dicarboxylic acids (oxalic, malic, succinic, adipic, malonic, fumaric, glutaric acid) and 1 monocarboxylic acid (caproic acid). Among organic acids, citric acid dominates - 98.41 mg/100 g (30.02% of the total acids), and the lowest content was in caproic acid (0.41 mg/100 g (0.13% of the total acids)).

Among phenolcarboxylic acids, 8 compounds were identified, namely: vanillic, benzoic, ferulic, p-hydroxybenzoic, gentisic, lilac, salicylic and phenylacetic acid. Vanillic acid prevails among all phenolcarboxylic acids (5.18 mg/100 g (1.57% of the total acids)), in turn, phenylacetic acid is found in raspberry shoots in the smallest amount (0.21 mg/100 g (0 .06% of the amount of acids)).

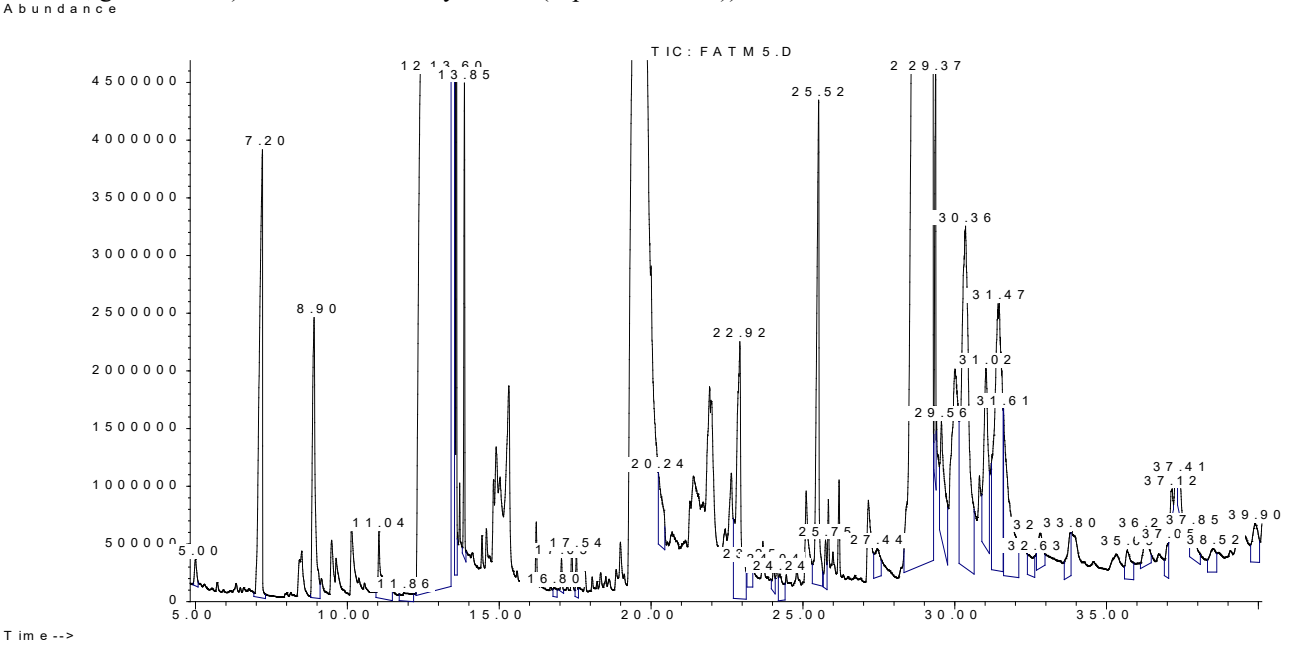

Figure 1. GC fingerprint of *Rubus idaeus* shoot extract

Ellagotannins and catechins are considered to be involved in plant defense mechanisms against insects like moths, viruses, bacteria, and herbivores. They achieve this by making the plant tissues unpalatable and non-nutritious, rendering them unsuitable as food sources [18]. A recent study of Krauze-Baranowska M. *et. al*. [10], they have studied the methanolic extract of *Rubus idaeus* shoots of cultivar "Willamette". They found that sum of polyphenols content was 2.39%, sanguiin H6 – 1.36%, ellagic acid – 0.29%, (+)-catechin – 0.03% and epicatechin – 0.02% in *Rubus idaeus* shoots extract.

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Table 3. Qualitative composition and quantitative content of organic (mono-, di-, tricarboxylic and fatty acids) and phenolcarboxylic acids in *Rubus idaeus* shoots extract

	Compound	Rt, min	Quantitative content in extract, mg/100 g	% out of sum acids
1	Citric acid	28.736	49.21±1.00	30.69
2	Malic acid	21.496	4.07±0.08	2.54
3	Succinic acid	13.812	3.32±0.08	2.07
4	Oxalic acid	8.959	2.76±0.08	1.72
5	Iso-citric acid	31.034	2.59±0.08	1.62
6	Glutaric acid	20.096	0.97±0.04	0.60
7	Malonic acid	11.394	0.95±0.04	0.59
8	Adipic acid	36.274	0.61±0.04	0.38
9	Fumaric acid	12.301	0.31±0.04	0.19
10	Caproic acid	5.056	0.21±0.04	0.13
	Total mono-, di-, tricarboxylic acids		**61.48**	38.34
11	Vanillic acid	31.664	2.59±0.08	1.62
12	Benzoic acid	14.076	1.51±0.08	0.94
13	Ferulic acid	39.815	0.79±0.04	0.49
14	*p*-hydroxybenzoic acid	36.935	0.61±0.04	0.38
15	Syringic acid	37.428	0.42±0.04	0.26
16	Gentisic acid	37.803	0.32±0.04	0.20
17	Salicylic acid	17.154	0.17±0.01	0.11
18	Phenylacetic acid	16.806	0.11±0.01	0.07
	Total phenolcarboxylic acids		**6.75**	4.21
19	Levulinic acid	12.689	64.47±1.00	40.21
20	Linoleic acid	30.294	8.50±0.08	5.30
21	Linolenic acid	31.580	6.80±0.08	4.24
22	Palmitic acid	25.433	4.06±0.08	2.53
23	Oleic acid	30.183	1.91±0.04	1.19
24	Stearic acid	30.061	1.68±0.04	1.05
25	Arachidic acid	32.659	0.64±0.04	0.40
26	Heneicosanoic acid	34.236	0.58±0.04	0.36
27	Behenic acid	35.601	0.58±0.04	0.36
28	Tetracosanoic acid	38.494	0.55±0.04	0.34
29	Heptadecanoic acid	26.410	0.54±0.04	0.34
30	2-hydroxypalmitic acid	32.043	0.43±0.04	0.27
31	Azelaic acid	24.764	0.41±0.04	0.26
32	Palmitoleic acid	25.776	0.34±0.04	0.21
33	Myristic acid	21.738	0.33±0.04	0.21
34	Lauric acid	17.634	0.22±0.01	0.14
35	Tricosanoic acid	37.173	0.21±0.01	0.13
36	Pentadecanoic acid	24.099	0.19±0.01	0.12
	Total Fatty acids		**92.11**	57.45
	TOTAL ACIDS		**160.34**	**100**

Compared to our results, in our research, the sum of polyphenols was in 20.25% lower, the content of sanguiin 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.* [19] 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 *Rubus idaeus* shoots collected in April or May, whereas we obtained extract from *Rubus idaeus* shoots collected in July. Mechanism of inflammation represents a chain of dynamic responses including both cellular

The next stage of our research was to conduct a theoretical study of the anti-inflammatory activity of the identified compounds using molecular docking in order to understand their promising capabilities for suppressing the inflammatory process. For a theoretical assessment of the

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anti-inflammatory effect, the following key enzyme structures were selected: COX-2. phospholipase A2. NF-kB, 5-LOX, NADPH oxidase, myeloperoxidase and xanthine oxidase. We chose diclofenac sodium as the standard of comparison for theoretical studies of the enzymes COX-2, phospholipase A2, NF-kB, 5-LOX, and for NADPH oxidase, myeloperoxidase and xanthine oxidase - epigallocatechin-3-O-gallate. To understand the level of selectivity of inhibition of the studied substances to the active centers of bacterial enzymes, we applied the following classification of selectivity [16]: IC50 < 0.001 mM (high selective); 0.05 > IC50 > 0.01 (medium selective); IC50 >0.05 mM (low selective).

Molecular modeling of the studied compounds was carried out with the active site of COX-2. The active site was represented by the following amino acids: Ala202. Tyr348. Thr206. Tyr385. Trp387. Glu203. Ala199 and Leu390. According to the results of the study, it was found that the following compounds had a high affinity for the active site compared to the standard: (+)-catechin, citric acid, iso-citric acid, epicatechin, ellagic acid, linoleic acid, stearic acid and arachidic acid. When comparing the results obtained with the reference standard, (+)-catechin was better by 46%, citric acid - by 41%, iso-citric acid - by 39%, epicatechin - by 25%, ellagic acid - by 14%, linolenic acid - by 10%, stearic acid by 7%, and arachidic acid by 4%, while other compounds performed worse than the comparison standard. (Table 4)

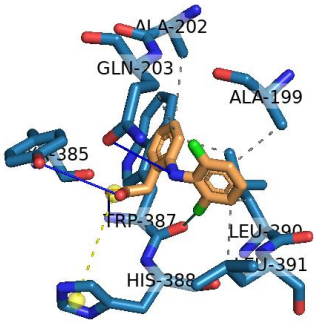

Fig. 2. Molecular interaction analysis of diclofenac sodium with active center of COX-2 structure

Table 4. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the COX-2 structure

№	Ligand	COX-2[a]	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
	Diclofenac sodium	**-5.76**	**0.060**	—	Low selective
1	(+)-Catechin	-8.40	0.00069	+46%	High selective
2	Citric acid	-8.11	0.0011	+41%	Medium selective
3	Iso-citric acid	-8.00	0.001	+39%	Medium selective
4	Epicatechin	-7.20	0.005	+25%	Medium selective
5	Ellagic acid	-6.55	0.016	+14%	Medium selective
6	Linoleic acid	-6.32	0.023	+10%	Medium selective
7	Stearic acid	-6.14	0.032	+7%	Medium selective
8	Arachidic acid	-6.00	0.050	+4%	Medium selective
9	Tetracosanoic acid	-5.32	0.065	-8%	Medium selective
10	Linolenic acid	-5.17	0.16	-10%	Low selective
11	2-hydroxypalmitic acid	-5.15	0.18	-11%	Low selective
12	Heneicosanoic acid	-5.12	0.20	-11%	Low selective
13	Heptadecanoic acid	-5.12	0.21	-11%	Low selective
14	Behenic acid	-5.1	0.22	-11%	Low selective

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№	Ligand	COX-2[a]	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
15	Glutaric acid	-5.00	0.26	-13%	Low selective
16	Succinic acid	-4.85	0.28	-16%	Low selective
17	Vanillic acid	-4.70	0.36	-18%	Low selective
18	Malic acid	-4.63	0.40	-20%	Low selective
19	Palmitic acid	-4.50	0.41	-22%	Low selective
20	Oleic acid	-4.31	0.56	-25%	Low selective
21	Phenylacetic acid	-4.25	0.65	-26%	Low selective
22	Benzoic acid	-4.04	1.09	-30%	Low selective
23	Salicylic acid	-4.00	1.18	-31%	Low selective
24	Azelaic acid	-3.98	1.67	-31%	Low selective
25	Levulinic acid	-3.71	1.92	-36%	Low selective
26	Gentisic acid	-3.50	2.64	-39%	Low selective
27	Palmitoleic acid	-3.50	2.96	-39%	Low selective
28	Pentadecanoic acid	-3.35	3.34	-42%	Low selective
29	Myristic acid	-3.33	3.68	-42%	Low selective
30	Tricosanoic acid	-3.30	3.83	-43%	Low selective
31	Syringic acid	-3.25	3.96	-44%	Low selective
32	Lauric acid	-3.23	5.05	-44%	Low selective
33	Ferulic acid	-3.20	5.55	-44%	Low selective
34	Oxalic acid	-3.07	5.64	-47%	Low selective
35	*p*-hydroxybenzoic acid	-3.00	6.42	-48%	Low selective
36	Malonic acid	-2.97	6.66	-48%	Low selective
37	Adipic acid	-2.00	30.53	-65%	Low selective
38	Caproic acid	-1.50	75.60	-74%	Low selective
39	Fumaric acid	-1.47	84.34	-74%	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme that was studied was NF-kB. The active center of this enzyme was represented by the following amino acids: Lys147. His144. Tyr60. Leu210. Thr146. Lys148. Val145. According to the results shown in Table. 5 the following compounds had high binding energies compared to diclofenac sodium: ellagic acid, epicatechin, fumaric acid, salicylic acid, oxalic acid, benzoic acid, (+)-catechin, vanillic acid, citric acid, ferulic acid, iso- citric acid and *p*-hydroxybenzoic acid.

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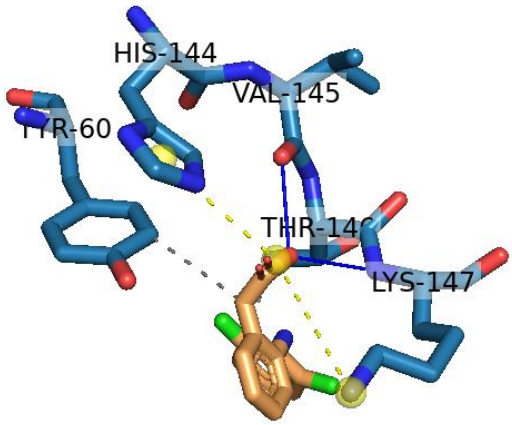

Fig. 3. Molecular interaction analysis of diclofenac sodium with active center of NF-kB structure

Table 5. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the NF-kB structure

№	Ligand	NF-kB	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
	Diclofenac sodium	**-3.90**	**1.38**	—	Low selective
1.	Fumaric acid	-5.35	0.120	+37%	Low selective
2.	Salicylic acid	-5.31	0.127	+36%	Low selective
3.	Oxalic acid	-5.27	0.130	+35%	Low selective
4.	Benzoic acid	-5.18	0.16	+33%	Low selective
5.	(+)-Catechin	-4.82	0.29	+24%	Low selective
6.	Vanillic acid	-4.72	0.34	+21%	Low selective
7.	Citric acid	-4.6	0.42	+18%	Low selective
8.	Epicatechin	-4.58	0.44	+17%	Low selective
9.	Ferulic acid	-4.45	0.55	+14%	Low selective
10.	Iso-Citric acid	-4.3	0.60	+10%	Low selective
11.	*p*-hydroxybenzoic acid	-4.10	0.62	+5%	Low selective
12.	Ellagic acid	-3.93	1.23	+3%	Low selective
13.	Phenylacetic acid	-3.89	1.98	0%	Low selective
14.	Syringic acid	-3.4	2.70	-13%	Low selective
15.	Myristic acid	-3.1	7.84	-21%	Low selective
16.	Gentisic acid	-3	8.98	-23%	Low selective
17.	Malonic acid	-2.69	10.73	-31%	Low selective
18.	Heneicosanoic acid	-2.65	11.32	-32%	Low selective
19.	Malic acid	-2.64	11.69	-32%	Low selective
20.	Arachidic acid	-2.6	13.33	-33%	Low selective
21.	Palmitoleic acid	-2.45	15.35	-37%	Low selective
22.	Levulinic acid	-2.42	16.87	-38%	Low selective
23.	Oleic acid	-2.41	16.99	-38%	Low selective

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№	Ligand	NF-kB	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
24.	Behenic acid	-2.41	16.99	-38%	Low selective
25.	Heptadecanoic acid	-2.40	17.23	-38%	Low selective
26.	2-hydroxypalmitic acid	-2.39	17.54	-38%	Low selective
27.	Tetracosanoic acid	-2.38	18.12	-39%	Low selective
28.	Succinic acid	-2.37	18.28	-39%	Low selective
29.	Linolenic acid	-2.37	18.31	-39%	Low selective
30.	Lauric acid	-2.33	20.54	-40%	Low selective
31.	Glutaric acid	-2.32	21.79	-41%	Low selective
32.	Palmitic acid	-2.31	22.65	-41%	Low selective
33.	Adipic acid	-2.2	25.57	-44%	Low selective
34.	Azelaic acid	-2.1	28.87	-46%	Low selective
35.	Linoleic acid	-2.0	34.37	-49%	Low selective
36.	Stearic acid	-1.95	36.90	-50%	Low selective
37.	Pentadecanoic acid	-1.63	48.86	-59%	Low selective
38.	Caproic acid	-1.43	58.87	-64%	Low selective
39.	Tricosanoic acid	-1.40	66.98	-64%	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds was carried out with the active site of 5-LOX. The active center was represented by the following amino acids: Thr66. Ile119. Leu120. Phe123. According to the results of the study, it was found that the following compounds had a high affinity for the active site compared to diclofenac sodium: epicatechin and ellagic acid. When comparing the results obtained with the reference standard, epicatechin by 37%, and ellagic acid by 9%, while other compounds performed worse than the reference standard. (Table 6)

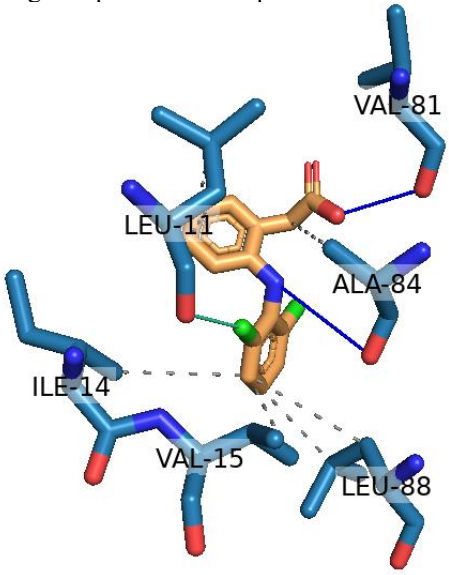

Fig. 4. Molecular interaction analysis of diclofenac sodium with active center of 5-LOX structure

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Table 6. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the 5-LOX structure

№	Ligand	5-LOX	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
I.	**Diclofenac sodium**	**-6.00**	**0.03982**	—	Low selective
	Epicatechin	-8.23	0.00093	+37%	High selective
	Ellagic acid	-6.55	0.016	+9%	Medium selective
	(+)-Catechin	-4.55	0.46	-24%	Low selective
	Vanillic acid	-4.69	0.37	-22%	Low selective
	Citric acid	-4.43	0.57	-26%	Low selective
	Glutaric acid	-4.30	0.60	-28%	Low selective
	Phenylacetic acid	-4.30	0.61	-28%	Low selective
	Iso-Citric acid	-4.12	0.71	-31%	Low selective
	Benzoic acid	-3.44	3.02	-43%	Low selective
	Gentisic acid	-3.31	3.35	-45%	Low selective
	Syringic acid	-3.30	3.56	-45%	Low selective
	Ferulic acid	-3.20	5.55	-47%	Low selective
	p-hydroxybenzoic acid	-3.00	6.55	-50%	Low selective
	Levulinic acid	-2.96	6.78	-51%	Low selective
	Malonic acid	-2.60	12.41	-57%	Low selective
	Fumaric acid	-2.58	12.93	-57%	Low selective
	Salicylic acid	-2.35	19.00	-60%	Low selective
	Arachidic acid	-2.30	20.34	-62%	Low selective
	Tetracosanoic acid	-2.20	24.45	-63%	Low selective
	2-hydroxypalmitic acid	-2.16	26.54	-64%	Low selective
	Heptadecanoic acid	-2.13	28.23	-65%	Low selective
	Heneicosanoic acid	-2.12	29.34	-65%	Low selective
	Behenic acid	-2.10	30.54	-65%	Low selective
	Palmitoleic acid	-1.85	40.65	-69%	Low selective
	Stearic acid	-1.83	45.57	-70%	Low selective
	Adipic acid	-1.80	48.68	-71%	Low selective
	Azelaic acid	-1.80	48.33	-71%	Low selective
	Linolenic acid	-1.79	48.68	-70%	Low selective
	Linoleic acid	-1.71	56.11	-72%	Low selective
	Succinic acid	-1.55	73.06	-74%	Low selective
	Palmitic acid	-1.50	85.64	-75%	Low selective
	Oxalic acid	-1.40	94.60	-77%	Low selective
	Oleic acid	-1.31	101.03	-78%	Low selective
	Lauric acid	-1.31	101.03	-78%	Low selective
	Myristic acid	-1.30	108.45	-78%	Low selective
	Tricosanoic acid	-1.28	110.66	-79%	Low selective
	Pentadecanoic acid	-1.25	126.67	-79%	Low selective
	Malic acid	-1.20	133.02	-80%	Low selective
	Caproic acid	1.30	145.45	-78%	Low selective

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Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme that was studied was phospholipase A2. The active center of this enzyme was represented by the following amino acids: Tyr28. Gly30. Gly32. Asp49. Ca501. Cys45. Phe22. Pro18. Arg6. Ile9. Phe5. Phe106. His48. Cys29. According to the results shown in Table. 6. the following compounds had high binding energies compared to diclofenac sodium: epicatechin, (+)-catechin and ellagic acid (Table 7)

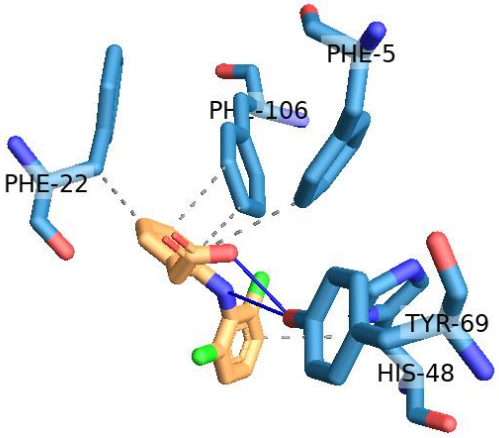

Fig. 5. Molecular interaction analysis of diclofenac sodium with active center of 5-LOX structure

Table 7. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the phospholipase A2 structure

№	Ligand	Phospholipase A2	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
	Diclofenac sodium	**-7.65**	**0.00248**	—	Medium selective
1.	Ellagic acid	-9.52	0.000098	24%	High selective
2.	(+)-Catechin	-9.34	0,00014	22%	High selective
3.	Epicatechin	-9.01	0.00025	18%	High selective
4.	Citric acid	-7.08	0.00648	-7%	Medium selective
5.	Stearic acid	-6.67	0.011	-13%	Medium selective
6.	Iso-Citric acid	-6.56	0.023	-14%	Medium selective
7.	Linoleic acid	-6.44	0.032	-16%	Medium selective
8.	Arachidic acid	-6.10	0,050	-20%	Medium selective
9.	Ferulic acid	-5.92	0.062	-23%	Medium selective
10.	Linolenic acid	-5.74	0.067	-25%	Medium selective
11.	Salicylic acid	-5.66	0.068	-26%	Medium selective
12.	Vanillic acid	-5.50	0.069	-28%	Medium selective
13.	Tetracosanoic acid	-5.50	0.069	-28%	Medium selective
14.	Glutaric acid	-5.45	0.071	-29%	Medium selective
15.	Heneicosanoic acid	-5.42	0.073	-29%	Medium selective
16.	Heptadecanoic acid	-5.40	0.075	-29%	Medium selective
17.	2-hydroxypalmitic acid	-5.38	0.080	-30%	Medium selective
18.	Behenic acid	-5.30	0.096	-31%	Medium selective
19.	Benzoic acid	-4.98	0.16	-35%	Low selective
20.	Malic acid	-4.67	0.38	-39%	Low selective
21.	Palmitic acid	-4.60	0.44	-40%	Low selective
22.	Oleic acid	-4.40	0.54	-42%	Low selective

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№	Ligand	Phospholipase A2	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
23.	Oxalic acid	-4.39	0.60	-43%	Low selective
24.	Levulinic acid	-4.36	0.65	-43%	Low selective
25.	Phenylacetic acid	-4.30	0.69	-44%	Low selective
26.	Azelaic acid	-4.25	0.81	-44%	Low selective
27.	*p*-hydroxybenzoic acid	-4.00	0.89	-48%	Low selective
28.	Malonic acid	-3.99	1.12	-48%	Low selective
29.	Palmitoleic acid	-3.98	1.30	-48%	Low selective
30.	Succinic acid	-3.91	1.35	-49%	Low selective
31.	Gentisic acid	-3.80	1.90	-50%	Low selective
32.	Pentadecanoic acid	-3.70	2.23	-52%	Low selective
33.	Syringic acid	-3.65	2.80	-52%	Low selective
34.	Myristic acid	-3.60	3.01	-53%	Low selective
35.	Lauric acid	-3.58	3.61	-53%	Low selective
36.	Tricosanoic acid	-3.47	4.12	-55%	Low selective
37.	Adipic acid	-3.00	6.11	-61%	Low selective
38.	Caproic acid	-2.10	8.22	-73%	Low selective
39.	Fumaric acid	-2.00	9.11	-74%	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The following theoretical studies were carried out on a group of enzymes that are responsible for the formation of free radicals and oxidative stress. These include NADPH oxidase, myeloperoxidase and xanthine oxidase.

Molecular modeling of the studied compounds was carried out with the active site of NADPH oxidase. The active center was represented by the following amino acids: Glu691. Ser522. Glu443. Thr462. Cys668. Phe667. Pro521. Thr520. Tyr445. Pro542. Asp444. Phe693.

According to the results of the study, it was found that the following compounds had a high affinity for the active site compared to epigallocatechin-3-O-gallate: citric acid, epicatechin, (+)-catechin, ellagic acid and vanillic acid. When comparing the results obtained with the reference standard, citric acid was better by 27%, epicatechin - by 19%, (+)-catechin - 11%, ellagic acid - 4% and vanillic acid - by 1%, while other compounds had worse results than the comparison standard. (Table 8)

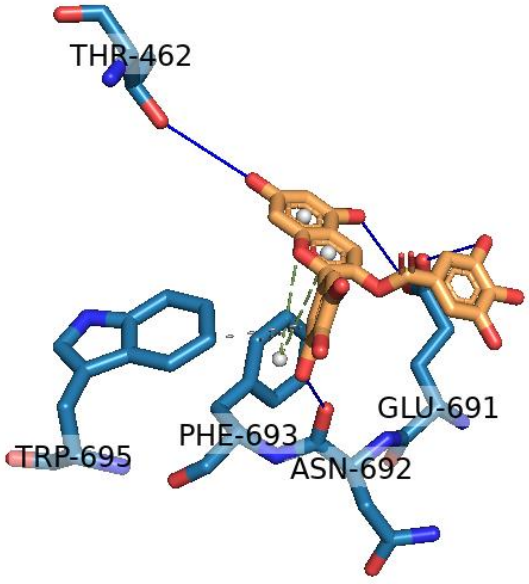

Fig. 6. Molecular interaction analysis of epigallocatechin-3-O-gallate with active center of NADPH oxidase structure

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Table 8. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the NADPH oxidase structure

№	Ligand	NADPH oxidase	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)		%	
	Epigallocatechin-3-O-gallate	**-5.97**	**0.042**	—	Medium selective
1	Citric acid	-7.57	0.0028	+27%	Medium selective
2	Epicatechin	-7.11	0.0062	+19%	Medium selective
3	(+)-Catechin	-6.60	0.014	+11%	Medium selective
4	Ellagic acid	-6.18	0.030	+4%	Medium selective
5	Vanillic acid	-6.02	0.038	+1%	Medium selective
6	Ferulic acid	-4.99	0.22	-16%	Low selective
7	Salicylic acid	-4.71	0.35	-21%	Low selective
8	Levulinic acid	-4.48	0.52	-25%	Low selective
9	Benzoic acid	-4.37	0.62	-27%	Low selective
10	*p*-hydroxybenzoic acid	-4.12	0.73	-31%	Low selective
11	Phenylacetic acid	-4.00	1.15	-33%	Low selective
12	Succinic acid	-3.92	1.34	-34%	Low selective
13	Linoleic acid	-3.82	1.59	-36%	Low selective
14	Linolenic acid	-3.53	2.58	-41%	Low selective
15	Stearic acid	-3.46	2.92	-42%	Low selective
16	Malonic acid	-3.22	4.40	-46%	Low selective
17	Palmitic acid	-3.18	4.80	-47%	Low selective
18	Gentisic acid	-3.15	5.10	-47%	Low selective
19	Oleic acid	-3.14	5.66	-47%	Low selective
20	Malic acid	-3.13	6.08	-48%	Low selective
21	Syringic acid	-3.12	6.43	-48%	Low selective
22	Glutaric acid	-3.10	6.98	-48%	Low selective
23	Iso-Citric acid	-3.00	7.56	-50%	Low selective
24	Arachidic acid	-2.98	8.12	-50%	Low selective
25	Fumaric acid	-2.90	8.78	-51%	Low selective
26	Heneicosanoic acid	-2.88	9.00	-52%	Low selective
27	Lauric acid	-2.83	9.66	-53%	Low selective
28	Behenic acid	-2.75	11.86	-54%	Low selective
29	Myristic acid	-2.71	12.02	-55%	Low selective
30	Palmitoleic acid	-2.61	12.30	-56%	Low selective
31	Heptadecanoic acid	-2.41	15.89	-60%	Low selective
32	Azelaic acid	-2.40	16.10	-60%	Low selective
33	Oxalic acid	-2.36	18.66	-60%	Low selective
34	2-hydroxypalmitic acid	-2.36	19.01	-60%	Low selective
35	Pentadecanoic acid	-2.36	20.33	-60%	Low selective
36	Tetracosanoic acid	-2.35	21.67	-61%	Low selective
37	Caproic acid	-2.30	23.88	-61%	Low selective
38	Tricosanoic acid	-2.19	25.67	-63%	Low selective
39	Adipic acid	-2.11	27.23	-65%	Low selective

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Note: a – free-binding energy; b – inhibition constant, IC50, mmol

The next enzyme that was studied was myeloperoxidase. The active center of this enzyme was represented by the following amino acids: Leu420. Pro145. Arg42. Met411. Ile9. Leu425. Arg333. Leu417. Phe146. According to the results shown in Table. 7. the following compounds had high binding energies compared to epigallocatechin-3-O-gallate: salicylic acid, oxalic acid, benzoic acid, (+)-catechin, ellagic acid, fumaric acid, vanillic acid, epicatechin, phenylacetic acid, ferulic acid. (Table 9)

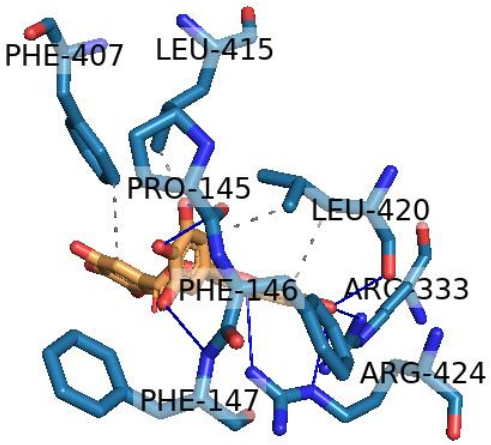

Fig. 6. Molecular interaction analysis of epigallocatechin-3-O-gallate with active center of myeloperoxidase structure

Table 9. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the myeloperoxidase structure

№	Ligand	Myeloperoxidase	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
	Epigallocatechin-3-O-gallate	**-4.52**	**0.0423**	—	Low selective
1	Salicylic acid	-6.06	0.036	34%	Medium selective
2	Oxalic acid	-5.98	0.041	32%	Medium selective
3	Benzoic acid	-5.86	0.051	30%	Medium selective
4	(+)-Catechin	-5.57	0.083	23%	Medium selective
5	Ellagic acid	-5.51	0.091	22%	Medium selective
6	Fumaric acid	-5.47	0.097	21%	Medium selective
7	Vanillic acid	-5.19	0.158	15%	Low selective
8	Epicatechin	-5.04	0.202	12%	Low selective
9	Phenylacetic acid	-4.86	0.312	8%	Low selective
	Citric acid	-4.79	0.310	5%	Low selective
10	Ferulic acid	-4.65	0.391	3%	Low selective
12	Iso-Citric acid	-4.32	0.560	-4%	Low selective
13	Glutaric acid	-4.11	0.680	-9%	Low selective
14	*p*-hydroxybenzoic acid	-4.00	0.984	-12%	Low selective
15	Gentisic acid	-3.88	1.34	-14%	Low selective
16	Stearic acid	-3.85	1.50	-15%	Low selective
17	Syringic acid	-3.78	1.68	-16%	Low selective
18	Linolenic acid	-3.73	1.83	-17%	Low selective
19	Palmitic acid	-3.61	2.23	-20%	Low selective
20	Tetracosanoic acid	-3.56	2.65	-21%	Low selective
21	Linoleic acid	-3.50	2.73	-23%	Low selective

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№	Ligand	Myeloperoxidase	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
22	Heneicosanoic acid	-3.43	3.01	-24%	Low selective
23	Heptadecanoic acid	-3.43	3.02	-24%	Low selective
24	Oleic acid	-3.42	3.11	-24%	Low selective
25	2-hydroxypalmitic acid	-3.38	3.13	-25%	Low selective
26	Caproic acid	-3.36	3.65	-26%	Low selective
27	Behenic acid	-3.36	3.71	-26%	Low selective
28	Azelaic acid	-3.25	4.12	-28%	Low selective
29	Adipic acid	-3.16	4.46	-30%	Low selective
30	Arachidic acid	-3.16	4.6	-30%	Low selective
31	Malonic acid	-3.03	5.42	-33%	Low selective
32	Palmitoleic acid	-2.98	6.42	-34%	Low selective
33	Levulinic acid	-2.96	6.71	-35%	Low selective
34	Tricosanoic acid	-2.80	7.88	-38%	Low selective
35	Pentadecanoic acid	-2.78	8.21	-38%	Low selective
36	Lauric acid	-2.63	10.23	-42%	Low selective
37	Myristic acid	-2.61	11.00	-42%	Low selective
38	Succinic acid	-2.60	12.44	-42%	Low selective
39	Malic acid	-2.24	22.70	-50%	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Molecular modeling of the studied compounds was carried out with the active site of xanthine oxide. The active center was represented by the following amino acids: Ala255. Leu257. Glu402. Leu398 and hydrophobic bonds: Pro400. Thr396. Gly399. Lys256. Ile403. Lys249. According to the results of the study, it was found that the following compounds had a high affinity for the active site compared to epigallocatechin-3-O-gallate: (+)-catechin, epicatechin. When comparing the results obtained with the reference standard, (+)-catechin was better by 5%, and epicatechin - by 2%, while other compounds had worse results than the reference standard. (Table 10)

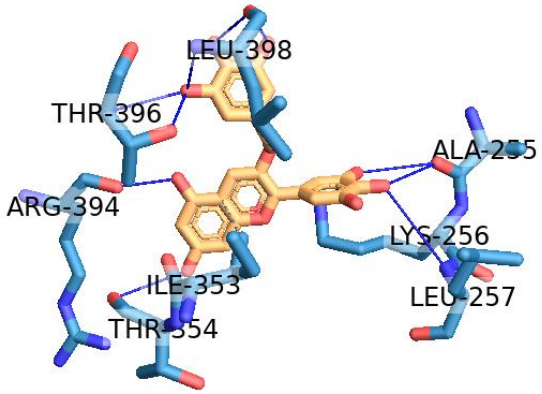

Fig. 7. Molecular interaction analysis of epigallocatechin-3-O-gallate with active center of xanthine structure

Table 10. Results of molecular docking of the compounds identified by the HPLC and GC in the *Rubus idaeus* shoots extract with the xanthine oxidase structure

№	Ligand	Xanthine oxidase	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
	Epigallocatechin-3-O-gallate	-7.10	**0.00445**		

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№	Ligand	Xanthine oxidase	Ki[b]	Difference compared to standard	Level of selectivity
		ΔGbind[a] (kcal/mol)	mmol	%	
1	(+)-Catechin	-7.43	0.00360	+5%	High selective
2	Epicatechin	-7.21	0.00523	+2%	High selective
3	Ellagic acid	-6.85	0.00952	-4%	Medium selective
4	Phenylacetic acid	-5.86	0.010	-17%	Medium selective
5	Citric acid	-5.28	0.135	-26%	Low selective
6	Iso-Citric acid	-5.11	0.240	-28%	Low selective
7	Glutaric acid	-4.88	0.358	-31%	Low selective
8	Gentisic acid	-4.58	0.543	-35%	Low selective
9	*p*-hydroxybenzoic acid	-4.36	0.657	-39%	Low selective
10	Fumaric acid	-4.28	0.731	-40%	Low selective
11	Salicylic acid	-4.25	0.773	-40%	Low selective
12	Oxalic acid	-4.24	0.780	-40%	Low selective
13	Benzoic acid	-4.24	0.776	-40%	Low selective
14	Vanillic acid	-4.03	1.12	-43%	Low selective
15	Ferulic acid	-4.01	1.16	-44%	Low selective
16	Syringic acid	-3.96	1.30	-44%	Low selective
17	Palmitic acid	-3.86	1.60	-46%	Low selective
18	Heneicosanoic acid	-3.83	1.70	-46%	Low selective
19	Linoleic acid	-3.71	1.91	-48%	Low selective
20	Linolenic acid	-3.68	2.01	-48%	Low selective
21	Behenic acid	-3.66	2.10	-48%	Low selective
22	Azelaic acid	-3.65	2.30	-49%	Low selective
23	Caproic acid	-3.56	2.50	-50%	Low selective
24	Heptadecanoic acid	-3.53	2.70	-50%	Low selective
25	Levulinic acid	-3.44	2.99	-52%	Low selective
26	Tetracosanoic acid	-3.41	4.23	-52%	Low selective
27	Arachidic acid	-3.36	4.80	-53%	Low selective
28	Oleic acid	-3.22	5.11	-55%	Low selective
29	2-hydroxypalmitic acid	-3.18	5.40	-55%	Low selective
30	Malonic acid	-3.08	5.52	-57%	Low selective
31	Adipic acid	-3.06	6.00	-57%	Low selective
32	Tricosanoic acid	-2.91	6.10	-59%	Low selective
33	Lauric acid	-2.88	7.60	-59%	Low selective
34	Palmitoleic acid	-2.86	9.00	-60%	Low selective
35	Succinic acid	-2.73	9.96	-62%	Low selective
36	Malic acid	-2.54	13.81	-64%	Low selective
37	Stearic acid	-2.49	14.97	-65%	Low selective
38	Pentadecanoic acid	-2.23	16.80	-69%	Low selective
39	Myristic acid	-2.12	18.80	-70%	Low selective

Note: a – free-binding energy; b – inhibition constant, IC50, mmol

Mechanism of inflammation represents a chain of dynamic responses including both cellular and vascular events with specific humoral secretions. These pathways involve changing physical location of white blood cells, and a group of secreted mediators at inflamed site. A

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variety of chemical mediators released: vasoactive amines (histamine and serotonin), peptide (bradykinin), and eicosanoids (prostaglandins, leukotrienes) [20]. Moreover, recent research has revealed that non-phagocytic cells, like interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and eNOS can also produce free radicals by expressing NADPH oxidase, and myeloperoxidase [21]. Importantly, oxidative stress not only arises from inflammation but can also provoke it. Studies have shown that hydrogen peroxide free radicals can initiate inflammation by activating transcriptional enzymes like NF-kB [22]. Therefore, in order to calculate theoretical doses of ionized and non-ionized (+)-catechin and epicatechin for suppress inflammation, we chose following crucial enzymes that play a pivotal role in developing inflammation: COX-2. phospholipase A2. NF-kB, 5-LOX, NADPH oxidase, myeloperoxidase, and xanthine oxidase.

Phospholipase A2 is an import enzyme that activate every inflammation pathway as phospholipase A2 catalyzes hydrolysis of glycerophosphate followed by liberation of arachidonic acid. Arachidonic acid is a precursor of eicosanoids, such as: prostaglandins and leukotrienes [23]. Thus, phospholipase A2 is a primary reason for developing inflammation. The next import enzyme that take part in 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 [24]. Leukotrienes can be produced instead of prostaglandins by the action of LOX-5 with liberated arachidonic acid. Moreover, LOX-5 contributes a big part in occurrence lipid peroxidation, in cell death including apoptosis, pyroptosis, and ferroptosis, a newly discovered type of cell death. Thus, COX-2 and LOX-5 are an important target for studying the anti-inflammatory activity of drugs. The next crucial enzyme in occurrence and developing of inflammation is transcriptional factor of NF-kB. The role of this enzyme is based on expression proinflammatory cytokines, such as IL-6. IL-10. and TNF-α. The factors that activate NF-kB signal pathway are excess of reactive oxygen species (ROS), and cytokines [25]. Thus, in order to suppress effect of "vicious circle" of inflammation, activation of NF-kB should have prevented. Oxidative stress is the main "partner" of inflammation as they directly related between each other, and can be the reason of developing each other. One of the crucial enzymes that causes formation ROS are NADPH oxidase, myeloperoxidase, and xanthine oxidase. Among the enzymes responsible for generating ROS, NADPH oxidase stands out as the sole enzyme devoted exclusively to this function [26]. 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. The second most important enzyme that form ROS is myeloperoxidase. This enzyme to a large extent is predominantly found in the neutrophils. The main function of myeloperoxidase had been mainly associated with its ability as a catalyzer of reactive oxidants that help to clear pathogens. However, according to compelling evidence in the case when an exacerbate infiltration of leukocytes can favor a poorly-controlled production and excess of release of myeloperoxidase and its oxidants that lead to tissue damage and the development and propagation of acute and chronic vascular inflammation [27]. So, myeloperoxidase was chosen as a target for inhibition oxidative stress. The next enzyme that has a significant on a production of ROS is xanthine oxidase. Xanthine oxidase is a dimeric metalloflavoprotein comprising two identical subunits, each enzyme includes one molybdenum-containing molybdopterin cofactor (Mo-co) and FAD cofactor. The purine oxidation occurs at the Mo-co site, while the FAD site is the oxidized nicotinamide adenine dinucleotide and O_2 reduction sites. The main role of xanthine oxidase is oxidation of hypoxanthine to xanthine and xanthine to uric acid. However, except taking part in purine metabolism, xanthine oxidase produces following forms of ROS: superoxide anion ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) [28]. Therefore, xanthine oxidase is target for decreasing a number of ROS inflammation. For further development of technologies for obtaining the extract, it is necessary to select chemical compounds that must be present in the extract to obtain maximum anti-inflammatory effect. A literature search showed that today there is no criterion for selecting compounds based on the level of binding energies during molecular docking. This problem is relevant for modern science. In our research, the criterion for selecting compounds will be as follows: “the value of the binding energies of the studied compounds must be greater than the binding energy of the standard for each presented mechanism of action.” Thus, taking into account the above study results, we can conclude that only two compounds meet this criterion: (+)-catechin and epicatechin. Other compounds work perfectly according to one mechanism, but in another case they practically do not inhibit it. Such compounds will only increase the dose of the extract, and will not in any way affect the effectiveness of the extract. These “ballast” compounds include organic acids (mono-, di-, tricarboxylic and fatty acids) and phenolcarboxylic acids, which in no way actively influence the suppression of the inflammatory process. Therefore, these groups of compounds must be removed from the native extract. To do this, we used an organic solvent - chloroform, followed by acidification with sulfate acid to pH = 3 to destroy possible salts, extraction was carried out twice within 15 minutes. Thus, the new raspberry shoot extract contains only catechin derivatives.

Conclusions

Rubus idaeus shoot extract was dominated by (+)-catechin, epicatechin, levulinic acid, citric acid and vanillic acid. During the study, it was found that derivatives of organic (mono-, di-, tricarboxylic and fatty acids) and phenolcarboxylic acids do not have a high level of anti-inflammatory effect. It was established that (+)-catechin and epicatechin have a higher level of affinity than other identified phenolic compounds and organic acids for the active centers of phospholipase A2. COX-2. LOX-5. NF-kB, NADPH oxidase, myeloperoxidase and xanthine oxidase. The optimal technology for obtaining an extract with the maximum level of anti-inflammatory effect is the removal of organic and phenolcarboxylic acids.

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Molecular docking studies of anti-inflammatory, antioxidant activity and phytochemical composition of *Rubus idaeus* shoot extract

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Introduction. Currently, the primary treatments for inflammation include steroidal drugs (e.g., prednisolone) and non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac and indomethacin, which are widely used to manage both acute and chronic conditions like rheumatoid arthritis and osteoarthritis. However, these medications are often associated with numerous adverse effects. As a result, the search for novel anti-inflammatory agents derived from natural herbal sources has become a pressing need. **The aim of the work** was carried out molecular docking studies of anti-inflammatory, antioxidant activity of identified compounds and investigation phytochemical composition of *Rubus idaeus* shoot extract by HPLC and GC. **Material and methods.** The quantification of phenolic compounds was accomplished through HPLC, the content of organic and phenolcarboxylic acids was determined by GC, molecular docking of the cyclooxygenase-2 (COX-2), phospholipase A2, nuclear factor kB (NF-kB), 5-lypoxygenase (5-LOX), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase, xanthine oxydase enzymes was carried out using the AutoDockTools 1.5.6 software. **Results and Discussion.** The 11 compounds were identified by the HPLC and 36 compounds were detected by GC. The epicatechin (882.00 mg/100 g), (+)-catechin (480.00 mg/100 g), ellagic acid and its derivatives (459.00 mg/100 g), citric acid (49.21 mg/100 g), vanillic acid (2.59 mg/100 g) and levulinic acid (64.67 mg/100 g) were dominated in the obtained extract of raspberry shoots. The free energy of (+)-catechin and epicatechin were the highest for the active sites of COX-2. phospholipase A2, NF-kB, 5-LOX, NADPH oxidase, myeloperoxidase, xanthine oxidase enzymes. **Conclusion**. *Rubus idaeus* shoot extract was dominated by (+)-catechin, epicatechin, levulinic acid, citric acid and vanillic acid. During the study, it was found that derivatives of organic (mono-, di-, tricarboxylic and fatty acids) and phenolcarboxylic acids do not possess a high level of anti-inflammatory effect. The optimal technology for obtaining an extract with the maximum level of anti-inflammatory effect is to remove organic and phenolcarboxylic acids and leave catechins derivatives.

Key words: Raspberry, Shoot extract, HPLC, GC-MS, Molecular docking, Anti-inflammatory activity, Antioxidant activity

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