UDC 615.322:616-002 https://doi.org/10.24959/nphj.26.211

S. R. Petroshchuk[1], L. V. Derymedvid[1], O. Yu. Maslov[1], M. A. Komisarenko[1], I. O. Lebedinets[2], T. K. Yudkevych[2], S. V. Kolisnyk[1]

[1] National University of Pharmacy of the Ministry of Health of Ukraine
[2] Research Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy of the Ministry of Health of Ukraine

The study of the anti-inflammatory activity of a thick lingonberry fruit extract in the carrageenan-induced rat paw edema model and molecular docking

Aim. To assess the anti-inflammatory activity of a concentrated lingonberry fruit extract using the carrageenan-induced rat paw edema model, complemented by the molecular docking analysis.

Materials and methods. The study object was a concentrated lingonberry fruit extract. Molecular docking analyses were conducted using AutoDockTools 1.5.6, and the anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in rats.

Results. The theoretical assessment of the anti-inflammatory activity of the lingonberry fruit extract showed that lingonberry anthocyanins, such as cyanidin-3-galactoside, cyanidin-3-arabinoside, blocked three of them highly selectively, such as cyclooxygenase (COX-2), phospholipase A_2 and 5-lipoxygenase (5-LOX), and medium-selective nuclear factor kappa B (NF-kB). No highly selective inhibitor was found among anthocyanins of NF-kB. At the same time, cyanidin-3-glucoside blocked three out four targets, such as COX-2, phospholipase A2, 5-LOX. Although the widely used reference compounds in medicine and research – the synthetic drug diclofenac sodium and the natural flavonoid quercetin – demonstrated the inhibitory activity against pro-inflammatory enzymes, their binding affinities were moderate to low. The molecular docking analysis showed the following binding energy values (kcal/mol) for diclofenac sodium and quercetin: COX-2 (–5.76 and –4.59), phospholipase A_2 (–7.65 and –6.79), 5-LOX (–6.00 and –6.45), and NF-κB (–3.00 and –3.61), respectively. Experimental studies have shown a thick lingonberry fruit extract in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) reduces edema in 1, 2, 3 and 4 hours by 45 %, 35 %, 25 % and 24 %compared to the control group, respectively.

Conclusions. A comprehensive theoretical and experimental study of the anti-inflammatory properties of the lingonberry fruit extract has been conducted using the molecular docking analysis and the carrageenan-induced rat paw edema model *in vivo*. The results *in silico* demonstrated that lingonberry anthocyanins exhibited a strong binding affinity toward key pro-inflammatory targets, including COX-2, phospholipase A_2, 5-LOX, and NF-κB. The findings *in vivo* showed that administration of a thick lingonberry fruit extract in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) significantly inhibited inflammatory responses in all phases of the carrageenan-induced paw edema.

Keywords: *lingonberry; fruit; inflammation; in silico; extract; carrageenan model.*

С. Р. Петрощук[1], Л. В. Деримедвідь[1], О. Ю. Маслов[1], М. А. Комісаренко[1], І. О. Лебединець[2], Т. К. Юдкевич[2], С. В. Колісник[1]

[1] Національний фармацевтичний університет Міністерства охорони здоров'я України
[2] Навчально-науковий інститут прикладної фармації Національного фармацевтичного університету Міністерства охорони здоров'я України

Дослідження протизапальної активності густого екстракту плодів брусниці на карагенан-індукованій моделі набряку лапи щурів та молекулярний докінг

Мета – оцінка протизапальної активності концентрованого екстракту плодів брусниці за допомогою моделі набряку лапи щурів, індукованого карагенаном, доповненої молекулярним докінговим аналізом**.**

Матеріали та методи. Об'єктом дослідження був густий екстракт плодів брусниці. Молекулярний докінг проводили за допомогою AutoDockTools 1.5.6. Протизапальний ефект оцінювали на моделі набряку лапи, індукованого карагенаном у щурів.

Результати та їхнє обговорення. Теоретична оцінка протизапальної активності екстракту плодів брусниці показала, що антоціани брусниці, такі як ціанідин-3-галактозид, ціанідин-3-арабінозид, блокували три з них високоселективно, такі як циклооксигеназа-2 (ЦОГ-2), фосфоліпаза A_2 та 5-ліпооксигеназа (5-ЛОГ), та середньоселективно ядерного фактора каппа B (NF-kB). Не було виявлено жодного високоселективного інгібітора серед антоціанів NF-kB. Тоді як ціанідин-3-глюкозид блокував три з чотирьох мішеней, таких як ЦОГ-2, фосфоліпаза A_2, 5-ЛОГ. Незважаючи на те, що широко застосовувані референтні сполуки в медицині та наукових дослідженнях – синтетичний препарат диклофенак натрію та природний флавоноїд кверцетин – виявляли інгібувальну активність щодо про-запальних ферментів, їхні показники зв'язування були помірними або низькими. Молекулярний докінг показав такі значення енергії зв'язування (ккал/моль) для диклофенаку натрію та кверцетину відповідно: COX-2 (–5,76 та –4,59), фосфоліпаза A_2 (–7,65 та –6,79), 5-LOX (–6,00 та –6,45) та NF-κB (–3,00 та –3,61). Експериментальні дослідження показали, що густий екстракт плодів брусниці в дозі 13,0 мг/кг (у перерахунку на суму

антоціанів, виражено як ціанідин-3-глікозид) зменшує набряки через 1, 2, 3 та 4 години на 45, 35, 25 та 24 % порівняно з контрольною групою відповідно.

Висновки. Було проведено комплексне теоретичне та експериментальне дослідження протизапальних властивостей екстракту плодів брусниці з використанням молекулярного докінг-аналізу та моделі набряку лапи щурів, індукованого карагенаном, *in vivo*. Результати *in silico* продемонстрували, що антоціани брусниці демонструють сильну спорідненість зв'язування з ключовими прозапальними мішенями, включно з ЦОГ-2, фосфоліпазою A_2, 5-ЛОГ та NF-κB. Результати *in vivo* показали, що введення густого екстракту плодів брусниці в дозі 13,0 мг/кг (у перерахунку на суму антоціанів, виражено як ціанідин-3-глікозид) значно пригнічувало запальні реакції на всіх фазах набряку лапи, індукованого карагенаном.

Ключові слова: *брусниця; плоди; запалення; in silico; екстракт; карагенанова модель.*

Introduction. Inflammation is a key biological defense mechanism that enables the body to respond to external challenges, such as microbial infections, chemical exposures, and other injurious stimuli. The immune system is central to this process as it detects foreign or harmful agents and triggers a cascade of pro-inflammatory signaling events. These events result in the enhanced production of cytokines and the activation of immune cells, including macrophages and lymphocytes [1]. The inflammatory response is tightly regulated by multiple interconnected signaling pathways, notably mitogen-activated protein kinase (MAPK) pathways, activation of the nuclear factor-κB (NF-κB), and the biosynthesis of prostaglandins.

The inflammatory cascade consists of a series of coordinated events. Upon exposure to external stimuli, neutrophils and macrophages are rapidly recruited to the site of the tissue injury. This cellular activation initiates the synthesis of prostaglandins through cyclooxygenase enzymes (COX-1 and COX-2) and stimulates the release of key pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and the tumor necrosis factor-α (TNF-α) [2].

Current anti-inflammatory treatments are largely based on steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). Although these agents are effective, their cli-nical use is limited by significant adverse effects. Steroidal anti-inflammatory drugs are associated with immunosuppression and decreased bone mineral density, while NSAIDs are commonly linked to the gastrointestinal ulceration and a bronchospasm [3]. As a result, the identification and development of new anti-inflammatory agents derived from natural products have become a major focus in contemporary medicine and pharmaceutical research.

Today, medicinal plants that are rich sources of anthocyanins have attracted considerable attention from the scientific community [4]. This interest is primarily due to the potent antioxidant activity of natural compounds, as well as their generally favorable safety profiles, with adverse effects occurring less frequently compared to those associated with synthetic drugs.

Lingonberry fruits were selected as a promising source of anthocyanins, while green tea leaves were chosen as a source of catechins. Lingonberry (*Vaccinium vitis-idaea* L.) is an evergreen shrub belonging to the *Ericaceae* family and is widely distributed across the Baltic countries, northern regions of Ukraine and Belarus, as well as Canada [5]. The chemical composition of *V. vitis-idaea* fruits is characterized primarily by anthocyanins, notably cyanidin-3-*O*-galactoside, along with organic acids, such as citric acid [6].

Numerous studies have investigated the pharmacological activity of lingonberry fruits [7]. Anthocyanins derived from lingonberry have been shown to exhibit a wide range of biological effects, including anti-inflammatory, antioxidant, antimicrobial, antihyperglycemic, anticancer, and neuroprotective activities [8, 9]. In addition, lingonberry has been traditionally used in folk medicine for the treatment of fever, infections, diabetes, and liver disorders [10]. Taken together, these findings suggest that lingonberry anthocyanins represent promising candidates for the development of novel antimicrobial and antioxidant pharmaceutical agents.

Numerous studies have investigated the anti-inflammatory activity of lingonberry fresh fruit extracts [11, 12]. However, there is currently not enough data on their effects in the carrageenan-induced inflammation model and on the molecular interactions of lingonberry anthocyanins with pro-inflammatory targets.

Therefore, the aim of this study was to assess the anti-inflammatory activity of a concentrated lingonberry fruit extract using the carrageenan-induced rat paw edema model, complemented by the molecular docking analysis of its anthocyanins against key pro-inflammatory targets.

Materials and methods. Lingonberry fruits was collected in October 2022 in the Kostivtsi village, Zhutomyr region, Ukraine (50.329417, 29.536861).

A 100.0 g sample of lingonberry (accurate weight) was pressed, and the resulting material was extracted with 96 % ethanol in the ratio of three times the mass of the raw material. After filtration, the extract obtained was concentrated using a vacuum evaporator at 50–60 °C until the mass ratio of the extract to the raw material reached 1:0.35.

The experiment used 25 male outbred white rats, each weighing 180–220 g, received from the National University of Pharmacy (NUPh) vivarium. Rats were housed in pairs in Macrolon cages with *ad libitum* access to food and water, which were replenished daily. Bedding was changed every three days. The animals were maintained under controlled conditions with a temperature of 22 ± 2 °C, relative humidity of 60 ± 5 %, and a 12-hour light/dark cycle.

All study procedures were consistent with the National Institute of Health guidelines for the care and use of laboratory animals and the European Council for the Care and Use of Laboratory Animals (86/609/EEC) of November 24, 1986 [13]. The study protocol was sanctioned by the Local Ethics Committee.

The anti-inflammatory activity of the extract was evaluated in 25 male outbred rats weighing 180–220 g. The acute inflammation was induced by a subplantar injection of 0.1 mL of 1 % carrageenan (Fluka, Switzerland) into the right hind paw. The edema formation was measured 1, 2, 3, and 4 hours after injection [14].

All animals were randomly divided into five groups. The first group served as the control pathology (positive control animals received no treatment). The second group was administered sodium diclofenac in the dose of 8 mg/kg. The third group received Quertin (Public Joint-Stock Company "Scientific and Production Center Borshchagov Chemical and Pharmaceutical Plant") in the dose of 50 mg/kg. The fourth group was treated with the lingonberry extract in the dose of 6.5 mg/kg calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside, and the fifth group received the lingonberry extract in the dose of 13.0 mg/kg calculated with reference to the total anthocyanins expressed as cyaniding-3-glycoside.

Molecular docking studies were performed using AutoDockTools version 1.5.6 [15]. The three-dimensional structures of cyclooxygenase-2 (COX-2; PDB ID: 1DDX), phospholipase A_2 (PLA_2; PDB ID: 3HSW), 5-lipoxygenase (5-LOX; PDB ID: 2Q7M), and NF-κB (PDB ID: 1SVC) were retrieved from the Protein Data Bank (PDB) [16]. The resolutions of the protein structures were 3.00 Å for 1DDX, 2.50 Å for 3HSW, 4.25 Å for 2Q7M, and 2.60 Å for 1SVC. The ligand structures of cyanidin-3-galactoside (CID: 176457), cyanidin-3-arabinoside (CID: 91810602), cyanidin-3-glycoside (CID: 197081), cyanidin-3,5-diglucoside (CID: 5158757), cyanidin-3-(6″-acetylglucoside) (CID: 15714477), quercetin (CID: 5280343), and diclofenac sodium (CID: 5018304) were obtained from the PubChem database [17]. The active binding sites of the target proteins were identified using the Computed Atlas of Surface Topography of Proteins (CASTp) server [18].

To obtain statistical results, the Statistica 10 program was used, the results were analyzed using Mann-Whitney test. Differences were considered significant at $p<0.05$.

Results and discussion. At the initial stage of the study, the molecular docking analysis was conducted to assess the anti-inflammatory potential of the lingonberry fruit extract. Inflammatory responses are regulated by multiple signaling pathways that trigger the activation and release of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. For the evaluation *in silico*, COX-2, 5-LOX, phospholipase A2, and NF-κB were selected as key pro-inflammatory targets.

To benchmark the anti-inflammatory potential of the lingonberry fruit extract, sodium diclofenac was included as a reference "gold-standard" drug as it is widely recommended in official clinical protocols for the management of both acute and chronic inflammatory conditions. In addition, quercetin was selected as a representative natural anti-inflammatory compound, taking into account its pharmacological profile and its clinical use in the form of the marketed drug Quertin for the treatment of cardiovascular, neurological, and renal disorders.

In recent research of Vilkickyte et al. [19], the composition of anthocyanins was estimated in the lingonberry fruit extract using high performance liquid chromatography. According to this study, the following anthocyanins was identified: cyanidin-3-galactoside (80.51 % out of total anthocyanins), cyanidin-3-arabinoside (13.20 % out of total anthocyanins), cyanidin-3-glucoside (5.69 % out of total anthocyanins), cyanidin-3,5-diglucoside (1.22 % out of total anthocyanins) and cyanidin 3-(6"-acetylglucoside) (0.10 % out of total anthocyanins). These compounds were evaluated using the molecular docking study to realize the anti-inflammatory potential of the lingonberry fruit extract. Furthermore, the selectivity of the compound inhibition was classified as follows: high selectivity for $IC_{50} < 0.001$ mM, medium selectivity for IC_{50} between 0.01 and 0.05 mM, and low selectivity for $IC_{50} > 0.05$ mM [20].

According to the results presented in Table 1, cyanidin-3-arabinoside, cyanidin-3-galactoside, and cyanidin-3-glucoside were identified as highly selective inhibitors of the COX-2 enzyme. Cyanidin-3-(6″-acetylglucoside) exhibited moderate selectivity, whereas sodium diclofenac, while quercetin demonstrated low selectivity toward COX-2. In contrast, cyanidin-3,5-diglucoside showed no inhibitory activity against the COX-2 enzyme. The active site of the COX-2 enzyme comprises the following amino acid residues: ALA199, ALA202, GLU203, THR206, TYR385, TRP387, HIS388, LEU390, and LEU391 (Table 1).

Furthermore, the inhibitory activity of compounds from the lingonberry fruit extract against phospholipase A2 was evaluated. The results indicated that cyanidin-3,5-diglucoside, cyanidin-3-arabinoside, cyanidin-3-glucoside, cyanidin-3-(6″-acetylglucoside), and cyanidin-3-galactoside acted as highly selective inhibitors, whereas sodium diclofenac and quercetin exhibited moderate selectivity. The active site involved in the phospholipase A_2 inhibition includes the amino acid residues LYS147, VAL145, HIS144, THR146, TYR60, PHE22, and TYR28 (Table 2).

Another key pro-inflammatory enzyme studied was 5-lipoxygenase (5-LOX). The results demonstrated that cyanidin-3-arabinoside, cyanidin-3-galactoside, cyanidin-3,5-diglucoside, cyanidin-3-glucoside, and cyanidin-3-(6″-acetylglucoside) exhibited high selectivity as 5-LOX inhibitors. In contrast, quercetin and sodium diclofenac showed moderate selectivity toward 5-LOX. The active site of the 5-LOX enzyme involved in ligand binding consists of the amino acid residues VAL81, ALA84, LEU11, ILE14, VAL34, and LEU88 (Table 3).

Another significant enzyme involved in the chronic inflammatory process is nuclear factor kappa B (NF-κB). No highly selective inhibitors were identified; however, cyanidin-3-galactoside and cyanidin-3-arabinoside demonstrated moderate selectivity toward NF-κB. In contrast, the remaining compounds exhibited low selectivity for inhibition of the NF-κB active site, which may reflect the structural complexity and critical regulatory

Table 1

The molecular docking of anthocyanins and the anti-inflammatory drug standards – diclofenac sodium and quercetin with the COX-2

Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki[b] mmol	Binding site	Level of selectivity
Cyanidin-3-arabinoside	-12.12	0.0000011	A: ALA199, LEU391, TRP387, TYR385, GLU203	High selectivity
Cyanidin-3-galactoside	-11.11	0.00000714	A: ALA199, ALA202, TYR385, TRP387, HIS388, LEU390, LEU391	High selectivity
Cyanidin-3-glycoside	-10.85	0.0000112	A: TYR385, HIS386, TRP387, HIS388, TRP387, LEU390, LEU391, VAL447	High selectivity
Cyanidin 3-(6″-acetylglucoside)	-7.50	0.00318	A: CYS36, ASN39, CYS41, PRO154, ALA156	Middle selectivity
Diclofenac sodium	-5.76	0.05977	A: ALA199, ALA202, GLN203, TRP387, LEU390, LEU390, TYR385, HIS388	Low selectivity
Quercetin	-4.59	0.42855	A: CYS36, ASN39, CYS41, PRO154, ALA156	Low selectivity
Cyanidin-3,5-diglucoside	64.58			Inactive

Notes: ΔGbind: free-binding energy, Ki: 50 % enzyme inhibition concentration, green/yellow/red: high/moderate/low selectivity.

Table 2

The molecular docking of anthocyanins and the anti-inflammatory drug standards – diclofenac sodium and quercetin with phospholipase A2

Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki[b] mmol	Binding site	Level of selectivity
Cyanidin-3,5-diglucoside	-14.55	0.00000002158	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin-3-arabinoside	-12.54	0.0000006414	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin-3-glucoside	-11.52	0.0000036	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin 3-(6″-acetylglucoside)	-11.36	0.00000473	A:PHE22, PHE106, GLY30, CYS45, HIS48, TYR69	High selectivity
Cyanidin-3-galactoside	-10.45	0.00002177	A: PRO18, PHE22, LEU31, CYS45, HIS48, ASP49, TYR69, HIS48, PHE22, ASN23	High selectivity
Diclofenac sodium	-7.65	0.00248	A:PHE5, PHE22, HIS48, PHE106, TYR69	Middle selectivity
Quercetin	-6.79	0.01062	A: PHE5, ILE9, PHE22, GLY30, CYS45, HIS48, ASP49	Middle selectivity

Notes: ΔGbind: free-binding energy, Ki: 50 % enzyme inhibition concentration, green/yellow/red: high/moderate/low selectivity.

role of this enzyme in inflammatory signaling pathways. The active site of NF-κB comprises the amino acid residues LYS147, LYS148, THR146, TYR60, LEU210, and HIS144 (Table 4).

Furthermore, all data obtained were summarized, and the compounds were conditionally classified into three categories. The first category comprised compounds with high selectivity for the active site, the second one included compounds with moderate selectivity, while the third category consisted of compounds with low selectivity. This classification approach was applied to clearly identify compounds interacting most effectively with pro-inflammatory targets, as well as those exhibiting lower levels of interaction.

Table 5 shows the summarized results of the molecular docking of the pro-inflammatory enzyme inhibition of anthocyanins of the lingonberry fruit extract. The results demonstrate that none of the compounds,

Table 3

The molecular docking of anthocyanins and the anti-inflammatory drug standards – diclofenac sodium and quercetin with 5-LOX

Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki[b] mmol	Binding site	Level of selectivity
Cyanidin-3-arabinoside	-11.03	0.00000818	A: PHE123, VAL70, ILE119, THR66	High selectivity
Cyanidin-3-galactoside	-9.34	0.0001427	A: VAL70, ILE119, PHE123	High selectivity
Cyanidin-3,5-diglucoside	-8.99	0.00025536	A: PHE123, ILE110, THR66	High selectivity
Cyanidin-3-glucoside	-8.65	0.000458	A: VAL70, ILE119, THR66	High selectivity
Cyanidin 3-(6″-acetylglucoside)	-7.99	0.0014	A: VAL70, ILE119, THR66	High selectivity
Quercetin	-6.45	0.01857	A: ILE119, THR66	Middle selectivity
Diclofenac sodium	-6.00	0.03982	A: VAL81, ALA84, LEU11, ILE14, VAL15, LEU88	Middle selectivity

Notes: ΔGbind: free-binding energy, Ki: 50 % enzyme inhibition concentration, green/yellow/red: high/moderate/low selectivity.

Table 4

The molecular docking of anthocyanins and anti-inflammatory drug standards – diclofenac sodium and quercetin with Nf-kB

Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki[b] mmol	Binding site	Level of selectivity
Cyanidin-3-galactoside	-6.41	0.02013	A: LYS244, TYR60, HIS144, THR146, LYS147	Middle selectivity
Cyanidin-3-arabinoside	-6.04	0.03708	A: LYS244, TYR60, HIS144, THR146, LYS147	Middle selectivity
Cyanidin-3-glucoside	-5.38	0.1148	A: LYS244, PRO246, ALA245, TYR60, HIS144, SER211, LEU210, LYS147, ASP209, MET208	Low selectivity
Cyanidin 3-(6″-acetylglucoside)	-4.20	0.82741	A: LEU210, TYR60, HIS144, LYS147	Low selectivity
Diclofenac sodium	-3.90	1.38	A: TYR50, HIS144, LEU210, VAL145, THR146, LYS147	Low selectivity
Cyanidin-3,5-diglucoside	-3.89	1.40	A: LYS147, LEU210, THR146, HIS144, TYR60	Low selectivity
Quercetin	-3.61	2.28	A: LYS145, LYS147, LEU210, THR146, THR60, HIS144	Low selectivity

Notes: ΔGbind: free-binding energy, Ki: 50 % enzyme inhibition concentration, green/yellow/red: high/moderate/low selectivity.

both anthocyanins and drug standards, were found to inhibit all the mentioned pro-inflammatory targets with high selectivity. However, cyanidin-3-galactoside, cyanidin-3-arabinoside blocked three of them, such as high selective COX-2, phospholipase A2 and 5-LOX, and middle selective NF-kB. Cyanidin-3-glucoside blocked three out four targets, such as COX-2, phospholipase A2, 5-LOX. It has been found that widespread "gold standards" in medicine and science, such as diclofenac sodium and quercetin, are not as effective at suppressing the crucial targets of inflammation.

In the study *in vivo* using the carrageenan-induced rat paw edema model, the lingonberry fruit extract administration in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) resulted in a significant reduction of the paw edema by 45 % compared to the control group during the first hour of inflammation. A sustained anti-inflammatory effect was observed at subsequent time points with the edema inhibition of 35.0 %, 25.0 %, and 24.0 % in 2, 3, and 4 hours, respectively, relative to the control group (Table 6).

In contrast, the anti-inflammatory activity of lingonberry fruit extract in the lower dose of 6.5 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) was significantly lower ($p < 0.05$) than that observed in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside). When compared to the reference drug diclofenac sodium, the lingonberry fruit extract in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) exhibited a non-significant difference ($p > 0.05$) in the edema reduction at the 2-hour time point. However,

Table 5

A schematic overview of the classification of anti-inflammatory drug standards and principal compounds identified in the lingonberry fruit extract

Compound	COX-2	phospho lipase A2	5-LOX	Nf-kB	Number of closed key enzyme of inflammation
Drug standard					
Diclofenac sodium	#	&	&	#	2
Quercetin	#	&	&	#	2
Compounds in the lingonberry fruit extract					
Cyanidin-3-galactoside	✓	✓	✓	&	4
Cyanidin-3-arabinoside	✓	✓	✓	&	4
Cyanidin-3-glucoside	✓	✓	✓	#	3
Cyanidin-3,5-diglucoside	#	✓	✓	#	2
Cyanidin 3-(6″-acetylglucoside)	&	✓	✓	#	2

Notes: ✓ – high level of selectivity; & – medium level of selectivity; # – lower of selectivity.

Table 6

The anti-inflammatory activity of the lingonberry extract in the carrageenan edema model n = 5, (M ± m)

Experimental conditions	Dose, mg/kg	Parameter	Dynamics of the inflammation development, hours			
			1	2	3	4
Control pathology (CP)	–	ΔV, mL	0.47	0.84	1.10	1.16
Diclofenac Sodium	8.0	ΔV, mL	0.20* ± 0.01	0.52* ± 0.04	0.71* ± 0.04	0.73* ± 0.04
		AA, %	**58.0**	**38.0**	**35.0**	**37.0**
Quertin	50.0	ΔV, mL	0.27* ± 0.01	0.51* ± 0.04	0.84* ± 0.04	0.81* ± 0.04
		AA, %	**43.0**	**39.0**	**24.0**	**30.0**
Lingonberry fruit extract	6.5	ΔV, mL	0.33*/**/# ± 0.03	0.69*/**/# ± 0.05	0.95*/**/# ± 0.07	0.12*/**/# ± 0.01
		AA, %	**30.0**	**18.0**	**14.0**	**10.0**
Lingonberry fruit extract	13.0	ΔV, mL	0.26*/**/$ ± 0.02	0.55*/$ ± 0.04	0.83*/**/$ ± 0.06	0.88*/**/$ ± 0.06
		AA, %	**45.0**	**35.0**	**25.0**	**24.0**

Notes: 1) * $p < 0.05$ – The level of statistical significance of the CP group; 2) ** $p < 0.05$ – reliable values for the drug diclofenac sodium; 3) # $p < 0.05$ – reliable values of Quertin; 4) $ $p < 0.05$ – reliable values of the lingonberry fruit extract in the of dose 6.5 mg/kg, calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside; 5) The dose of the lingonberry fruit extract was calculated with reference to the total anthocyanins expressed as cyaniding-3-glycoside; 6) AA – the anti-inflammatory activity; 7) ΔV – the size of the edema; 8) n – the number of animals in the group.

diclofenac sodium demonstrated a significantly higher anti-inflammatory effect in 1, 3, and 4 hours (Table 6).

Comparison of the lingonberry fruit extract (13.0 mg/kg, calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) with the reference drug Quertin revealed no statistically significant differences ($p > 0.05$) in the anti-inflammatory activity at any of the evaluated time points. Conversely, the lower dose of the lingonberry fruit extract (6.5 mg/kg, calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) showed a significantly weaker anti-inflammatory effect compared to both diclofenac sodium and Quertin at all time points assessed (1–4 hours), with the reference drugs exhibiting significantly greater activity ($p < 0.05$) (Table 6).

The inflammation represents a complex biological response to external or internal stimuli and is closely associated with the development of oxidative stress. To evaluate the anti-inflammatory activity, the carrageenan-induced rat paw edema model is employed as it reliably reflects the key mechanisms underlying the inflammatory process. According to the canonical progression of inflammation in this model, histamine and serotonin predominate during the first hour, pro-inflammatory cytokines during the second hour, and prostaglandins, particularly COX-2, from the third to the fifth hour. Based on the results obtained, the lingonberry fruit extract demonstrated the inhibitory activity across all phases of inflammation.

The molecular docking results obtained in the present study demonstrated a binding energy of –5.76 kcal/mol for diclofenac sodium against COX-2 (PDB ID: 1DDX), with the calculated IC_{50} value of 0.05977 mmol. Notably, this binding affinity appears less favorable compared to the previously published data. For example, Ibrahim et al. (2018) [21] reported a docking score of –9.549 kcal/mol for the diclofenac interaction with COX-2.

Such discrepancies may be attributed to differences in docking protocols, including the selected protein structure, grid box parameters, ligand preparation procedures, scoring functions, and validation strategies. Even when the same enzyme COX-2 is studied, variations in crystallographic conformations, resolution, or active site configuration can substantially influence calculated binding energies. Therefore, docking scores should be interpreted as relative rather than absolute indicators of the biological activity.

Importantly, despite the moderate binding energy observed *in silico*, diclofenac exhibited a pronounced anti-inflammatory activity in the carrageenan-induced paw edema model. This apparent inconsistency may reflect the multifactorial nature of pharmacological responses *in vivo*. The anti-inflammatory efficacy of diclofenac is not exclusively mediated through the COX-2 inhibition, but also involves additional mechanisms, including the modulation of NF-κB signaling, suppression of the phospholipase A_2 activity, interference with the leukocyte migration, and reduction of the reactive oxygen species generation.

It should be noted, however, that the pharmacological response observed *in vivo* is influenced by multiple physiological factors, including the degree of gastrointestinal absorption, bioavailability, distribution within tissues, metabolic transformation, and the elimination rate of the administered compounds. Variability in these parameters may significantly affect the overall efficacy of both the extract under research and the reference drug. In addition, plant-derived polyphenols may undergo an extensive biotransformation, leading to the formation of active or inactive metabolites that can modulate the final biological response.

Furthermore, while diclofenac is primarily recognized as a cyclooxygenase inhibitor, its anti-inflammatory activity may also involve additional mechanisms beyond COX-2 inhibition, including modulation of NF-κB signaling, inhibition of phospholipase A_2 activity, and effects on reactive oxygen species generation. Therefore, the anti-inflammatory effects of both the lingonberry extract and diclofenac *in vivo* may reflect a multifactorial interaction with several molecular targets rather than the selective action on a single pathway.

Thus, the efficacy *in vivo* represents the integrated outcome of both pharmacodynamic and pharmacokinetic determinants.

Conclusions and prospects of further research. A comprehensive theoretical and experimental study of the anti-inflammatory properties of the lingonberry fruit extract has been conducted using the molecular docking analysis and the carrageenan-induced rat paw edema model *in vivo*. The results *in silico* demonstrated that lingonberry anthocyanins exhibited a strong binding affinity toward key pro-inflammatory targets, including COX-2, phospholipase A_2, 5-LOX, and NF-κB. The findings *in vivo* showed that administration of a thick lingonberry fruit extract in the dose of 13.0 mg/kg (calculated with reference to the total anthocyanins expressed as cyanidin-3-glycoside) significantly inhibited inflammatory responses in all phases of the carrageenan-induced paw edema.

Conflict of interests: authors have no conflict of interest to declare.

REFERENCES

1. Nesci S., Spagnoletta A., Oppedisano F. Inflammation, mitochondria and natural compounds together in the circle of trust. *International Journal of Molecular Sciences*. 2023. Vol. 24(7). P. 6106. DOI: 10.3390/ijms24076106.
2. Flavonoids as potential anti-inflammatory molecules: a review / J. M. Al-Khayri et al. *Molecules*. 2022. Vol. 27(9). P. 2901. DOI: 10.3390/molecules27092901.
3. Azab A., Nassar A., Azab A. Anti-inflammatory activity of natural products. *Molecules*. 2016. Vol. 21(10). P. 1321. DOI: 10.3390/molecules21101321.
4. Plant anthocyanins: classification, biosynthesis, regulation, bioactivity, and health benefits / Z. Lu et al. *Plant Physiology and Biochemistry*. 2024. Vol. 217. P. 109268. DOI: 10.1016/j.plaphy.2024.109268.
5. The study of the qualitative composition and the quantitative content of phenolic compounds in dietary supplements with lingonberry / O. Y. Maslov et al. *Journal of Organic and Pharmaceutical Chemistry*. 2021. Vol. 19(4/76). P. 40–46. DOI: 10.24959/ophcj.21.243782.
6. Chemical composition and biological activities of the nord-west romanian wild bilberry (Vaccinium myrtillus L.) and lingonberry (Vaccinium vitis-idaea L.) leaves / B.-E. Ștefănescu et al. *Antioxidants*. 2020. Vol. 9(6). P. 495. DOI: 10.3390/antiox9060495.
7. Kowalska K. Lingonberry (Vaccinium vitis-idaea L.) fruit as a source of bioactive compounds with health-promoting effects–a review. *International Journal of Molecular Sciences*. 2021. Vol. 22(10). P. 5126. DOI: 10.3390/ijms22105126.
8. Edible berries-an update on nutritional composition and health benefits-part II / P. Thorakkattu et al. *Current Nutrition Reports*. 2025. Vol. 14(1). P. 10. DOI: 10.1007/s13668-024-00608-x.
9. Nutritional and physicochemical properties of wild lingonberry (Vaccinium vitis-idaea L.)–effects of geographic origin / D. Urbonaviciene et al. *Molecules*. 2023. Vol. 28(12). P. 4589. DOI: 10.3390/molecules28124589.
10. Діуретична активність фітосубстанцій із листя брусниці звичайної / К. В. Цеменко та ін. *Актуальні питання фармацевтичної і медичної науки та практики.* 2018. Т. 11, № 3(28). С. 312–317. DOI: 10.14739/2409-2932.2018.3.145276.
11. Anthocyanin-rich lingonberry extract improves intestinal homeostasis based on intestinal stem cells in high-fat-diet-fed mice via gut microbiota-mediated Wnt/PPAR signaling / J. She et al. *Food Bioscience*. 2024. Vol. 62. P. 105411. DOI: 10.1016/j.fbio.2024.105411.
12. Vaccinium as potential therapy for diabetes and microvascular complications / H. Huang et al. *Nutrients*. 2023. Vol. 15(9). P. 2031. DOI: 10.3390/nu15092031.
13. Approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes : Council Directive of 24.11.1986 no. 86/609/EEC : as of 9 May 2013.
14. Доклінічні дослідження лікарських засобів : метод. рек. / за ред. О. В. Стефанова. Київ : Авіцена, 2001. 527 с.

15. Morris G. M., Huey R., Olson A. J. Using autodock for ligand-receptor docking. *Current Protocols in Bioinformatics*. 2008. Vol. 24(1). DOI: 10.1002/0471250953.bi0814s24.

16. Updated resources for exploring experimentally-determined pdb structures and computed structure models at the RCSB protein data bank / S. K. Burley et al. *Nucleic Acids Res*. 2025. Vol. 53(D1). P. D564–D574. DOI: 10.1093/nar/gkae1091.

17. PubChem 2025 update / S. Kim et al. *Nuc. Ac. Res.* 2025. Vol. 53(D1). P. D1516–D1525. DOI: 10.1093/nar/gkae1059.

18. CASTp 3.0: computed atlas of surface topography of proteins / W. Tian et al. *Nuc. Ac. Res*. 2018. Vol. 46(W1). P. W363–W367. DOI: 10.1093/nar/gky473.

19. Kondža M., Brizić I., Jokić S. Flavonoids as CYP3A4 inhibitors in vitro. *Biomedicines*. 2024. Vol. 12(3). P. 644. DOI: 10.3390/biomedicines12030644.

20. Vilkickyte G., Raudone L., Petrikaite V. Phenolic fractions from Vaccinium vitis-idaea L. and their antioxidant and anticancer activities assessment. *Antioxidants*. 2020. Vol. 9(12). P. 1261. DOI: 10.3390/antiox9121261.

21. Ibrahim M. M., Elsaman T., Al-Nour M. Y. Synthesis, anti-Inflammatory activity, and in Silico study of novel diclofenac and isatin conjugates. *International Journal of Medicinal Chemistry*. 2018. Vol. 2018. P. 1–11. DOI: 10.1155/2018/9139786.

REFERENCES

1. Nesci, S., Spagnoletta, A., & Oppedisano, F. (2023). Inflammation, mitochondria and natural compounds together in the circle of trust. *International Journal of Molecular Sciences*, *24*(7), 6106. https://doi.org/10.3390/ijms24076106

2. Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as potential anti-inflammatory molecules: a review. *Molecules*, *27*(9), 2901. https://doi.org/10.3390/molecules27092901

3. Azab, A., Nassar, A., & Azab, A. (2016). Anti-Inflammatory Activity of Natural Products. *Molecules*, *21*(10), 1321. https://doi.org/10.3390/molecules21101321

4. Lu, Z., Wang, X., Lin, X., Mostafa, S., Zou, H., Wang, L., & Jin, B. (2024). Plant anthocyanins: classification, biosynthesis, regulation, bioactivity, and health benefits. *Plant Physiology and Biochemistry*, *217*, 109268. https://doi.org/10.1016/j.plaphy.2024.109268

5. Maslov, O. Y., Komisarenko, M. A., Kolisnyk, S. V., Antonenko, O. V., Kolisnyk, O. V., & Kostina, T. A. (2021). The study of the qualitative composition and the quantitative content of phenolic compounds in dietary supplements with lingonberry. *Journal of Organic and Pharmaceutical Chemistry*, *19*(4/76), 40–46. https://doi.org/10.24959/ophcj.21.243782

6. Ștefănescu, B.-E., Călinoiu, L. F., Ranga, F., Fetea, F., Mocan, A., Vodnar, D. C., & Crișan, G. (2020). Chemical composition and biological activities of the nord-west romanian wild bilberry (Vaccinium myrtillus L.) and lingonberry (Vaccinium vitis-idaea L.) leaves. *Antioxidants*, *9*(6), 495. https://doi.org/10.3390/antiox9060495

7. Kowalska, K. (2021). Lingonberry (Vaccinium vitis-idaea L.) fruit as a source of bioactive compounds with health-promoting effects–a review. *International Journal of Molecular Sciences*, *22*(10), 5126. https://doi.org/10.3390/ijms22105126

8. Thorakkattu, P., Jain, S., Sivapragasam, N., Maurya, A., Tiwari, S., Dwivedy, A. K., Koirala, P., & Nirmal, N. (2025). Edible berries-an update on nutritional composition and health benefits-part II. *Current Nutrition Reports*, *14*(1), 10. https://doi.org/10.1007/s13668-024-00608-x

9. Urbonaviciene, D., Bobinaite, R., Viskelis, P., Viskelis, J., Petruskevicius, A., Puzeryte, V., Cesoniene, L., Daubaras, R., Klavins, L., & Bobinas, C. (2023). Nutritional and physicochemical properties of wild lingonberry (Vaccinium vitis-idaea L.)–effects of geographic origin. *Molecules*, *28*(12), 4589. https://doi.org/10.3390/molecules28124589

10. Tsemenko, K. V., Kireiev, I. V., Komisarenko, M. A., & Koshovyi, O. M. (2018). Diuretychna aktyvnist fitosubstantsii iz lystia brusnytsi zvychainoi. *Aktualni pytannia farmatsevtychnoi i medychnoi nauky ta praktyky, 11*(3/28), 312–317. https://doi.org/10.14739/2409-2932.2018.3.145276

11. She, J., Hu, X., Deng, J., Fan, S., Zhang, Q., Nie, F., Zhang, J., Christian, M., Wang, Z., & Dai, X. (2024). Anthocyanin-rich lingonberry extract improves intestinal homeostasis based on intestinal stem cells in high-fat-diet-fed mice via gut microbiota-mediated Wnt/PPAR signaling. *Food Bioscience*, *62,* 105411. https://doi.org/10.1016/j.fbio.2024.105411

12. Huang, H., Luo, Y., Wang, Q., Zhang, Y., Li, Z., He, R., Chen, X., & Dong, Z. (2023). Vaccinium as potential therapy for diabetes and microvascular complications. *Nutrients*, *15*(9), 2031. https://doi.org/10.3390/nu15092031

13. Approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. (2013, May 9). Council Directive № 86/609/EEC.

14. Stefanov, O. V. (Red). (2001). *Doklinichni doslidzhennia likarskykh zasobiv*: metod. rek. Avitsena.

15. Morris, G. M., Huey, R., & Olson, A. J. (2008). Using autodock for ligand-receptor docking. *Current Protocols in Bioinformatics*, *24*(1). https://doi.org/10.1002/0471250953.bi0814s24

16. Burley, S. K., Bhatt, R., Bhikadiya, C., Bi, C., Biester, A., & Biswas, P. (2025). Updated resources for exploring experimentally-determined pdb structures and computed structure models at the RCSB protein data bank. *Nucleic Acids Res*, *53*(D1), 564–574. https://doi.org/10.1093/nar/gkae1091

17. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., & He, S. (2025). PubChem 2025 update. *Nucleic Acids Res*, *53*(D1), 1516–1525. https://doi.org/10.1093/nar/gky473

18. Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res*, *46*(W1), 363–367. https://doi.org/10.1093/nar/gkae1059

19. Kondža, M., Brizić, I., & Jokić, S. (2024). Flavonoids as CYP3A4 inhibitors in vitro. *Biomedicines*, *12*(3), 644. https://doi.org/10.3390/biomedicines12030644

20. Vilkickyte, G., Raudone, L., & Petrikaite, V. (2020). Phenolic fractions from Vaccinium vitis-idaea L. and their antioxidant and anticancer activities assessment. *Antioxidants*, *9*(12), 1261. https://doi.org/10.3390/antiox9121261

21. Ibrahim, M. M., Elsaman, T., & Al-Nour, M. Y. (2018). Synthesis, anti-inflammatory activity, and in silico study of novel diclofenac and isatin conjugates. *International Journal of Medicinal Chemistry*, *2018*, 1–11. https://doi.org/10.1155/2018/9139786

Information about the authors:
S. R. Petroshchuk, postgraduate student of the Department of Pharmacology and Clinical Pharmacy, National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: chemistry29@meta.ua. ORCID: https://orcid.org/0009-0001-0105-2967
L. V. Derymedvid, Doctor of Medicine (Dr. habil.), Professor of the Department of Pharmacology and Clinical Pharmacy, National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: derimedved67@gmail.com. ORCID: https://orcid.org/0000-0002-5064-6550
O. Yu. Maslov, PhD, Teaching Assistant of the Department of General Chemistry, National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: alexmaslov392@gmail.com. ORCID: https://orcid.org/0000-0001-9256-0934
M. A. Komisarenko, Candidate of Pharmacy (Ph.D.), Associated Professor of the Department of Pharmacognosy and Nutriciology, National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: a0503012358@gmail.com. ORCID: https://orcid.org/0000-0002-1161-8151
I. O. Lebedinets, Specialist, Research Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: jude5@ukr.net. ORCID: https://orcid.org/0000-0001-6901-0045
T. K. Yudkevych, Deputy Director, Research Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: yudkevich66@ukr.net. ORCID: https://orcid.org/0000-0001-6173-2780
S. V. Kolisnyk, Doctor of Pharmacy (Dr. habil.), Professor, Head of the Department of General Chemistry, National University of Pharmacy of the Ministry of Health of Ukraine. E-mail: s_kolesnik@nuph.edu.ua. ORCID: https://orcid.org/0000-0002-4920-6064
Відомості про авторів:
С. Р. Петрощук, аспірантка кафедри фармакології і клінічної фармації, Національний фармацевтичний університет Міністерства охорони здоров'я України. E-mail: chemistry29@meta.ua. ORCID: https://orcid.org/0009-0001-0105-2967
Л. В. Деримедвідь, доктор медичних наук, професор кафедри фармакології і клінічної фармації, Національний фармацевтичний університет Міністерства охорони здоров'я України. E-mail: derimedved67@gmail.com. ORCID: https://orcid.org/0000-0002-5064-6550
О. Ю. Маслов, доктор філософії, асистент кафедри загальної хімії, Національний фармацевтичний університет Міністерства охорони здоров'я України. E-mail: alexmaslov392@gmail.com. ORCID: https://orcid.org/0000-0001-9256-0934
М. А. Комісаренко, кандидат фармацевтичних наук, доцент кафедри фармакогнозії і нутриціології, Національний фармацевтичний університет Міністерства охорони здоров'я України. E-mail: a0503012358@gmail.com. ORCID: https://orcid.org/0000-0002-1161-8151
І. О. Лебединець, фахівчиня, Навчально-науковий інститут прикладної фармації Національного фармацевтичного університету Міністерства охорони здоров'я України. E-mail: jude5@ukr.net. ORCID: https://orcid.org/0000-0001-6901-0045
Т. К. Юдкевич, заступник директора з наукової роботи, Навчально-науковий інститут прикладної фармації Національного фармацевтичного університету Міністерства охорони здоров'я України. E-mail: yudkevich66@ukr.net.
ORCID: https://orcid.org/0000-0001-6173-2780
С. В. Колісник, доктор фармацевтичних наук, професор, завідувач кафедри загальної хімії, Національний фармацевтичний університет Міністерства охорони здоров'я України. E-mail: s_kolesnik@nuph.edu.ua. ORCID: https://orcid.org/0000-0002-4920-6064

Дата першого надходження: 10.02.2026 р.
Дата прийняття до друку: 07.03.2626 р.
Дата публікації: 31.03.2026 р.