ISSN 2786-6661

Antioxidant potential of blackberry extract: integrated in vitro, in vivo and in silico molecular docking analysis

UDC: 582.281.615.322.4. 573.26

DOI: https://doi.org/10.32345/USMYJ.1(160).2026.120-129

Received: January 10, 2026
Accepted: March 12, 2026
Published online: March 31, 2026

Mykola Komisarenko, Artem Marchenko, Oleksandr Maslov, Iryna Lebedinets, Tetiana Yudkevych, Sergii Kolisnyk, Alla Koval

National University of Pharmacy, Kharkiv, Ukraine

ORCID:

Mykola Komisarenko: 0000-0002-1161-8151
Artem Marchenko: 0009-0002-8616-0410
Oleksandr Maslov: 0000-0001-9256-0934
Iryna Lebedinets: 0000-0001-6901-0045
Tetiana Yudkevych: 0000-0001-6173-2780
Sergii Kolisnyk: 0000-0002-4920-6064
Alla Koval: 0000-0001-9491-0459

Corresponding author:

Artem Marchenko

E-mail: chemistry29@meta.ua

Abstract: in recent years, increasing attention has been paid to natural compounds as potential therapeutic agents due to their broad spectrum of biological activities. Plant-derived compounds with antioxidant properties are of particular interest, as they may suppress pathological pathways. Therefore, the search for novel natural compounds capable of attenuating oxidative stress remains a promising area of biomedical research. The aim of this work was to evaluate the antioxidant potential of a thick blackberry fruit extract using in vitro, in vivo, and in silico molecular docking models. The study investigated a thick blackberry fruit extract. Docking simulations were performed using AutoDockTools 1.5.6. The antioxidant potential was assessed in vitro by the ferric reducing antioxidant potential (FRAP) assay, potentiometric method, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay; in vivo, on mice via a carrageenan-induced paw edema assay. The in silico evaluation of antioxidant properties indicated that the major blackberry anthocyanins—cyanidin-3-glucoside, cyanidin-3-(3''-malonyl glucoside), and cyanidin-3-xyloside—blocked two out of three prooxidant targets. In contrast, cyanidin-3,3'-diglucoside and cyanidin-3-rutinoside blocked only one out of three targets, namely xanthine oxidoreductase. In vivo experiments revealed that administration of blackberry thick fruit extract at doses of 60.0 and 120.0 mg/kg possessed high antioxidant potential, where the extract significantly increased catalase levels to 51.31 ± 3.00 and 70.56 ± 3.40 μmol/min·L and decreased malondialdehyde (MDA) levels to 0.140 ± 0.016 and 0.120 ± 0.016 μmol/L in serum compared with the control group, respectively. Antioxidant profiling showed that the blackberry fruit extract exhibited the highest activity in the FRAP, potentiometric, and ABTS assays. According to the theoretical results, it was shown that due to the high content of anthocyanins in the blackberry fruit extract, the extract possesses the ability to inhibit crucial prooxidant enzymes. In vivo and in vitro models showed that the blackberry fruit extract demonstrated predicted interactions with enzyme targets relevant to oxidative stress.

Keywords: Blackberry, Fruits, Anti-Oxidant Effect, Anthocyanins, Enzymes.

How to cite this article: Komisarenko M, Marchenko A, Maslov O, Lebedinets I, Yudkevych T, Kolisnyk S, Koval A. Antioxidant potential of blackberry extract: integrated in vitro, in vivo and in silico molecular docking analysis. Ukrainian Scientific Medical Youth Journal. 2026;1(160):120-129. doi:10.32345/USMYJ.1(160).2026.120-129

Introduction

Oxidative stress has been recognized by the scientific community as a key factor contributing to the development of cardiovascular, metabolic, neurodegenerative, and oncological diseases [1]. This condition arises when excessive production of reactive oxygen species (ROS) occurs, including superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH), and nitric oxide (NO•) [2]. The human body possesses an endogenous antioxidant defense system composed of enzymatic components such as superoxide dismutase, catalase, and glutathione peroxidase, which function to neutralize reactive species and maintain redox balance [3]. However, under the influence of various endogenous and exogenous factors, this defense system may become insufficient to completely eliminate free radicals, thereby increasing the risk of oxidative damage and associated diseases. For this reason, regular dietary intake of natural antioxidants derived from food sources, as well as specialized dietary supplements, is considered an important strategy for supporting antioxidant protection and reducing oxidative stress–related health risks [4].

Today, medicinal plants that are a rich source of anthocyanins have attracted high attention from the scientific community [5]. Above all, this is related to the fact that natural compounds have a potent antioxidant effect, and moreover, side effects rarely occur after the application of natural compounds compared to synthetic drugs.

Blackberry fruits were chosen as a promising source of anthocyanins. Blackberry is a shrub of the *Rosaceae* family. Its distribution area includes Europe, North America, and Asia [6]. The chemical composition of blackberry fruits is represented by anthocyanins, organic acids, and hydroxycinnamic acids [6].

Blackberry fruits were selected as a promising natural source of anthocyanins. Blackberry (family *Rosaceae*) is a widely distributed shrub found across Europe, North America, and Asia [6]. The phytochemical profile of blackberry fruits includes significant amounts of anthocyanins, organic acids, and hydroxycinnamic acid derivatives [7]. Numerous studies have investigated the pharmacological properties of blackberry fruits, demonstrating that their anthocyanins exhibit anti-inflammatory, antioxidant, antimicrobial, anti-hyperglycemic, immunomodulatory, and anticancer activities [8, 9]. In traditional medicine, blackberry preparations have been used for the management of fever, infectious conditions, diabetes, and liver disorders [10]. Considering these biological properties, blackberry anthocyanins may represent promising candidates for the development of novel antioxidant and antimicrobial agents.

Several investigations have examined the anti-inflammatory potential of fresh blackberry fruit extracts [11, 12]. However, to date, limited information is available regarding the evaluation of blackberry anthocyanins using a carrageenan-induced model or their molecular docking interactions with key prooxidant targets.

The aim of the work was to evaluate antioxidant potential of a blackberry thick fruit extract using *in vitro*, *in vivo* models and *in silico* molecular docking.

Materials and Methods

Blackberry fruits (*Rubus plicatus* Weihe & Nees) were harvested in July 2021 in the vicinity of Ternova village (50°19′31″ N, 36°66′93″ E).

Exactly 100.0 g of blackberry fruit was pressed and extracted with 96% ethanol using a threefold solvent-to-material ratio. After filtration, the resulting filtrate was concentrated under reduced pressure using a vacuum evaporator at 50–60 °C to obtain an extract with a final extract-to-raw material mass ratio of 1:0.35.

During the experimental period, mice were housed in pairs in Macrolon cages under standard laboratory conditions. Animals had unrestricted access to food and water, which were refreshed daily, and bedding material was replaced every three days. Environmental parameters were maintained at 22 ± 2°C with a relative humidity of 60 ± 5% and a 12 h light/12 h dark cycle.

All animal procedures were carried out following the principles for the humane care and use of experimental animals, in accordance with the Order of the Ministry of Education and Science of Ukraine "On approval of the Procedure for conducting experiments on animals by scientific institutions" (01 March 2012, No. 2012) and the Ukrainian Law “On Protection of Animals from Cruel Treatment” (21 February 2006, No. 344). The Bioethics Committee of the National University of Pharmacy reviewed the study materials and confirmed that no ethical violations were observed (Meeting Minutes No. 17, March 5, 2025).

The antioxidant activity in vitro was assessed by FRAP [13], ABTS [14] and potentiometric [15] assays.

The in vivo antioxidant activity of the blackberry fruit extract was evaluated using a carrageenan-induced paw edema model in outbred white male mice (18–25 g) [16]. A total of 30 animals were used in the study. All experimental procedures were conducted in accordance with established guidelines for the care and use of laboratory animals.

The animals were randomly divided into six groups (n = 5 per group). All treatments were administered

ISSN 2786-6661

once daily for three consecutive days. Group I (Intact control): animals received distilled water. Group II (Control pathology): animals received distilled water and were subjected to inflammation induction. Group III (Reference drug): animals were treated with the reference drug "Quertin." (Public Joint Stock Company "Research and Production Center "Borshchagov Chemical and Pharmaceutical Plant"). Group IV: animals received blackberry fruit extract at a dose of 20 mg/kg. Group V: animals received blackberry fruit extract at a dose of 60 mg/kg. Group VI: animals received blackberry fruit extract at a dose of 120 mg/kg. (Table 1)

On the third day, acute inflammation was induced in Groups II–VI by subplantar injection of 0.1 mL of 1% (w/v) carrageenan solution in 0.9% sodium chloride into the right hind paw.

At the end of the experiment (4 hours after carrageenan administration, corresponding to the peak of edema), the animals were humanely euthanized under appropriate anesthesia. Blood samples were collected immediately via decapitation. The collected blood was centrifuged to obtain serum. The serum samples were further applied for a biochemical analysis of antioxidant parameters as catalase activity [17] and MDA [18].

Table 1. Scheme of *in vivo* experiment

Group Number	Sample	Dose, mg/kg
1.	Intact	–
2.	Control Pathology	–
3.	"Quertin"	100,0 mg/kg expressed as quercetin 7.0 mg/kg
4.	Blackberry extract	20,0 mg/kg, expressed as the total content of polyphenolic compounds expressed as gallic acid
5.	Blackberry extract	60,0 mg/kg , expressed as the total content of polyphenolic compounds expressed as gallic acid
6.	Blackberry extract	120,0 mg/kg , expressed as the total content of polyphenolic compounds expressed as gallic acid

Molecular docking simulations were carried out with AutoDockTools v1.5.6 [14,15]. In Table 2 was showed a scheme of proteins applied for research.

Statistical processing of the data was conducted using Statistica 10 software. Group comparisons were performed with the Mann–Whitney U test, and results were considered statistically significant when

Table 2. Scheme of proteins applied for research

№	Protein Name	PDB ID	Resolutions, Å
1.	Nicotinamide adenine dinucleotide phosphate oxyreductase (NADPH oxidoreductase)	5o0x	2.20
2.	Myeloperoxidase	3f9p	2.93
3.	Xanthine oxidoreductase	1fiq	2.50

p-values were below 0.05. When studying in vitro antioxidant activity, the results were analyzed using one-way analysis of variance with Tukey's test, where differences were considered significant at $p < 0.05$.

Results

In the first stage of this research, a molecular docking study was conducted to evaluate the antioxidant potential of blackberry fruit extract. Various prooxidant pathways exist, including mitochondrial dysfunction, NADPH oxidase activation, and transition metal-mediated reactions. However, we consider NADPH oxidoreductase, myeloperoxidase, and xanthine oxidoreductase to be the key prooxidant enzymes. To benchmark the potential of the blackberry extract, it was compared to quercetin (a natural compound), as the quercetin-based drug "Quertin" is currently used in the treatment of cardiological, neurological, and renal diseases

The carrageenan-induced inflammation model is a well-established and classic model for studying acute inflammatory response, which is accompanied by the generation of reactive oxygen species and the development of oxidative stress [19]. It is known that induction of inflammation with carrageenan activates neutrophils and macrophages, leading to excessive formation of free radicals and activation of lipid peroxidation processes [20]. Thus, this model allows assessing the ability of the studied extract not only to indirectly influence the inflammatory process but also, which is key to our work, to counteract oxidative stress, which is an integral component of inflammation. Therefore, the main focus of our study was concentrated on biochemical markers of antioxidant defense, such as catalase activity, and oxidative damage, namely malondialdehyde, rather than on classical indicators of inflammation.

In our previous research [8], the anthocyanin composition of blackberry fruit extract was estimated by high performance liquid chromatography. According to this study, the following anthocyanins were identified: cyanidin-3-glucoside (84.10% of total anthocyanins), cyanidin-3,3'-diglucoside (7.38% of total anthocyanins), cyanidin-3-(3"-malonyl glucoside) (6.30% of total anthocyanins), cyanidin-3-xy-

loside (1.50% of total anthocyanins), and cyanidin-3-rutinoside (0.60% of total anthocyanins). These identified compounds were evaluated using a molecular docking study to realize the realize antioxidant potential of blackberry fruit extract. In addition, compound selectivity was categorized based on IC_{50} values using predefined criteria: IC_{50} < 0.001 mM indicated high selectivity; values within 0.001–0.05 mM were considered moderate selectivity; and IC_{50} > 0.05 mM corresponded to low selectivity [21]. It should be emphasized that this classification is arbitrary and used for approximate comparison only and does not represent a precise or rigorous pharmacological assessment of selectivity.

According to the results shown in Table 3, highly selective inhibitors of the NADPH oxidoreductase enzyme were not identified. Only cyanidin-3-glucoside exhibited medium selectivity, whereas quercetin was a low-selective inhibitor. The other anthocyanin compounds of blackberry fruit had positive values, which are associated with unfavorable binding and low ligand–receptor affinity to NADPH oxidoreductase (Table 3).

Further, the ability of blackberry fruit extract compounds to inhibit myeloperoxidase was assessed. According to the results, it was found that cyanidin-3-xyloside, cyanidin-3-glucoside, and cyanidin-3-(3''-malonyl glucoside) were medium-selective inhibitors, while quercetin, cyanidin-3-rutinoside, and cyanidin-3,3'-diglucoside were low-selective inhibitors (Table 4).

The next important prooxidant enzyme is xanthine oxidoreductase. According to the results of the study, cyanidin-3-xyloside was found to be a highly selective inhibitor, while cyanidin-3-(3''-malonyl glucoside),, cyanidin-3,3'-diglucoside, and cyanidin-3-rutinoside demonstrated moderate selectivity as inhibitors. Quercetin had low selectivity toward the prooxidant enzyme xanthine oxidoreductase (Table 5).

Furthermore, all obtained data were summarized, and the compounds were conditionally classified into four categories. The first category comprised compounds with high predicted selectivity for the active site, the second included compounds with moderate predicted selectivity, the third consisted of compounds with low predicted selectivity and fourth

Table 3. Results of molecular docking analysis of anthocyanins compared with reference antioxidant agent (quercetin) against NADPH oxidoreductase

№	Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki mmol	Predicted interaction strength
1.	Cyanidin-3-glucoside	-7.08	0,00644	Medium
2.	Quercetin	-5.51	0,09072	Low
3.	Cyanidin-3-(3''-malonyl glucoside)	49.76	–	Inactive
4.	Cyanidin-3-xyloside	70.27	–	Inactive
5.	Cyanidin-3-rutinoside	104.72	–	Inactive
6.	Cyanidin-3,3'-diglucoside	162.08	–	Inactive

Notes. ΔGbind: free-binding energy, Ki: 50 % enzyme inhibition concentration, green/yellow/red: high/moderate/low predicted interaction strength, blue color – positive docking values indicate unfavorable binding and low ligand–receptor affinity.

Table 4. Results of molecular docking analysis of anthocyanins compared with reference antioxidant agent (quercetin) against myeloperoxidase enzyme

№	Ligand	Binding energy ΔGbinda (kcal/mol)	Ki mmol	Predicted interaction strength
1.	Cyanidin-3-xyloside	-7.01	0.00731	Medium
2.	Cyanidin-3-glucoside	-6.51	0.01694	Medium
3.	Cyanidin-3-(3''-malonyl glucoside)	-6.08	0.03495	Medium
4.	Cyanidin-3-rutinoside	-5.85	0.0517	Low
5.	Cyanidin-3,3'-diglucoside	-5.34	0.12258	Low
6.	Quercetin	-3.30	3.79	Low

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

ISSN 2786-6661

Table 5. Results of molecular docking analysis of anthocyanins compared with reference antioxidant agent (quercetin) against xanthine oxidoreductase enzyme

№	Ligand	Binding energy ΔGbinda (kcal/mol)	Ki mmol	Predicted interaction strength
1.	Cyanidin-3-xyloside	-10.01	0.00004602	High
2.	Cyanidin-3-(3''-malonyl glucoside)	-8.05	0.00126	Medium
3.	Cyanidin-3,3'-diglucoside	-7.96	0.00146	Medium
4.	Cyanidin-3-rutinoside	-7.95	0.00149	Medium
5.	Cyanidin-3-glucoside	-6.45	0.01871	Low
6.	Quercetin	-5.80	0.05629	Low

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

one included compounds with unfavorable binding. This classification approach was applied to clearly identify compounds that interact most effectively with prooxidant targets, as well as those exhibiting lower levels of interaction.

Table 6 shows the summarized results of molecular docking of prooxidant enzyme inhibition by anthocyanins from blackberry fruit extract. The results demonstrate that none of the compounds, including both anthocyanins and drug standards, exhibited high selective inhibition of all the mentioned prooxidant targets. However, cyanidin-3-glucoside, cyanidin-3-(3''-malonyl glucoside) and, and cyanidin-3-xyloside blocked two out of three prooxidant targets. Whereas cyanidin-3,3'-diglucoside and cyanidin-3-rutinoside blocked only one out of three targets, namely xanthine oxidoreductase. Quercetin did not theoretically effectively inhibit the key oxidative targets in this study.

Table 7 presents the antioxidant activity of blackberry fruit extract evaluated in the carrageenan-induced edema model in mice (n = 5, M ± m). Antioxidant status was assessed by measuring catalase activity and MDA concentration, which reflect endogenous antioxidant defense and lipid peroxidation intensity, respectively.

Induction of inflammatory pathology caused pronounced oxidative stress compared with intact animals. In the control pathology group, catalase activity significantly decreased from 57.75 μmol/min·L in intact animals to 26.22 μmol/min·L, while MDA levels increased from 0.221 μmol/L to 0.267 μmol/L, indicating activation of lipid peroxidation processes. The reference preparation Quertin (7.0 mg/kg) also reduced lipid peroxidation; however, catalase activity remained lower than in intact animals.

Administration of blackberry fruit extract produced a dose-dependent antioxidant effect. At a dose of 20 mg/kg, moderate improvement was observed, characterized by increased catalase activity (35.12 μmol/min·L) and reduced MDA levels (0.200 μmol/L). Increasing the dose to 60 mg/kg

Table 6. Summary diagram illustrating the categorization of reference antioxidant agents and the major constituents detected in blackberry fruit extract

№	Compound	NADPH oxidoreductase	myeloperoxidase	xanthine oxidoreductase	Number of key oxidant enzymes affected
	Reference drug				
1.	Quercetin				0
	Compounds blackberry fruit extract				
1.	Cyanidin-3-glucoside				2
2.	Cyanidin-3,3'-diglucoside				1
3.	Cyanidin-3-(3''-malonyl glucoside)				2
4.	Cyanidin-3-xyloside				2
5.	Cyanidin-3-rutinoside				1

Notes. green – high level of predicted selectivity; yellow – medium level of predicted selectivity; red – lower of predicted selectivity; blue – unfavorable binding

resulted in a more pronounced normalization of oxidative parameters, with catalase activity reaching 51.31 μmol/min·L and MDA decreasing to 0.140 μmol/L. The highest dose (120 mg/kg) demonstrated the strongest antioxidant activity, significantly elevating catalase activity to 70.56 μmol/min·L and reducing MDA concentration to 0.120 μmol/L, exceeding the effects observed with reference treatments. Overall, the data indicate that blackberry fruit extract effectively attenuates oxidative stress under inflammatory conditions by enhancing antioxidant enzyme activity and suppressing lipid peroxidation in a dose-dependent manner.

The next stage of our study was the assessment of the antioxidant activity of blackberry fruit extract by the potentiometric method in comparison with the preparation "Quertin" at an equimolar concentration of 0.1 mol/L. For this purpose, we dissolved the calculated amount of the dosage form of the studied preparations ("Quertin") in equal volumes of distilled water at a temperature of 90°C, then filtered the prepared solutions and brought them to the mark with the same solvent. According to the study results shown in Table 8, it was found that the blackberry fruit extract has an 86%, 86%, and 89% higher level of antioxidant activity than the comparison preparation "Quertin".

Next, we evaluated the antioxidant effect of blackberry fruit extract by the FRAP method in comparison with the drug "Quertin" at an equimolar concentration of 0.1 mol/L. For this purpose, we dissolved the calculated amount of the dosage form of the studied drug "Quertin" in equal volumes of distilled water at a temperature of 90°C, then filtered the prepared solutions and brought them to the mark with the same solvent. According to the study results shown in Table 9, the blackberry fruit extract was found to have a higher level of antioxidant effect than the reference drug "Quertin".

Table 8. Antioxidant activity of the blackberry fruit extract determined by potentiometric method *in vitro*

Experimental conditions	Dose	mmol-eqv./$m_{dry.\ res}$
"Quertin"	0.10 mol/L	5.40±0.05
Blackberry fruit extract		220.70±1.03*

Notes. Values are presented as mean ± standard deviation. One-way ANOVA with Tukey's post-hoc test was used.
* indicates $p < 0.05$ compared to Quertin

Table 9. Antioxidant activity of the blackberry fruit extract determined by FRAP assay *in vitro*

Experimental conditions	Dose	nmol/mL-eq. EGCG
"Quertin"	0.10 mol/L	10.00±0.05
Blackberry fruit extract		54.70±0.33*

Notes. Values are presented as mean ± standard deviation. One-way ANOVA with Tukey's post-hoc test was used.
* indicates $p < 0.05$ compared to Quertin, EGCG – epigallocatechin-3-O-gallate

The antioxidant potential of "Quertin" and blackberry fruit extract was evaluated using the ABTS radical scavenging assay. IC_{50} values (μmol/L) represent the concentration required to inhibit 50% of ABTS radicals. Lower IC_{50} values indicate stronger antioxidant activity. The blackberry fruit extract exhibited the highest antioxidant activity among the tested compounds, with an IC_{50} value markedly lower than those of the standard antioxidant. "Quertin" showed moderate radical scavenging activity (Table 10).

Table 7. Antioxidant activity of the blackberry extract on the carrageenan edema model n = 5, (M ± m)

Experimental conditions	Dose, mg/kg	Catalase, μmol/min·L	MDA, μmol/L
Intact		57.75 ± 2.89	0.221 ± 0.013
Control pathology		26.22* ± 1.31	0.267* ± 0.011
"Quertin"	7.0[1]	26.04* ± 1.30	0.159*/** ± 0.008
Blackberry fruit extract	20.0[2]	35.12*/**/***/#/ α ± 2.10	0.200*/**/***/#/ α ± 0.016
Blackberry fruit extract	60.0[2]	51.31**/#/α/& ± 3.00	0.140*/**/***/#/ α ± 0.014
Blackberry fruit extract	120.0[2]	70.56*/**/***/#/α/&/$ ± 3.40	0.120*/**/***/#/α/&/$ ± 0.016

Notes. *: $p < 0.05$ denotes statistically significant differences with the intact group; **: $p < 0.05$ denotes statistically significant differences with the CP (control pathology) group; α: $p < 0.05$ denotes statistically significant differences with the Quertin; &: $p < 0.05$ denotes statistically significant differences with the blackberry fruit extract at a dose 20 mg/kg; $: $p < 0.05$ denotes statistically significant differences with the blackberry fruit extract at a dose 60 mg/kg; 1 – Dose of Quertin expressed in term of quercetin, dose of dosage form – 100 mg/kg; 2 – The dose is equivalent to the sum of phenolic compounds expressed as gallic acid.

ISSN 2786-6661

Table 10. Antioxidant activity of the blackberry fruit extract determined by ABTS assay *in vitro*

Experimental conditions	IC50, μmol/L
"Quertin "	15.0 ± 0.75
Blackberry fruit extract	0.50 ± 0.03*

Notes. Values are presented as mean ± standard deviation. One-way ANOVA with Tukey's post-hoc test was used.
* indicates $p < 0.05$ compared to Quertin

Discussion

Oxidative stress reflects an imbalance between free radicals and the antioxidant defense system. The main products of oxidative stress are the superoxide anion ($O_2•^-$), hydroxyl radical (•OH), and oxidized forms of unsaturated fatty acids. Free radicals disrupt normal cell signaling mechanisms and contribute to the subsequent development of chronic diseases. Catalase is a protective antioxidant enzyme responsible for reducing hydrogen peroxide levels and, consequently, oxidative stress in the body. According to the results presented in Table 7, it can be concluded that blackberry fruit extract at doses of 120.0 and 60.0 mg/kg possesses sufficient antioxidant activity to actively suppress oxidative stress during the inflammatory process and inactivate free radicals.

These results are consistent with the findings of Cho BO, who reported a significant ($p < 0.05$) increase in antioxidant enzyme activity and a reduction in oxidative stress markers following administration of blackberry extracts [22]. Their data support the observed statistically significant modulation of catalase and MDA in our study.

Similarly, Tony S. K. and co-authors demonstrated that anthocyanin-rich blackberry extract significantly reduces oxidative stress and lipid peroxidation while improving antioxidant status in experimental animals [23]. In their study, treatment with blackberry extract led to a decrease in oxidative stress markers and restoration of antioxidant balance, which is consistent with our findings showing reduced MDA levels and increased catalase activity.

The high antioxidant capacity of the extract in the FRAP (54.70 nmol/mL EGCG equivalents) and ABTS (0.50 ± 0.03 μmol/L) assays further supports these findings. The relatively low standard deviation in the ABTS assay indicates good reproducibility of the results. Similar results have been reported by Vázquez Medina et al., who demonstrated that blackberry fruit extracts exhibit high ferric reducing and radical scavenging capacities in vitro, as measured by FRAP and ABTS/TEAC assays, due to their rich phenolic and terpenoid content ($p < 0.05$). [24]

Importantly, the dose-dependent nature of the antioxidant effect observed in this study is supported by Skrovankova S., who noted that optimal antioxidant effects are typically achieved within a specific concentration range, with statistically significant improvements observed at moderate-to-high doses ($p < 0.05$) [25]. In our study, the 120 mg/kg dose demonstrated the most pronounced and statistically significant antioxidant effect, suggesting that this dose falls within the optimal therapeutic window.

The high antioxidant activity of the blackberry fruit extract observed in vivo, in vitro assays, in our view can be primarily attributed to the synergistic effects of multiple anthocyanins present in the extract. Key compounds contributing to this effect include cyanidin-3-glucoside, cyanidin-3,3′-diglucoside, cyanidin-3-(3″-malonyl glucoside), cyanidin-3-xyloside, and cyanidin-3-rutinoside. Acting together, we believe these anthocyanins enhance free radical scavenging and reducing capacity, producing a stronger overall antioxidant effect than any single component alone. This synergistic activity likely underlies the correlation observed between the high in vitro antioxidant capacity and the in vivo effects, including increased catalase activity and decreased MDA levels, indicating that the combined action of these anthocyanins plays a central role in mitigating oxidative stress.

Conclusions

A theoretical and experimental study of the antioxidant activity of blackberry fruit extract was conducted. According to the theoretical results, it was shown that due to the high content of anthocyanins in blackberry fruit extract, the extract possesses inhibit crucial prooxidant enzymes. In vivo and in vitro models showed that blackberry fruit extract showed predicted interactions with enzyme targets relevant to oxidative stress.

Funding. This study received no external funding.

Conflict of Interest. The authors declare no conflicts of interest in conducting the research, authorship, or publication of this article.

Consent for Publication. All authors have reviewed the manuscript text and provided consent for its publication.

Ethical Approval. The study was approved by The Bioethics Committee of the National University of Pharmacy reviewed the study materials and confirmed that no ethical violations were observed (Meeting Minutes No. 17, March 5, 2025) and complies with the requirements of the Law of Ukraine.

AI Disclosure. The authors used ChatGPT (OpenAI) for language editing of the English text. The authors reviewed and verified all AI-generated content to ensure accuracy and integrity.

Author Contributions (CRediT). Conceptualization – Marchenko Artem; Komisarenko Mykola; Maslov Oleksandr. Methodology – Lebedinets Iryna; Yudkevych Tetiana. Software – Lebedinets Iryna; Yudkevych Tetiana. Validation – Maslov Oleksandr. Formal Analysis – Maslov Oleksandr. Investigation – Marchenko Artem; Komisarenko Mykola; Maslov Oleksandr. Resources – Marchenko Artem. Data Curation – Marchenko Artem; Lebedinets Iryna; Yudkevych Tetiana. Writing – Original Draft – Marchenko Artem; Komisarenko Mykola; Maslov Oleksandr. Writing – Review & Editing – Kolisnyk Sergii; Koval Alla. Visualization – Not applicable. Supervision – Komisarenko Mykola. Project Administration – Marchenko Artem; Komisarenko Mykola; Maslov Oleksandr. Funding Acquisition – Marchenko Artem; Komisarenko Mykola; Maslov Oleksandr

REFERENCES

1. Maslov OY, Komisarenko MA, Kolisnyk SV, Golik MY, Tsapko YO, Akhmedov EY. Determination of the extraction frequency of green tea leaves by the antioxidant method. J Org Pharm Chem. 2022;20(1(77)):28-34. doi: 10.24959/ophcj.22.252320
2. Maslov OY, Komisarenko MA, Ponomarenko SV, Kolisnyk SV, Osolodchenko TP, Kostina TA, Kolisnyk YS, Moroz VP. Antioxidant, antimicrobial and antifungal activity of the obtained "Cachisept" tablets for resorption in the oral cavity for the treatment and prevention of dental caries. Curr Pharm Med. 2023;16(3):213-6. doi: 10.14739/2409-2932.2023.3.285425
3. Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladâcenco O, Roza E, Costăchescu B, Grumezescu AM, Teleanu RI. An overview of oxidative stress, neuroinflammation and neurodegenerative diseases. Int J Mol Sci. 2022;23(11):5938. doi: 10.3390/ijms23115938
4. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. Eur J Heart Fail. 2018;21(4):425-35. doi: 10.1002/ejhf.1320
5. Lu Z, Wang X, Lin X, Mostafa S, Zou H, Wang L, Jin B. Plant anthocyanins: Classification, biosynthesis, regulation, bioactivity, and health benefits. Plant Physiol Biochem. 2024;217:109268. doi: 10.1016/j.plaphy.2024.109268
6. Asnaashari M, Tajik R, Khodaparast MH. Antioxidant activity of raspberry (Rubus fruticosus) leaves extract and its effect on oxidative stability of sunflower oil. J Sci Technol. 2014;52(8):5180-7. doi: 10.1007/s13197-014-1564-7
7. Gil-Martínez L, Mut-Salud N, Ruiz-García JA, Falcón-Piñeiro A, Maijó-Ferré M, Baños A, De la Torre-Ramírez JM, Guillamón E, Verardo V, Gómez-Caravaca AM. Phytochemicals determination, and antioxidant, antimicrobial, anti-Inflammatory and anticancer activities of blackberry fruits. Foods. 2023;12(7):1505. doi: 10.3390/foods12071505
8. Maslov O, Komisarenko M, Marchenko A, Plis D, Ponomarenko S, Osolodchenko T, Kolisnyk S. Comparing chemical composition, antimicrobial, anti-fungi and antioxidant activities of blackberry fruit thick and green tea leaf extract. Hacet Univ J Fac Pharm. 2025;45(1):18-29. doi: 10.52794/hujpharm.1477950
9. Lee H, Wang Z, Deng Z, Wang Y. Assessment of six blackberry cultivars using a combination of metabolomics, biological activity, and network pharmacology approaches. Antioxidants. 2024;13(3):319. doi: 10.3390/antiox13030319
10. Cenk E, Schmutz C, Pahlke G, Oertel A, Kollarova J, Mock HP, Matros A, Marko D. Immunomodulatory properties of blackberry anthocyanins in THP-1 derived macrophages. Int J Mol Sci. 2021;22(19):10483.doi: 10.3390/ijms221910483
11. Varzaru I, Oancea AG, Vlaicu PA, Saracila M, Untea AE. Exploring the antioxidant potential of blackberry and raspberry leaves: phytochemical analysis, scavenging activity, and in vitro polyphenol bioaccessibility. Antioxidants. 2023;12(12):2125. doi: 10.3390/antiox12122125
12. Martins MS, Gonçalves AC, Alves G, Silva LR. Blackberries and mulberries: berries with significant health-promoting properties. Int J Mol Sci. 2023;24(15):12024. doi: 10.3390/ijms241512024
13. Al Qaisi Y, Alfarrayeh I, Alsarayreh A, Khleifat K, Abu-Nwas N. Assessment of antioxidant potential, cytotoxicity, and anticancer activity of methanolic extracts from selected wild medicinal plants. Phytomed Plus. 2024;4(2):100534. doi: 10.1016/j.phyplu.2024.100534
14. Cano A, Maestre AB, Hernández-Ruiz J, Arnao MB. ABTS/TAC methodology: main milestones and recent applications. Processes. 2023;11(1):185. doi: 10.3390/pr11010185

ISSN 2786-6661

15. Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI. Study and evaluation antioxidant activity of dietary supplements with green tea extract. Curr Issues Pharm Med. 2021;14(2):215-9. doi: 10.14739/2409-2932.2021.2.233306
16. Salem S, Leghouchi E, Soulimani R, Bouayed J. Reduction of paw edema and liver oxidative stress in carrageenan-induced acute inflammation by Lobaria pulmonaria and Parmelia caperata, lichen species, in mice. Int J Vitam Nutr Res. 2021;91(1-2):143-151. doi: 10.1024/0300-9831/a000620.
17. Ahmadi N, Khaki AA, Khodarahmi P, Baharara J, Zakerbostanabadi S. Effect of Anthocyanin on Oxidative Stress and Testis Structure in Streptozotocin-Induced Diabetic Rats. Crescent J Med Biol Sci. 2023;10(2):56-60. doi: 10.34172/cjmb.2023.09
18. Liu H, Zhao H, Che J, Yao W. Naringenin alleviates obesity-associated hypertension by reducing hyperlipidemia and oxidative stress. Kidney Blood Press Res. 2022;47(6):423-32. doi: 10.1159/000524172
19. Pradhan B, Ki JS. Biological activity of algal derived carrageenan: A comprehensive review in light of human health and disease. Int J Biol Macromol. 2023;238:124085. doi: 10.1016/j.ijbiomac.2023.124085
20. Alblihed MA. Astragalin attenuates oxidative stress and acute inflammatory responses in carrageenan-induced paw edema in mice. Mol Biol Rep. 2020;47(9):6611-20. doi: 10.1007/s11033-020-05712-z
21. Kondža M, Brizić I, Jokić S. Flavonoids as CYP3A4 Inhibitors in vitro. Biomedicines. 2024;12(3):644. doi: 10.3390/biomedicines12030644
22. Cho BO, Ryu HW, Jin CH, Choi DS, Kang SY, Kim DS, Byun MW, Jeong IY. Blackberry extract attenuates oxidative stress through up-regulation of Nrf2-Dependent antioxidant enzymes in carbon tetrachloride-treated rats. J Agric Food Chem. 2011;59(21):11442-8. doi: 10.1021/jf2021804
23. Tony SK, Hassan MS, Ismail HA, El-Naem GF, Gazwi HS. Effect of anthocyanin-rich blackberry juice on endoplasmic reticulum stress in streptozotocin-induced diabetic rats. Environ Sci Pollut Res. 2023;30(32):79067-81. doi: 10.1007/s11356-023-27827-z
24. Gil-Martínez L, Mut-Salud N, Ruiz-García JA, Falcón-Piñeiro A, Maijó-Ferré M, Baños A, De la Torre-Ramírez JM, Guillamón E, Verardo V, Gómez-Caravaca AM. Phytochemicals determination, and antioxidant, antimicrobial, anti-Inflammatory and anticancer activities of blackberry fruits. Foods. 2023;12(7):1505. doi: 10.3390/foods12071505
25. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015;16(10):24673-706. doi: 10.3390/ijms161024673

Антиоксидантний потенціал екстракту ожини: інтегрований аналіз in vitro, in vivo та in silico з молекулярним докінгом

Микола Комісаренко, Артем Марченко, Олександр Маслов, Ірина Лебединець, Тетяна Юдкевич, Сергій Колісник, Алла Коваль

Національний фармацевтичний університет, Харків, Україна

Corresponding author:

Artem Marchenko

E-mail: chemistry29@meta.ua

Анотація: в останні роки зростає інтерес до природних сполук як потенційних терапевтичних агентів завдяки їхньому широкому спектру біологічної активності. Особливу увагу привертають рослинні сполуки з антиоксидантними властивостями, оскільки вони можуть пригнічувати патологічні процеси. Тому пошук нових природних сполук, здатних послаблювати оксидативний стрес, залишається перспективним напрямом біомедичних досліджень. Метою роботи було оцінити антиоксидантний потенціал густого екстракту плодів ожини з використанням моделей in vitro, in vivo та молекулярного докінгу in silico. У дослідженні вивчали густий екстракт плодів ожини. Докінгові дослідження проводили з використанням програми AutoDockTools 1.5.6. Антиоксидантний потенціал оцінювали in vitro за допомогою методу визначення відновлювальної здатності заліза (FRAP), потенціометричного методу та тесту з 2,2'-азино-біс(3-етилбензотіазолін-6-сульфоновою кислотою) (ABTS); in vivo — на мишах на моделі набряку лапи, індукованого карагеніном. Оцінка in silico показала, що основні антоціани ожини — ціанідин-3-глюкозид, ціанідин-3-(3''-малонілглікозид) та ціанідин-3-ксилозид — блокували два з трьох прооксидантних ферментів-мішеней. Натомість ціанідин-3,3'-диглюкозид і ціанідин-3-рутинозид інгібували лише одну з трьох мішеней, а саме ксантиноксидоредуктазу. У дослідах in vivo встановлено, що введення густого екстракту плодів ожини в дозах 60,0 та 120,0 мг/кг проявляло виражений антиоксидантний ефект: екстракт достовірно підвищував рівень каталази до 51,31±3,00 та 70,56±3,40 мкмоль/хв·л відповідно та знижував рівень малонового діальдегіду (МДА) до 0,140±0,016 та 0,120±0,016 мкмоль/л у сироватці крові порівняно з контрольною групою. Профілювання антиоксидантної активності показало, що екстракт плодів ожини проявляв найвищу активність у тестах FRAP, потенціометричному та ABTS. Згідно з теоретичними результатами встановлено, що завдяки високому вмісту антоціанів екстракт плодів ожини здатний інгібувати ключові прооксидантні ферменти. Моделі in vivo та in vitro продемонстрували, що екстракт активно пригнічує утворення вільних радикалів під час перекисного окиснення ліпідів і генерації активних форм кисню.

Ключові слова: ожина, плоди, антиоксидантний ефект, антоціани, ензими.