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ОРИГИНАЛЬНАЯ СТАТЬЯ

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EVALUATION OF ANTIOXIDANT ACTIVITY LEVEL OF *ACTINIDIA ARGUTA* (SIEBOLD ET ZUCC.) PLANCH. EX MIQ. PLANT RAW MATERIAL, GROWN IN THE CAUCASIAN MINERAL WATERS REGION

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The aim of the study is the identification and evaluation of a new antioxidant activity in a potentially new medicinal raw material of *Actinidia arguta folia*.

Materials and methods. The total content of antioxidants was measured on a Tsvet Yauza-01-AA liquid chromatograph using the amperometric method. In parallel, the antioxidant activity of *Actinidia arguta* extracts was studied *in vitro* in the following dilution range: 62.5 μg/ml, 125 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml. Herewith, DPPH, superoxide, and hydroxyl radical inhibitory properties of the analyzed samples were evaluated.

The studies of the antioxidant activity with the determination of the activity of superoxide dismutase, glutathione peroxidase, catalase, the concentration of malondialdehyde and diene conjugates, have been conducted *in vivo*.

Results. When studying the antiradical activity (*in vitro* tests), it was found out that the highest radical-inhibiting activity comparable to the individual compound - quercetin, has the extraction from *Actinidia arguta folia*, obtained by the extraction with 40% ethyl alcohol. The IC_{50} value for the given extract in relation to DPPH; superoxide and hydroxyl radical, amounted to 537.6±23.924 μg/ml; 26.6±2.627 μg/ml and 72.6±3.264 μg/ml, respectively, which may indicate that this extract has reducing and radical scavenging properties. In parallel, the study of the total content of antioxidants in terms of quercetin and gallic acid has been carried out. It has also been found out that in the *Actinidia arguta folia* extract, obtained by the extraction with 40% ethyl alcohol, the content of the antioxidants is maximum.

Conclusion. The data obtained using the *in vitro* test were confirmed in the *in vivo* study, in which the course application of the *Actinidia arguta folia* extract, obtained by the extraction with 40% ethyl alcohol to the degree comparable to quercetin, contributed to an increase in the superoxide dismutase activity, a decrease in the lipid peroxidation products. The maximum content of antioxidants for *Actinidia arguta folia* was 0.73±0.007 and 0.47±0.005 mg/g in terms of quercetin and gallic acid, respectively. The extractant was 40% ethyl alcohol.

Keywords: *Actinidia arguta;* flavonoids; antioxidant activity; medicinal plant materials; ethnomedicine

Abbreviations: DPPH- 2,2-diphenyl-1-picrylhydrazyl; EDTA – ethylenediaminetetraacetic acid; DC – diene conjugates; TBA-AP – active products of thiobarbituric acid; ROS – reactive oxygen species; SOD – superoxide dismutase; GP – glutathione peroxidase; NADPH – reduced nicotinamide adenine dinucleotide phosphate; ERK – extracellular regulated kinase; MAPK – mitogen-activated protein kinase.

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RESEARCH ARTICLE

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ОЦЕНКА УРОВНЯ АНТИОКСИДАНТНОЙ АКТИВНОСТИ РАСТИТЕЛЬНОГО СЫРЬЯ ACTINIDIA ARGUTA (SIEBOLD ET ZUCC.) PLANCH. EX MIQ., ВЫРАЩИВАЕМОЙ В РЕГИОНЕ КАВКАЗСКИХ МИНЕРАЛЬНЫХ ВОД

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Цель. Выявление и оценка антиоксидантной активности нового лекарственного сырья – актинидии аргута листьев (*Actinidia arguta folia*).

Материалы и методы. Суммарное содержание антиоксидантов проводили на жидкостном хроматографе «Цвет Яуза-01-АА» амперометрическим методом. Параллельно изучали *in vitro* антиоксидантную активность извлечений *Actinidia argut*a в следующем диапазоне разведений: 62,5 мкг/мл, 125 мкг/мл, 250 мкг/мл, 500 мкг/мл и 1000 мкг/мл. При этом оценивались DPPH, супероксид и гидроксил-радикал ингибирующие свойства анализируемых образцов. Были проведены исследования *in vivo* антиоксидантной активности с определением активности супероксиддисмутазы, глутатионпероксидазы, каталазы, концентрации малонового диальдегида и диеновых конъюгатов.

Результаты. При изучении антирадикальной активности (*in vitro* тесты) установлено, что наиболее высокой радикал-ингибирующей активностью сопоставимой с индивидуальным соединением – кверцетином, обладает извлечение из актинидии аргута листьев, полученные экстракцией спиртом этиловым 40%. Величина IC_{50} для данного извлечения в отношении DPPH; супероксид и гидроксил-радикала составила 537,6±23,924 мкг/мл; 26,6±2,627 мкг/мл и 72,6±3,264 мкг/мл соответственно, что может свидетельствовать о наличии у данного извлечения восстановительных и радикал-скэвенджерных свойств. Параллельно проводилось изучение суммарного содержание антиоксидантов в пересчете на кверцетин и галловую кислоту. Также было обнаружено в извлечении актинидии аргута листьев, полученном экстракцией спиртом этиловым 40%, содержание антиоксидантов максимально.

Заключение. Данные, полученные с помощью испытания *in vitro*, были подтверждены в исследовании *in vivo*, в котором курсовое применение извлечения актинидии листьев, полученного экстракцией спиртом этиловым 40% в сопоставимой с кверцетином степени, способствовало увеличению активности супероксиддисмутазы, снижению продуктов липопероксидации. Максимальное содержание антиоксидантов для актинидии аргута листьев составило 0,73±0,007 и 0,47±0,005 мг/г в пересчете на кверцетин и галловую кислоту соответственно. Экстрагент – спирт этиловый 40%.

Ключевые слова: актинидии аргута; флавоноиды; антиоксидантная активность; лекарственное растительное сырье; этномедицина

Список сокращений: DPPH- 2,2-дифенил-1-пикрилгидразил; ЭДТА – этилендиаминтетрауксусная кислота; ДК – диеновые конъюгаты; ТБК-АП – активные продукты тиобарбитуровой кислоты; АФК – активные формы кислорода; СОД – супероксиддисмутаза; ГП – глутатионпероксидаза; НАДФН – никотинамидадениндинуклеотидфосфат восстановленный; ERK – внеклеточная регулируемая киназа; MAPK – митоген-активируемая протеинкиназа.

INTRODUCTION

The genus *Actinidia* Lindl. includes about thirty species, the places of natural growth of which are Central and East Asia, the island of Java. In Russia, these are relict plants of the Far East (species: *Actinidia giraldi* Diels; *Actinidia kolomikta* (Maxim.) Maxim; *Actinidia argut*a (Siebold et Zucc.) Planch. ex Miq.; *Actinidia polygama* (Siebold et Zucc.) Maxim. Breeding work with the Far Eastern actinidia was started in 1906 by I.V. Michurin, who created a selection fund of domestic actinidia [1].

One of the promising species is *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq., as it tolerates temperature changes well and is resistant to low temperatures (in the winter period), and it has a high yield. The following varieties were bred by Michurin I.V. from the samples of this species – “Early” and “Yeilding” [2]. At present, the growth of *Actinidia arguta* has spread far enough beyond its habitat, and every year it is more and more interesting as a promising species for horticulture and medicine [4]. Fruits are evaluated for the content of ascorbic acid

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[3, 8], as well as due to the high content of biologically active substances (BASs), which have antioxidant, adaptogenic and immunomodulatory properties [5–7]. *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. is a well-known Japanese plant called sarunushi. In its fruits, the content of biologically active substances such as catechins, ascorbic acid, anthocyanins, beta-carotenes and other polyphenols, which are well preserved in processed products, has been established. The studies by Japanese scientists show that *A. arguta* juice components are promising for a potential use as chemopreventive agents [8, 9]. There are studies that the genus *Actinidia* Lindl. species can be a potential source of natural antioxidants [10].

At present, *Actinidia* arguta (Siebold et Zucc.) Planch. ex Miq., successfully grown under the conditions of the Caucasian Mineral Waters, is of interest for scientific research. Over the past ten years, the specimens growing under the climatic conditions of Pyatigorsk, have been monitored. Their unpretentiousness in care, frost resistance, annual fruiting and good yields should be notified.

By a screening phytochemical analysis, the content of flavonoids and tannins, the presence of which predicts a possible antioxidant activity, has been established in the *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. *folia* [11].

THE AIM of the study is the identification and evaluation of a new antioxidant activity in a potentially new medicinal raw material of *Actinidia arguta folia*.

MATERIALS AND METHODS

Object of study

The object of the study is *Actinidia arguta folia* harvested in the fruiting phase (autumn) of the producing plant – *Actinidia arguta* of the *Actinidiaceae* family, from the specimens grown under the climatic conditions of the city of Pyatigorsk, the Stavropol Territory, in the open field near Novopyatigorsk-Skachki, the coordinatesare are: 44°01’07” N and 43°03’12” E, 545 m above the sea level. The identification of the raw materials was carried out by Vdovenko-Martynova N.N., an Associate Professor of the Department of Pharmacognosy, Botany and Technology of Phytopreparations of the Pyatigorsk Medical and Pharmaceutical Institute – a branch of the Volgograd State Medical University. *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. is a dioecious perennial twining vine up to 15 meters high with a stem, which eventually becomes woody. The leaves are large, up to twelve centimeters long, green, turn bright yellow in autumn and fall off in November. It bears fruits annually. The fruits are berries up to 3 cm long, juicy, sweet and sour, their taste reminds that of kiwi.

Obtaining extracts and determining the total content of antioxidants in terms of quercetin and gallic acid

The extracts for the research were prepared from the dried crushed raw materials. The total content of antioxidants was determined in terms of quercetin and gallic acid. Using a calibration plot of the output signal dependence on the quercetin and/or gallic acid concentration, the mass concentration of antioxidants was measured. The determination was carried out on a Tsvet Yauza-01-AA liquid chromatograph (OJSC “Khimavtomatika”, Russia) by the amperometric method [12–15].

The analyzed raw material was crushed to the size of particles passing through a sieve with a diameter of 0.5 mm. About 1.0 g (an accurately weighed portion) was placed in a conical flask with a thin section with a capacity of 100 ml, 30 ml of extractant (purified water, ethyl alcohol of various concentrations: 95%, 70%, 40%) was added, heated in the water bath under reflux for 30 minutes. After cooling, it was filtered through a paper filter into a 100 ml volumetric flask. The extraction was repeated twice, using 30 ml of the extractant. The extracts were combined and made up to the mark [12–14].

For each sample, five consecutive measurements of the output signal (the peak area) of the analyzed extraction were recorded. The dilutions were taken into account in the calculations.

The mass concentration X (mg/g) was calculated by the formula:

$$X = \frac{X_g * V_n * N}{m_n * 1000}, \quad (1)$$

where: X_g is the mass concentration of the antioxidants found from the calibration curve, mg/l; V_n is the volume (extraction) of the plant material, ml; m_n is a sample of the vegetable raw materials, g; N is the dilution factor of the analyzed sample.

Determination of pharmacological activity

The antioxidant activity of *Actinidia arguta* extracts was studied *in vitro* in the following dilution range: 62.5 μg/ml, 125 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml. In this case, the inhibitory properties of 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and the hydroxyl radical of the analyzed samples were evaluated. All the tests were performed in the triplet form.

DPPH test

The ability of the studied *Actinidia arguta* extracts to inhibit the formation of the DPPH radical in the model medium was evaluated according to the method described by Flieger J. et al. [16]. The mixture consisting of 1 ml of the analyzed extract at various concentrations and 0.5 ml of a 0.4 mM DPPH solution in methanol (the analytical grade, Vekton, Russia) was incubated for 30 min. at room temperature. Further, the change in the optical density of the studied samples was recorded at λ=518 nm relative to methyl alcohol. The methanolic solution of DPPH was taken as the positive control (A_0). Quercetin (Sigma-Aldrich, USA) at similar concentrations was used as a comparison. The percentage of the inhibition was calculated using the formula [16]:

$$\text{inhibition } \% = \frac{A_x * 100}{A_0}, \quad (2)$$

where: A_x is the sample optical density of the extraction reference standard; A_0 is the optical density of the positive control sample.

Evaluation of hydroxyl radical inhibitory activity

A method based on the spectrophotometric detection of a colored condensed complex of 2-thiobarbituric acid and degradation products of 2-deoxyribose degraded by the hydroxyl radical generated in the Fenton reaction, was used. The model medium included: 0.1 ml of a 2.8 mM deoxyribose solution; 0.1 ml of a 0.1 mM ethylenediaminetetraacetic acid (EDTA) solution; 0.1 ml of a 0.1 mm ascorbate solution; 0.1 ml of a phosphate buffer (pH 7.4) and 1 ml of the *Actinidia folia* assay extracts in the estimated concentration range. The resulting mixture was incubated at 37°C for one hour. Next, 1 ml of the 2.8% trichloroacetic acid solution and 1 ml of the 1% solution 2-thiobarbituric acid were added, heated for 20 minutes in the water bath (100°C). After cooling, the extinction of the samples was measured at λ=532 nm relative to the air. Fenton's medium without the addition of the studied extracts served as a positive control. As a comparison, quercetin of similar concentrations was used. The degree of hydroxyl radical formation inhibition was calculated by the formula (2) [17].

Evaluation of superoxide radical inhibitory activity

An analysis method based on the spectrophotometric detection of riboflavin photoconversion reaction products was used. The model medium included: 0.1 ml solution of the studied *Actinidia folia* extracts in various concentrations; 0.1 ml of a 1.5 mM nitro-blue tetrazolium solution; 0.2 ml of a 0.1 M EDTA solution; 0.05 ml of a 0.12 mM riboflavin solution and 2.55 ml of phosphate buffer (pH 7.4). The mixture was incubated for 5 min at room temperature. The sample extinction was measured at λ=560 nm relative to the air. The positive control was the incubation medium without the addition of the studied extracts. As a comparison, quercetin was used at similar concentrations. The percentage of the superoxide radical formation inhibition was calculated by formula (2) [18].

During the *in vitro* testing, the optical density was measured on a spectrophotometer PE-5400V (Promecolab, Russia).

Evaluation of "acute toxicity"

The toxicity study of *Actinidia folia* extracts in an acute experiment was carried out according to the "Up and Down" testing procedure, the main provisions of which are presented in the OECD Guideline for the evaluation of oral chemical compounds toxicity No. 425.

According to the principles of "the acute toxicity" evaluation, set out in OECD No. 425, the toxicity study experiment of the test samples involves two stages. The first stage is "a limit test", in which the studied objects were administered orally at the dose of 5000 mg/kg. When 3 animals died, the main testing was performed; otherwise, the value of LD_{50} was taken as 5000 mg/kg. The animals were observed for 14 days from the moment of the introduction of the studied objects. The "acute toxicity" study of the *Actinidia folia* analyzed extracts was performed on Balb/c male mice weighing 20–25 grams, obtained from the Rappolovo laboratory animal nursery (Leningrad region, Russia), which had undergone microbiological control and a 2-week quarantine. The animals were kept under standard conditions: the air temperature was 20±2°C; the relative humidity was 60±5% with a daily cycle of 12 hours day/12 hours night and a free access to food and water. The design of the study and the conditions under which the animals were kept, were in accordance with generally accepted standards of experimental ethics[1]. The concept of the work was approved by the local ethics committee of the Pyatigorsk Medical and Pharmaceutical Institute, a branch of Volgograd State Medical University (Protocol No. 2 dated 03/20/2019).

Antioxidant activity evaluation of studied *in vivo* extracts

An *in vivo* study of the antioxidant *Actinidia folia* properties of extracts was performed on 60 male Wistar rats weighing 200–230 grams. Keeping the animals corresponded to that in the assessment of "acute toxicity". The studied extracts (95% ethyl alcohol – code A95; 70% ethyl alcohol – code A70; 40% ethyl alcohol – code A40 and the water extract - code AB) were administered to the rats without pathology per os at the dose of 1/50 of LD50 for 10 days. After that, blood was taken from the animals' abdominal aorta into a citrate-coated syringe, then the rats were decapitated under chloral hydrate anesthesia (350 mg/kg intraperitoneally). The blood was centrifuged at 1000g for 15 minutes to obtain serum, in which the change in the pro/antioxidant balance was determined. The reference substance was quercetin at the dose of 100 mg/kg, which was administered according to the scheme similar to the studied extracts [19].

Determination of diene conjugates concentration

The content of diene conjugates (DCs) in the animals' blood serum was determined by a spectrophoto-

[1] Directive 2010/63 / EU of the European Parliament and of the councilon the protection of animals used for scientific purposes, September 22, 2010.

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metric method. DC was extracted with the mixture of heptane+isopropanol (1:1). The amount of DC was calculated by the molar extinction coefficient of conjugated dienes at λ=233 nm 2,2×10^5 M^1 · см$^{-1}$ and expressed in nmol/ml. The optical density was measured on a PE-5400V spectrophotometer (Promecolab, Russia) [20].

Determination of the TBA-active products (TBA-AP) concentration

The content of TBA-AP was determined by a spectrophotometric detection at λ=532 nm of the colored reaction products of the peroxide products condensation reaction with 2-thiobarbituric acid. In this case, the color of the resulting solution is proportional to the TBA-AP concentration. The content of TBA-AP was calculated by the molar extinction coefficient of malondialdehyde (1.56×10^5 lmol^{-1} · cm^{-1}). The results obtained were expressed in nmol/ml. The optical density was measured on a PE-5400V spectrophotometer [21].

Determination of catalase activity

A catalase activity was evaluated by the spectrophotometric method in the reaction of a hydrogen peroxide destruction determined by the interaction with a 4% ammonium molybdate solution. The color intensity of the reaction product was evaluated at λ=410 nm.

The catalase activity was calculated by the difference between the extinctions of the experimental and blank samples, using the molar extinction coefficient of hydrogen peroxide equal to 22.2×10^3 mM^{-1} · cm^{-1} and expressed in nmol/min/ml. The optical density was measured on a PE-5400V spectrophotometer [22].

Determination of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was evaluated by the xanthine oxidase method. The incubation medium contained: 0.05 mmol/l xanthine; 0.025 mmol/l 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride; 0.94 mmol/l EDTA, 80 U/l xanthine oxidase, 40 mmol/l CAPS buffer. The sample extinction was recorded at λ=505 nm. The SOD activity was expressed in U/l. The optical density was measured on a PE-5400V spectrophotometer [23].

Determination of glutathione peroxidase activity

The activity of glutathione peroxidase (GP) was determined in the coupled glutathione reductase reaction by the decrease in NADPH. The incubation medium included: 1 mmol/l EDTA, 50 mM K,Na-phosphate buffer, pH 7.4; 1 unit act./ml glutathioreductase; 20 mmol/l NADPH; 1 mmol/L glutathione (GSH); 30–60 μg of protein per 1 ml of medium. The sample extinction was recorded at λ=340 nm. The reaction was started by adding cumene hydroperoxide at the concentration of 1.5 mmol/l and carried out at the temperature of 25°C. The GP activity was expressed in U/l. The optical density was measured on a PE-5400V spectrophotometer [24].

Statistical analysis

The obtained results were statistically processed using the Statistica 6.0 software package (StatSoft, USA). The data were expressed as M±SEM. Statistically significant differences between the *in vivo* testing groups were determined by a one-way analysis of variance with the Newman-Keuls post-test. The IC_{50} value for *in vitro* tests was calculated by a probit analysis. The LD_{50} index was determined by the maximum likelihood method using the AOT425statpgm report software (OECD TG 425 2002, USA) [25].

RESULTS

Study of antioxidants total content

The total content of the antioxidants in terms of quercetin and gallic acid, the peak areas, the dilution ratio, are presented in Table 1.

The content of antioxidants in the studied extracts obtained by extracting *Actinidia arguta folia* with purified water and ethyl alcohol of various concentrations, was established. Analyzing the data in Table 1, it can be concluded that the maximum content of the total antioxidants in terms of quercetin and gallic acid was found out in the extract of *Actinidia arguta folia*, obtained by the extraction with 40% ethyl alcohol.

Evaluation of antioxidant activity *in vitro*

When studying the antioxidant properties of the studied extracts in *in vitro* tests, it was found out that, with respect to the DPPH radical, the analyzed extracts were characterized by an insignificant inhibitory activity, as evidenced by the IC50 value. For the objects under the codes A95, A70 and AB it made up 1150.9±52.321 μg/ml, 1660.9±45.954 μg/ml and 1918.5±85.617 μg/ml, respectively. At the same time, the IC_{50} for extracts from *Actinidia folia* obtained by the extraction with 40% ethyl alcohol and quercetin, was 537.6±23.924 μg/mL and 519.4±45.296 μg/mL, respectively (Fig. 1).

With regard to the superoxide radical, the most pronounced inhibitory activity was shown by the studied extracts under the codes A95, A40 and quercetin: the IC value was 30.7±1.238 μg/ml, 26.6±2.627 μg/ml and 11.3±1.974 μg/ml, respectively. At the same time, the extracts of A70 (IC_{50} = 204.3±9.114 μg/mL) and AB (IC_{50}=262.9±7.856 μg/mL) inhibited the generation of the superoxide radical in the model medium to a lesser extent (Fig. 2).

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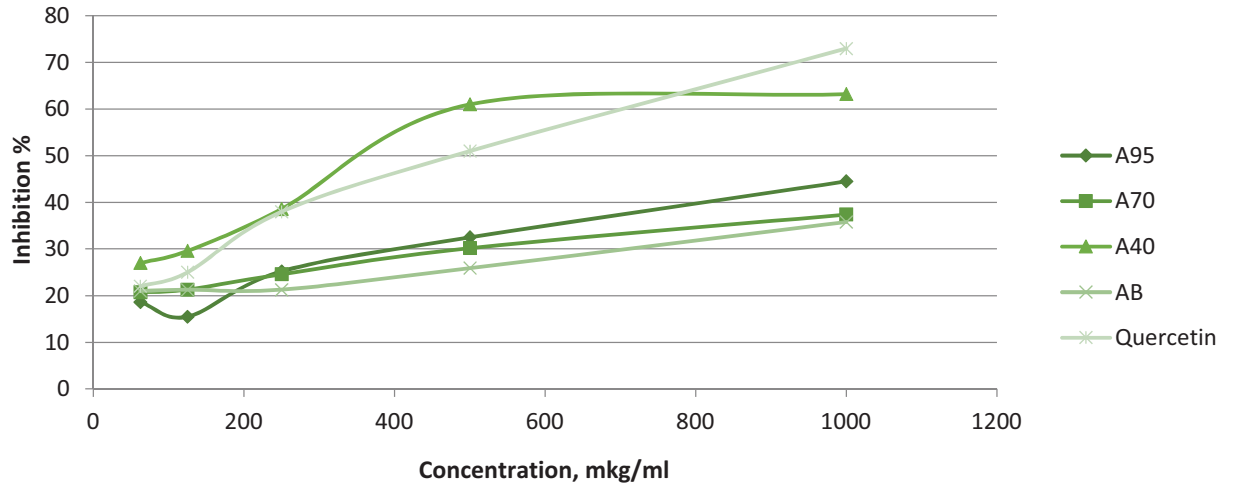

Figure 1 – Evaluation results of the DPPH-inhibitory activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

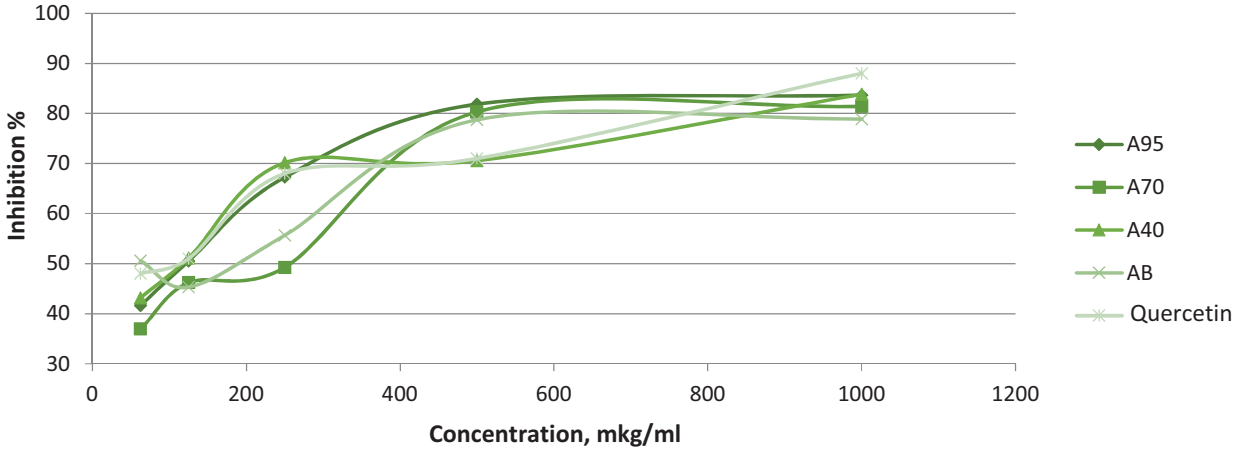

Figure 2 – Evaluation results of the superoxide-radical-inhibiting activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

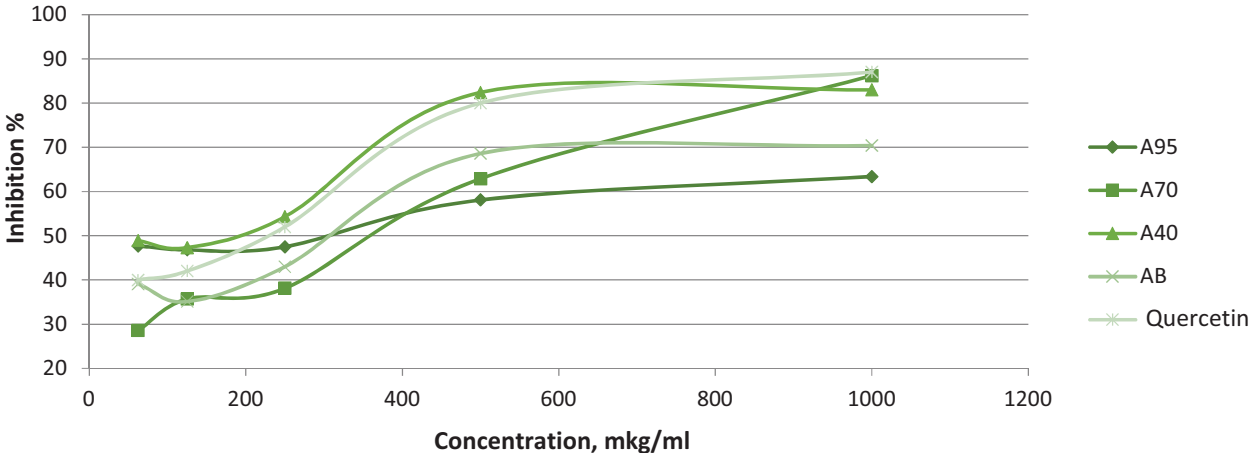

Figure 3 – Evaluation results of the hydroxyl-radical-inhibiting activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

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Table 1 – The content of antioxidants (in terms of quercetin and gallic acid) in extracts from *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. *folia*

Raw materials name	Extractants used	Peak areas (pA/sec)	Dilution ratio of analyzed samples	Total content of antioxidants, mg/g (n=6) in terms of	
				quercetin	gallic acid
Leaves	95% ethyl alcohol	2963.04	–	0.268±0,005	0.172±0.003
	70% ethyl alcohol	3210.95	2	0.584±0,009	0.375±0.004
	40% ethyl alcohol	4262.38	6	0.734±0,007	0.475±0.005
	Purified water	3991.70	2	2.215±0,007	1.436±0.006

Table 2 – Results of determining "the acute toxicity" of *Actinidia folia* investigated extracts

Animal No.	Weight, g	Investigated objects				Dose
		A95	A70	A40	AB	
1	22	O (S)				5000 mg/kg, *per os*
2	21	O (S)				
3	20	O (S)				
4	23		O (S)			
5	22		O (S)			
6	20		O (S)			
7	24			O (S)		
8	25			O (S)		
9	25			O (S)		
10	24				O (S)	
11	21				O (S)	
12	22				O (S)	

Note: O – no death on the first day of observation; (S) – no death during 14 days of observation.

Table 3 – Changes in the pro / antioxidant balance against the background of the course administration of the studied extracts and quercetin to the animals without pathology

Group	Intact animals (n=10)	A95 (n=10)	A70 (n=10)	A 40 (n=10)	AB (n=10)	Quercetin (n=10)
SOD, Unit/l	300.5±7.129 µ	304.4±8.432 µ	307.1±8.391µ	371±8.377*	309.5±7.973 µ	395.47±8.439*
GP, Unit/l	602.6±5.946	601.56±6.188	603.71±6.163	664.91±9.779*	604.75±9.050	657.29±6.129
Catalase, nmol/min/ml	0.863±0.0145	0.944±0.024	0.76±0.076 µ	1.188±0.084*	0.919±0.041	1.287±0.082*
TBA-AP, nmol/ml	5.4±0.524	4.36±0.611	4.43±0.298	3.75±0.173*	4.62±0.596	3.67±0.655*
DC, nmol/ml	10.8±0.696	9.71±0.31	9.74±0.454	7.31±0.271*	9.55±0.21	7.52±0.481*

Note: * is statistically significant relative to the intact animals ($p<0.05$; Newman-Keuls test); µ is statistically significant relative to the animals treated with A40 extract ($p<0.05$; Newman-Keuls test).

The formation of a hydroxyl radical in the model mixture most significantly inhibited the addition of the studied extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol (IC_{50} = 72.6±3.264 µg/ml) into the medium. At the same time, the IC_{50} values for the studied objects A95; A70; AV and quercetin amounted to 245.6±10.237 µg/ml; 382.5±11.974 µg/ml; 356.0±12.987 µg/ml and 192.2±7.515 µg/ml, respectively (Fig. 3).

"Acute toxicity" evaluation of *Actinidia folia* investigated extracts

In the course of evaluating "the acute toxicity" (Table 2) of the studied extracts, it was found out that during "the limit test" (the administration of the studied objects at the dose of 5000 mg/kg, per os), neither early nor delayed death of animals was noted. At the same time, there were no significant deviations in the general condition of the animals, their behavioral activity and sensorimotor perception. Thus, based on the data obtained during the implementation of "the limit test", the main testing was not started, and the value of LD_{50} for all the studied objects was taken as 5000 mg/kg, which allows us to attribute the studied extracts of *Actinidia folia* to the 5th toxicity class according to the GSH-classification[2].

[2] Ibid

Antioxidant activity evaluation of the studied Actinidia folia extracts *in vivo*

Based on the results of determining "the acute toxicity" of the studied *Actinidia folia* extracts, when evaluating the antioxidant activity *in vivo*, the administered dose of the studied objects was 100 mg/kg (p. o.). The results of this block of the experimental work are presented in Table 2.

Against the background of a 10-day administration of quercetin to the animals without a pathological background, an increase in the activity of SOD and catalase was notified compared to the intact animals by 31.6% ($p<0.05$) and 49.1% ($p<0.05$). At the same time, the catalytic properties of HP in the rats treated with quercetin did not statistically significantly differ from those in the intact animals (Table 3). It should also be notified that when using quercetin, there was a decrease (relative to the intact rats) in the concentration of DC and TBA-AP in the blood serum of the animals by 30.3% ($p<0.05$) and 32% ($p<0.05$), respectively. As can be seen from the data obtained (Table 3), the course administration of extracts under the codes A95, A70 and AB to the animals without pathology did not have a significant effect on the change in the pro/antioxidant balance. At the same time, when using A40 extract, an increase in the activity of SOD, GP and catalase was notified in comparison with the intact rats by 23.5% ($p<0.05$); 10.3% ($p<0.05$) and 37.7% ($p<0.05$), respectively, accompanied by a decrease in the concentration of TBA-AP and DC by 30.5% ($p<0.05$) and 32.3% ($p<0.05$), respectively.

At the same time, the indicators characterizing the state of the pro/antioxidant balance in the animals treated with quercetin and the studied extracts of *Actinidia folia* obtained by the extraction with 40% ethyl alcohol, did not differ statistically significantly. It should be notified that the activity of SOD in the blood serum of the rats that had been injected with A40 extract, was 21.9% ($p<0.05$); 20.8% ($p<0.05$) and 19.8% ($p<0.05$) higher than the same indicator in the animals treated with the studied objects under codes A95, A70 and AB, respectively. A catalase activity with A40 extracts was also superior to that of the animals that received A70 extract, by 56.3% ($p<0.05$).

DISCUSSION

Reactive oxygen species (ROS) are formed in cells during metabolism and perform many physiological processes, such as a regulation of cell proliferation, a microcirculatory blood flow, apoptosis reactions, and the gene expression [26].

Within certain physiological limits, the endogenous antioxidant defense system is designed to reduce the negative effect of oxidants on the body. Endogenous antioxidant enzymes, such as catalase, superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, and glutathione reductase, provide a timely inactivation of ROS, preventing their negative effect on the cells [28].

At the same time, a shift in the redox status towards prooxidants and the insufficient activity of endogenous antioxidant defense enzymes contribute to the development of the oxidative stress, which plays a significant role in the pathogenesis of a number of diseases: oncopathology, diabetes mellitus, cardiovascular diseases, alcoholic liver dystrophy, dementia, atherosclerosis, Parkinson's disease [29].

It is known that in the series of ROS, a superoxide radical and its derivatives (peroxonitrite), as well as the hydroxyl radical, have the highest cytotoxicity, which, through a direct destructive action and indirect reactions (usually involving secondary effector systems in the pathological cascade, for example, ERK and MAPK kinase) lead to the cell death [30].

An insufficient activity of the endogenous antioxidant defense and the associated increase in the amount of ROS, require the administration of exogenous antioxidants, among which the agents of the natural origin are especially prominent [31].

This research was devoted to the antioxidant activity study of the extracts obtained from *Actinidia folia.* The antioxidant activity of 95%, 70% and 40% alcohol extracts, as well as water extracts, was evaluated using *in vitro* and *in vivo* approaches. Thus, when studying the antiradical activity (*in vitro* tests), it was found out that the highest (among the studied objects) radical-inhibiting activity, comparable with the individual compound – quercetin, is the extraction from *Actinidia arguta folia,* obtained by the extraction with 40% ethyl alcohol. In relation to DPPH; superoxide and hydroxyl radical, the IC_{50} values for the given extract were 537.6±23.924 μg/mL, 26.6±2.627 μg/mL, and 72.6±3.264 μg/mL, respectively, which may indicate that this extract has reducing and radical scavenger properties [32].

In parallel, the study of the total content of the antioxidants in terms of quercetin and gallic acid, was carried out. In the *Actinidia arguta folia* extract obtained by the extraction with 40% ethyl alcohol, the maximum content of antioxidants was also found out.

It is known that the DPPH test is the most common approach for evaluating the acceptor properties of phenolic compounds. The analysis principle is based on the principle of the DPPH free radical reduction by accepting a hydrogen atom from a donor compound and converting the color from violet to yellow. Thus, this approach makes it possible to evaluate the acceptor properties of biologically active substances with a sufficiently high reliability [33].

At the same time, *in vitro* tests on superoxide and hydroxyl radicals are based on the ability of the analyzed object to suppress the formation of ROS in a model medium and, accordingly, make it possible to evaluate the scavenger activity [34, 35].

CONCLUSION

The maximum content of antioxidants in *Actinidia arguta folia* is 0.73±0.007 and 0.47±0.005 mg/g in terms

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of quercetin and gallic acid, respectively. The extractant is 40% ethyl alcohol.

The data obtained in the *in vitro* test were confirmed in the *in vivo* study in which the course application of the *Actinidia arguta folia* extract obtained by the extraction with 40% ethyl alcohol, to a degree comparable to quercetin, contributed to an increase in the activity of SOD, GP and catalase. This extract also contributed to the reduction of lipid peroxidation products in the animals without a pathological background, which suggests that the studied A40 extract has a high antioxidant activity. All of the above, combined with a low toxicity (LD_{50}≥5000 mg/kg, *per os*), makes this extract a promising object for a further study in order to create a drug with an antioxidant effect.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Dmitry I. Pozdnyakov – the study concept development, the *in vivo* experiment setting up, statistical processing of the study results, preparing the manuscript final version; Similla L. Adzhiakhmetova - the study concept development, the analyzed extracts obtaining, conducting *in vitro* studies, preparing the manuscript final version; Natalia N. Vdovenko-Martynova – the literature analysis, raw materials identification, the analyzed extracts obtaining, preparing the manuscript final version.

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