BIOTECHNOLOGY

STUDY OF THE BIOFUNCTIONAL PROPERTIES OF CEDAR PINE OIL WITH THE USE OF *IN VITRO* TESTING CULTURES

Aleksandr Yu. Prosekov, Lyubov' S. Dyshlyuk, Irina S. Milent'eva, Valery A. Pavsky, Svetlana A. Ivanova*, and Sergey Yu. Garmashov

Kemerovo State University,
Krasnaya Str. 6, Kemerovo 650043, Russian Federation

* e-mail: pavvm2000@mail.ru

Received February 20, 2018; Accepted in revised form April 20, 2018; Published June 20, 2018

Abstract: Cedar pine (*Pinus sibirica*) nuts are an environmentally friendly natural product that contains a unique set of useful biologically active substances. Due to its composition, pine nuts and their derivative products are widely used in a comprehensive therapy and prevention program for a lot of diseases. The objects of the study were cedar oil and oil emulsions (the cedar oil concentration was 1.0, 5.0 and 10.0%). The antimicrobial properties were determined using the diffusion method and by measuring the optical density. The prebiotic properties were estimated according to the ability to stimulate the bifidobacteria growth. The antioxidant activity was determined using the fluorescent ORAC method. The antihypertensive activity was estimated according to the ability to inhibit the angiotensin-1-converting enzyme. All the studied experimental oil emulsion samples, regardless of a pressing method (cold or heat), showed high antimicrobial characteristics without suppressing only *Candida albicans* EMTC 34 and *Proteus vulgaris* ATCC 63 from the studied 10 strains of the main testing cultures. The prebiotic properties of the emulsions obtained with the addition of cedar oil have been determined. The number of cells of the bifidobacteria cultivated in nutrient media with the addition of cedar oil (with the concentration from 5.0%) is almost 3 times as large as the amount when cultivated without it. Antioxidant cedar oil properties have been revealed. The hypotensive characteristics of cedar oil can be observed even at a concentration of 5.0%, the percentage of inhibition of the angiotensin-1-converting enzyme is up to 69%. With an increase in the concentration of cedar oil to 10.0% inhibition increases to 70%. The carried out studies of the functional properties of experimental cedar oil samples have confirmed its high quality and an opportunity to use it as the basis of biologically active food supplements and dietary, medical-preventive and sports nutrition.

Keywords: Cedar pine nuts (*Pinus sibirica*), Oil, Antimicrobial, prebiotic, antioxidant properties

DOI 10.21603/2308-4057-2018-1-136-143 *Foods and Raw Materials,* 2018, vol. 6, no. 1, pp. 136–143.

INTRODUCTION

Cedar pine has always been highly demanded by the local population in any place of its growth thanks to extremely useful qualities. The most consumed pine nuts are the fruits of Korean pine (*Pinus koraiensis*), cedar pine (*Pinus sibirica*), umbrella pine (*Pinus pinea*) and chilgoza pine (*Pinus gerardiana*) [1, 2]. As raw materials, cedar is very convenient for a non-waste product manufacturing process [3–6]. The more northern the latitudes of cedar pine habitats, the higher the value of cedar products. The high demand for pine nuts has resulted in an increase in their world output, according to FAO, China, Korea, Pakistan and Russia (Siberia and the Far East) are the main exporting countries [7].

Traditionally, it is believed that the most valuable cedar product is cedar oil, which is produced from cedar kernels [8, 9]. The oil yield depends on a pressing method (cold and heat pressing, solvent extraction), but about 45 to 65 g of butter per 100 g of nuts is usually reported about [10, 11]. Cedar nut oil is a high-quality natural and ecologically friendly food supplement [12, 13] that is necessary for the normal functioning of the human body [14, 15].

Cedar oil is a real natural storehouse of useful biologically active substances. Its composition includes proteins, carbohydrates, saturated and unsaturated fatty acids, phosphatides, vitamins A, B1, B2, B3 (PP), E, D, lecithin, essential oils, amino acids and minerals [16–21]. The vitamin E content in pine nut oil is several times more than that in olive oil. The use of cedar oil in the diet makes it possible to restore working capacity and cope with psychoemotional disorders. The beneficial effect of cedar oil in the complex of both therapeutic and preventive measures is well known [22–28]. The limited consumption of oil, as part of dietary nutrition, is recommended for faster satiation [29].

The clear antioxidant effect of both nuts themselves and their derivative products has been noted. The study [12] of rats has found an increase in the antioxidant capacity in the blood of the animals that used Far-Eastern pine oil. Lin et al. [30] derived four peptides from deodar cedar nuts with pronounced

Copyright © 2018, Prosekov et al. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license. This article is published with open access at http://jfrm.ru/.

antioxidant properties that were enhanced to 90% by an impulse action and an electric field. The ability of cedar oil to reduce cellular activity is planned to be used in the inhibition of cancer metastases [1, 31].

Cedar needles, which are widely represented in traditional Chinese medicine, primarily with their antimicrobial properties, as well as their biologically active characteristics, are of particular interest to researchers [32]. A lot of types of cedar needles are added into functional foods and food supplements to enhance a nutritional and / or pharmaceutical effect [33, 34]. If needles, with their antimicrobial properties, are also noted for their biologically active characteristics, nuts are probably noted for the similar effect, too, i.e., in addition to their nutritional and functional properties, they also have antimicrobial characteristics.

The study was aimed at the *in vitro* biofunctional properties (antibacterial, antioxidant, prebiotic, etc.) of the oil derived from the nuts of cedar pine that grows in the Kemerovo region as the basis of biologically active food supplements and dietary, therapeutic, preventive and sports nutrition.

STUDY OBJECTS AND METHODS

The experimental studies were carried out at the Research Institute of Biotechnology of the Kemerovo State University.

The objects of the study were the samples of the oil made with the use of two methods (cold and heat pressing) from nut kernels of cedar pine that grows on the territory of the Kemerovo Region (Tashtagol District, the crop of 2016 and 2017) and 1.0%, 5.0% and 10.0% O/W emulsions of this cedar oil.

Testing cultures: opportunistic and pathogenic strains of microorganisms: *Pseudomonas aeruginosa* ATCC 9027 is an opportunistic bacterium that induces nosocomial infections in humans; *Candida albicans* EMTC 34 is a microscopic fungus, the causative agent of opportunistic human infections; *Alcaligenes faecalis* EMTC 1882 is an opportunistic bacterium that induces itraabdominal infections, septicemia and meningitis in humans; *Leuconostoc mesenteroides* EMTC 1865 is an opportunistic bacterium that induces infectious diseases in humans; *Escherichia coli* ATCC 25922 is an opportunistic bacterium that induces gastroenteritis in humans; *Enterococcus casseliflavus* EMTC 1866 is a pathogenic bacterium that induces sepsis in humans; *Salmonella enterica* ATCC 14028 is a pathogenic bacterium that induces gastroenteritis in humans; *Staphylococcus aureus* ATCC 25923 is a pathogenic bacterium that induces pneumonia, meningitis, osteomyelitis, endocarditis, infectious toxic shock and sepsis in humans; *Bacillus mycoides* EMTC 9 are permanent contaminating agents of food raw materials and food products that induce food toxic infections in humans; *Proteus vulgaris* ATCC 63 is an opportunistic bacterium that induces intestinal infections in humans; as well as the *Bifidobacterium adolescentis* MC 42 bifidobacteria strain and the MDCK1 canine kidney epithelial cell line.

Sample preparation. The shell was preliminarily separated from the pine nut kernel, Sample No. 1 is from the crop of 2016, Sample No. 2 is from the crop of 2017. To prepare the samples of seeds from the cedar pine layers different in height (upper, middle and lower), 3 single samples weighing 100 g each were taken from each batch.

To obtain particles of 2 mm in size, the pine nut kernel was grinded in an electric mill for each of the pressing methods (cold or heat). In the case of cold pressing, the pine nut kernel was put under a UP-20 hydraulic press (Nizhny Novgorod, Russia) and the oil was pressed out by means of a gradual increase in the load. Heat pressing is washing the heated pine nut kernels with hot water and then the heat pressing thereof.

The 1.0%, 5.0% and 10.0% cedar O/W emulsions were prepared by adding cedar oil (1 g, 5 g or 10 g) slowly to water (100 g) in the presence of an emulsifier (0.01 g, 0.05 and 0.10 g of soya lecithin, respectively) stirring continuously (the stirring rate is 500 rpm).

The antibacterial (antimicrobial) properties of the *in vitro* oil obtained from pine nuts were determined in terms of the growth of the opportunistic and pathogenic test strains of microorganisms using the ***diffusion method*** and a ***method based on the measurement of optical density***.

Diffusion method [35, 36]**.** The testing culture was inoculated on a dense nutrient medium as lawn. The microorganisms were grown at the values of pH and temperature optimal for each test strain for 24 hours. The culture liquid was centrifuged at 7000 rpm for 10 minutes and the supernatant was separated. To separate the cells, the supernatant was filtered through Millex-GV filters (0.22 μm, Nihon "Millipore", USA). The antimicrobial activity was estimated by measuring the inhibition zones with respect to the testing culture of a microorganism [37]. The paper discs were dipped in the emulsions (cedar oil) / suspensions (a protein-vitamin complex, a carbohydrate-mineral complex) containing the tested food ingredients squeezing out the excesses. The discs were put on the agar with a testing culture observing the rules of asepsis. The disks were arranged so that the distance between their centers was not less than 24 mm. After placing the discs on the agar, they were pressed with a sterile needle or pincers until they completely contacted with the surface of the medium.

After 15 minutes after placing the discs, the cups were inverted and incubated at the pH and temperature optimal for each test strain for 24 hours. After incubation, the diameter of the zones of complete incubation was measured (according to the observation with the naked eye), including the diameter of the disk, to the nearest whole millimeter using a caliper, ruler or stencil designed for these purposes.

Optical density measurement. The method for determining antimicrobial activity based on the measurement of optical density is as follows. To estimate the antibacterial effect of the food ingredients, the testing cultures were co-incubated with the studied ingredients in 96-well culture plates.

The night broth cultures were resuspended in a nutrient medium corresponding to the species of microorganisms bringing the number of microorganisms to an inoculation dose of ~10^5 CFU/ml. A cell suspension and the studied food ingredients were simultaneously added in the wells in an amount of 1/10 of the total volume. Control is a liquid nutrient medium with no cedar oil added. The reference drug (Control 1) is the antibiotic ciprofloxacin (10 μg/ml). The total volume of suspension in a well is 200 μl. The number of repetitions is 2. The incubation was carried out at a temperature corresponding to the optimal growth temperature for each test strain using a shaker (580 rpm). After 24 hours, the optical density (OD) was measured using a multireader at a wavelength of 595 nm. The presence of antimicrobial properties was estimated by a change in OD as compared with the control [38]. In the wells where the cell growth stopped or slowed down, OD was lower than that in the wells with the normal growth of microorganisms.

To estimate the prebiotic properties of *in vitro* oil derived from pine nuts, the ability to stimulate selectively the growth of protective populations (bifidobacteria) of normal intestinal microflora was determined; as the test strain, *Bifidobacterium adolescentis* MC 42 was used. The test strain was inoculated in Petrie dishes followed by thermostating under anaerobic conditions (using an anaerobic culture apparatus and anaerobic agent) into the agarized selective culture media with the addition of a certain amount of a food ingredient and / or the dilution thereof. The Petrie dishes were thermostated at (37 ± 1)°C for (72 ± 3) h under anaerobic conditions. After incubation, the number of the colonies grown in the Petrie dishes was counted.

The *in vitro* **antioxidant activity** of the test oil samples obtained from pine nuts was estimated using the ORAC fluorescence method with the generation of a peroxyl radical in the reaction medium using a BioTek Synergy 2 microplate photometer-fluorometer (USA). The strategy for determining antioxidant activity *in vitro* in the culture test model included the incubation of MDCK1 canine kidney epithelial line cells in the presence of a potential antioxidant agent, the further effect of a free radical oxidation initiator and methods for detecting radical activity in a cell. The free radicals in the cultured cells were detected fluorimetrically according to the fluorescence intensity of dichlorodihydro-fluorescein diacetate (DCFH-DA) fluorochrome that penetrates into living cells where, being affected by cellular enzyme systems, a non-fluorescing compound is formed, which, in turn, being affected by free radicals, turns into fluorochrome.

The passage of 100 μl of cell suspension with the cytosis of 10^6 cells/ml was provided in the wells of sterile black 96-well plates. The edge wells of the plate were filled with a cell-free culture medium to provide the same moisture conditions in all the experimental wells of the plate. The plate was incubated in a thermostat for 12...24 h to allow the cells to precipitate and attach to the surface of the culture plastic. Then the contents of the wells were aspirated and 100 μl of potential antioxidant agent solutions (emulsions) at concentrations of 1.0%, 5.0% and 10.0% was added to each well. As the control, the cells cultured in 100 μl of a saline solution without the addition of food ingredients were used. The plate was incubated in a thermostat for 2 hours, then the contents of the wells were aspirated and 100 μl of a working DCFH-DA solution (30 min) was added, after which the solution was aspirated and the peroxide oxidation was induced by adding 100 μl of a working 2,2'-azobis 2-amidino-propane) dihydrochloride (AAPH) solution into the wells.

The fluorescence intensity was determined at an excitation wavelength of 485 nm and an emission wavelength of 528 nm using a Synergy 2 spectrophotometer-fluorometer immediately after the addition of AAPH ("0") in 30 minutes, 60 minutes and 90 minutes.

The anti-hypertensive (hypotensive) activity of the *in vitro* food ingredients derived from pine nuts was determined in vitro according to their ability to inhibit the angiotensin-1-converting enzyme (ACE), a key link in the renin-angiotensin system that regulates human blood pressure. The most sensitive method for determining the ACE inhibitory activity of a substance is a method with the use of internally-quenched ACE substrates. As an internally-quenched substrate, o-aminobenzoyl-phenylalanyl-arginyl-lysyl(dinitrophenyl)- proline was used, the measurements were carried out using a BioTek Synergy 2 microplate photometer-fluorometer (USA). The reaction time was 30 minutes at 37°C.

Statistical analysis. All the experiments were carried out *n*-fold, $n = 5$. The data were processed using mathematical statistics standard methods. The homogeneity of the sampling effects was checked using Student's t-test. The differences between the averages were considered significant if the confidence interval was less than 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

The previous studies of the nut kernels of the crop of 2016–2017 of the cedar pines that grow in the Kemerovo region confirmed the nutritional value of the samples studied [39, 40]. Oil was derived from the cedar kernels by heat and cold pressing as well as emulsions with the addition thereof.

Table 1 shows the results of the determination of antimicrobial properties of the examples of cedar oil emulsions using the diffusion method. All the experimental samples of cedar oil obtained both by cold and heat pressing are characterized by high antimicrobial activity against all the tested strains except for *Candida albicans* EMTC 34 and *Proteus vulgaris* ATCC 63. It should be noted that the antimicrobial properties are determined by cedar oil concentration (up to a concentration of 5.0%). The further increase in the content of cedar oil is not followed by a significant increase in the diameter of inhibition zones of pathogenic test strains.

Table 1. Results of the determination of antimicrobial properties of the examples of cedar oil emulsions using the diffusion method

Inhibition zone diameter, mm										
Test strain / Samples	TC1	TC2	TC3	TC4	TC5	TC6	TC7	TC8	TC9	TC10
1.0% emulsion										
No. 1	8.0 ± 1.6	0.0 ± 0.0	10.0 ± 1.0	12.2 ± 0.8	7.4 ± 1.1	7.2 ± 0.8	14.0 ± 1.0	11.4 ± 1.1	8.2 ± 1.3	0.0 ± 0.0
No. 2	8.4 ± 1.1	0.0 ± 0.0	11.2 ± 1.3	10.4 ± 1.1	10.4 ± 1.1	10.4 ± 1.1	15.0 ± 0.7	14.6 ± 0.9	7.6 ± 1.1	0.0 ± 0.0
5.0% emulsion										
No. 1	16.6 ± 1.1	4.2 ± 0.8	23.0 ± 1.6	22.4 ± 1.1	19.6 ± 0.9	19.8 ± 0.8	24.8 ± 0.8	18.8 ± 1.3	21.6 ± 1.1	5.8 ± 0.8
No. 2	22.6 ± 1.1	5.6 ± 1.5	24.8 ± 0.8	20.6 ± 1.1	22.6 ± 1.1	20.6 ± 1.1	24.8 ± 0.8	20.2 ± 0.8	22.0 ± 1.0	6.0 ± 1.0
10.0% emulsion										
No. 1	16.6 ± 1.1	4.4 ± 1.1	23.4 ± 1.8	22.6 ± 1.1	19.8 ± 0.8	20.0 ± 1.0	25.0 ± 0.7	19.0 ± 1.2	21.6 ± 1.1	5.8 ± 0.8
No. 2	23.0 ± 1.4	5.8 ± 1.3	24.8 ± 0.8	21.0 ± 1.6	23.0 ± 1.4	21.0 ± 1.4	25.0 ± 0.7	20.8 ± 0.8	22.2 ± 0.8	6.4 ± 0.9
Control 1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control 2	22.0 ± 1.1	24.0 ± 1.2	21.0 ± 1.1	23.0 ± 1.2	20.0 ± 1.0	19.0 ± 1.0	23.0 ± 1.2	26.0 ± 1.3	24.0 ± 1.2	22.0 ± 1.1

Note. TC1 is *Pseudomonas aeruginosa* ATCC 9027, TC2 is *Candida albicans* EMTC 34, TC3 is *Alcaligenes faecalis* EMTC 1882, TC4 is *Leuconostoc mesenteroides* EMTC 1865, TC5 is *Escherichia coli* ATCC 25922, TC6 is *Enterococcus casseliflavus* EMCC 1866, TC7 is *Salmonella enterica* ATCC 14028, TC8 is *Staphylococcus aureus* ATCC 25923, TC9 is *Bacillus mycoides* EMTC 9, TC10 is *Proteus vulgaris* ATCC 63; Control 1 is the antibiotic ciprofloxacin; Control 2 is a nutrient medium without oil.

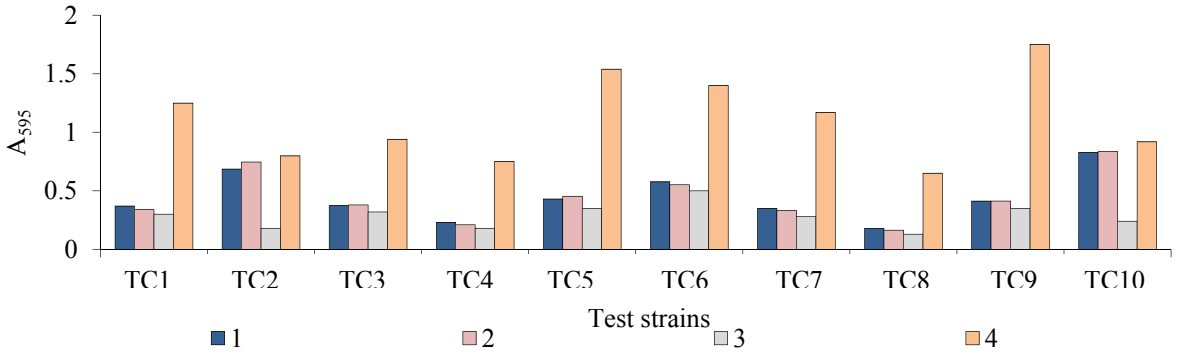

Fig. 1. Results of the determination of antimicrobial properties of cedar oil in a liquid nutrient medium: (1) oil (cold pressing); (2) oil (heat pressing); (3) Control 1 (the antibiotic ciprofloxacin); (4) Control 2 (a nutrient medium without oil). The data are expressed as a mean ± standard deviation (n = 5).
TC1 is *Pseudomonas aeruginosa* ATCC 9027, TC2 is *Candida albicans* EMTC 34, TC3 is *Alcaligenes faecalis* EMTC 1882, TC4 is *Leuconostoc mesenteroides* EMTC 1865, TC5 is *Escherichia coli* ATCC 25922, TC6 is *Enterococcus casseliflavus* EMTC 1866, TC7 is *Salmonella enterica* ATCC 14028, TC8 is *Staphylococcus aureus* ATCC 25923, TC9 is *Bacillus mycoides* EMTC 9, TC10 is *Proteus vulgaris* ATCC 63.

ISSN 2310-9599. Foods and Raw Materials, 2018, vol. 6, no. 1

The antimicrobial activity of cedar oil in a liquid nutrient medium was determined for the samples of 5.0% O/W cedar emulsions. The results are shown in Fig. 1.

The analysis of the data (Fig. 1) shows that the results of determining the antimicrobial activity of cedar oil in a liquid nutrient medium are consistent with the data obtained using the diffusion method (Table 1). Cedar oil shows high antimicrobial activity with regard to the test strains of *Pseudomonas aeruginosa* ATCC 9027, *Alcaligenes faecalis* EMTC 1882, *Leuconostoc mesenteroides* EMTC 1865, *Escherichia coli* ATCC 25922, *Enterococcus casseliflavus* EMTC 1866, *Salmonella enterica* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Bacillus mycoides* EMTC 9.

Figures 2–4 show the diagrams of a change in the number of bifidobacteria cells in the nutrient media that contain the oil derived from pine nuts.

The oil emulsions obtained with the addition of cedar nut oil have prebiotic properties since the number of cells of the bifidobacteria cultivated in nutrient media with oil (Fig. 2–4) increases compared to the amount of *Bifidobacterium adolescentis* cultivated in a nutrient medium with no addition of cedar oil. When cultivating bifidobacteria for 48 hours in a nutrient medium that contains cedar oil (5.0 and 10.0% emulsions in water), the number of cells increases up to 3.0 times compared to the control.

Figures 5–7 show the results of the determination of the antioxidant properties of the test cedar oil samples. Cedar oil shows pronounced antioxidant properties; there is a decrease for all the test samples in the intensity of fluorescence after 90 minutes of cell incubation (from 100% to 81.8%). Antioxidant activity grows with an increase in the concentration of ingredients up to 5.0%. The further increase in the concentration of cedar oil is followed by a slight increase in antioxidant activity.

Table 2 shows the results of the determination of the antihypertensive activity of the *in vitro* oil obtained from pine nuts. All the test emulsion samples with oil obtained from pine nuts have shown hypotensive properties. An oil emulsion, at a concentration of 5.0% already, has the highest ability to inhibit the angiotensin-1-converting enzyme, approximately by 70%, the further increase in the oil content practically does not change the inhibitory characteristics. The hypotensive properties of cedar oil are probably due to the presence of vitamins E and PP in its composition.

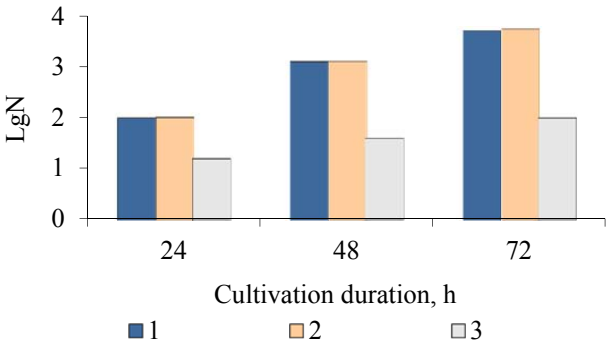

Fig. 2. Dynamics of the number of *Bifidobacterium adolescentis* MC 42 (lgN) cells in the nutrient medium that contains cedar oil (1.0% emulsion in water) after 24 h, 48 h and 72 h of fermentation: (1) oil (cold pressing); (2) oil (heat pressing); (3) control (a culture medium that does not contain any potential antioxidant agents). The data are expressed as a mean ± standard deviation (n = 5).

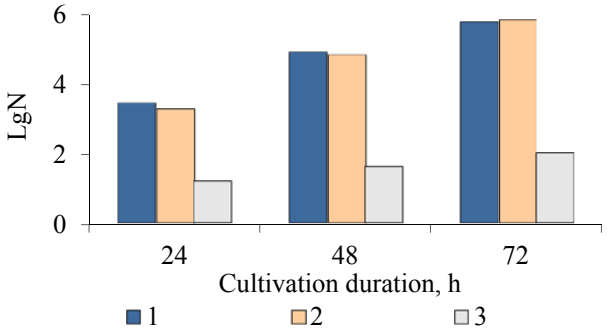

Fig. 3. Dynamics of the number of *Bifidobacterium adolescentis* MC 42 (lgN) cells in the nutrient medium that contains cedar oil (5.0% emulsion in water) after 24 h, 48 h and 72 h of fermentation: (1) oil (cold pressing); (2) oil (heat pressing); (3) control (a culture medium that does not contain any potential antioxidant agents). The data are expressed as a mean ± standard deviation (n = 5).

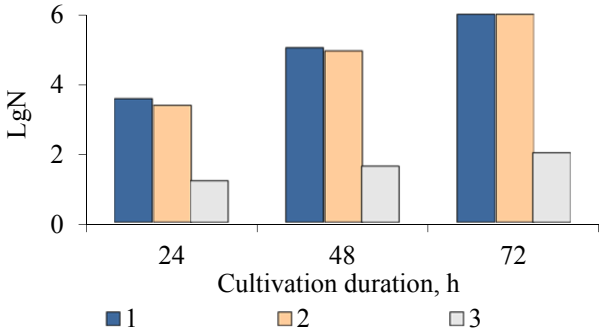

Fig. 4. Dynamics of the number of *Bifidobacterium adolescentis* MC 42 (lgN) cells in the nutrient medium that contains cedar oil (10.0% emulsion in water) after 24 h, 48 h and 72 h of fermentation: (1) oil (cold pressing); (2) oil (heat pressing); (3) control (a culture medium that does not contain any potential antioxidant agents). The data are expressed as a mean ± standard deviation (n = 5).

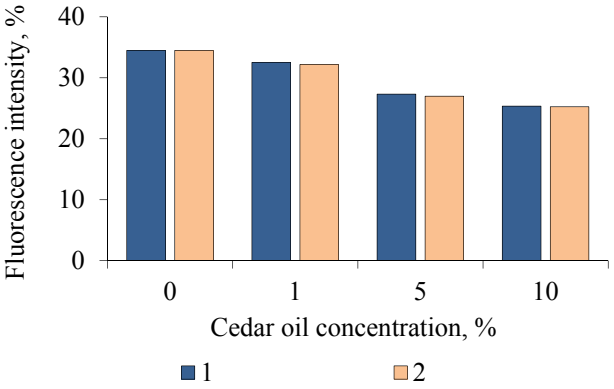

Fig. 5. Results of *in vitro* determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 30 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

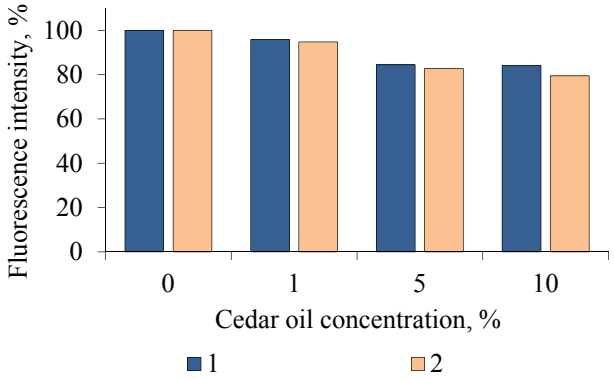

Fig. 7. Results of *in vitro* determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 90 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

Table 2. Results of the determination of the antihypertensive activity of the *in vitro* oil obtained from pine nut kernels

Test sample	ACE inhibition degree, %		
	1.0 %	5.0 %	10.0 %
Oil (cold pressing)	29.2 ± 0.9	68.2 ± 0.9	69.7 ± 1.2
Oil (heat pressing)	28.9 ± 1.2	69.5 ± 0.9	70.8 ± 0.8

CONCLUSIONS

According to the results of the study of the oil produced from the nuts of cedar pine that grows in the Kemerovo region, all the test samples obtained by both cold and heat pressing are characterized by high antimicrobial activity in relation to all the test strains, except for *Candida albicans* EMTC 34 and *Proteus vulgaris* ATCC 63. The antimicrobial characteristics depend on the concentration of cedar oil and can be observed at a concentration of up to 5.0%.

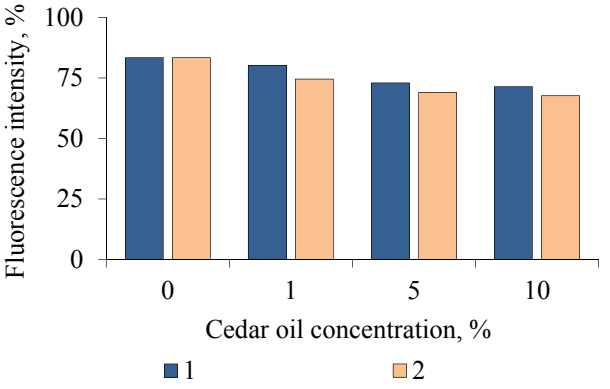

Fig. 6. Results of *in vitro* determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 60 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

Pine cedar oil, regardless of a production method, has a pronounced prebiotic effect. The number of the *Bifidobacterium adolescentis* cells cultivated in nutrient media with cedar oil exceeds the amount when cultivated in a nutrient medium without cedar oil from 1.7 to 3.1 times. The highest value (the difference is about 3 times) of the yield of bifidobacterial cells was observed during the cultivation in a nutrient medium supplemented with cedar oil for 48 hours (a 5.0% and 10.0% emulsion in water). The *in vitro* study of the antioxidant effect of cedar oil using the cultured MDCK1 cells has shown that cedar oil is characterized by pronounced antioxidant properties after 90 minutes of cell incubation for all the test samples (a decrease in fluorescence intensity). There was a decrease in antioxidant activity with an increase in the concentration of ingredients up to 5.0% for all the samples. The further increase in the concentration of food ingredients results in a slight increase in antioxidant activity.

The determination of antihypertensive activity of *in vitro* cedar oil shows that all the test samples have revealed antihypertensive characteristics. Cedar oil in a 5.0% emulsion inhibits angiotensin-1 by 68.9%, in a 10.0% emulsion – up to 70.2%, which is explained by the presence of vitamins E and PP therein.

The confirmation *in vitro* of the availability of biofunctional properties of the oil obtained from the nuts of cedar pine that grows in the Kemerovo region allows it to be used as the basis of not only biologically active food supplements, but also of dietary, therapeutic, preventive and sports nutrition.

ACKNOWLEDGMENTS

The present study has been carried out under the Federal Target Program "Research and Development in Priority Development Areas of Scientific and Technological Complex of Russia for 2014–2020 (Agreement No. 14.577.21.0255, the unique identifier of the Agreement is RFMEFI57717X0255).

REFERENCES

1. Xie K., Miles E.A., and Caldera P.C. A review of the potential health benefits of pine nut oil and its characteristic fatty acid pinolenic acid. *Journal of Functional Foods*, 2016, vol. 23, pp. 464–473. DOI: 10.1016/j.jff.2016.03.003.
2. Destaillats F., Cruz-Hernandez C., Giuffrida F., and Dionisi F. Identification of the botanical origin of pine nuts found in food products by gas−liquid chromatography analysis of fatty acid profile. *Journal of Agricultural and Food Chemistry*, 2010, vol. 58, no. 4, pp. 2082–2087. DOI: 10.1021/jf9041722.
3. Avstrievskikh A.N., Vekovtsev A.A., and Poznyakovskiy V.M. *Produkty zdorovogo pitaniya: novye tekhnologii, obespechenie kachestva, effektivnost' primeneniya* [Healthy food products: new technologies, quality assurance, application efficiency]. Novosibirsk: Sib. Univ. Publ., 2005. 413 p.
4. Mel'nichenko L.V., Vrubel' M.E., Aliev M.U., Huseynov A.K., and Strugovshchik Yu.S. Biologically active compounds of Siberian cedar pine fatty oil. *Modern high technologies*, 2014, no. 10, pp. 59–60. (In Russian).
5. Zabrodina S.V., Goncharov D.A., and Efremov A.A. Scientific approaches to the complex processing of non-wood Siberian pine (cedar) products. *Tne Bulletin of the KrasGAU*, 2006, no. 10, pp. 318–327. (In Russian).
6. Vorob'ev V.N. *Biologicheskie osnovy kompleksnogo ispol'zovaniya kedrovykh lesov* [Biological basis of complex use of pine forests]. Novosibirsk: Nauka Publ., 1983. 154 p.
7. *Non-wood forest products from conifers*. Available at: http://www.fao.org/docrep/x0453e/x0453e12.htm (accessed 20 April 2018).
8. Mazalevskiy V.B., Nitsievskaya K.N., and Motovilov O.K. Developing foodstuffs from pine nut kernels. *Siberian Herald of Agricultural Sciences*, 2015, vol. 6, no. 247, pp. 76–81. (In Russian).
9. Kim K.K., Kang Y.H., Kim D.J., Kim T.W., and Choe M. Analysis of Pine Nut Oil Composition and Its Effects on Obesity. *Korean Journal of Food Science and Technology*, 2014, vol. 46, no. 5, pp. 630–635. DOI: 10.9721/KJFST.2014.46.5.630.
10. Zadernowski R., Naczk M., and Czaplicki S. Chemical composition of Pinus Sibirica nut oils. *European Journal of Lipid Science and Technology*, 2009, vol. 111, no. 7, pp. 698–704. DOI: 10.1002/ejlt.200800221.
11. Chen X.Q., Zhang Y., Wang Z.Y., and Zu Y.G. In vivo antioxidant activity of *Pinus Koraiensis* nut oil obtained by optimised supercritical carbon dioxide extraction. *Natural Product Research*, 2011, vol. 25, no. 19, pp. 1807–1816. DOI: 10.1080/14786419.2010.483229.
12. Carcel L.M., Bon J., Acuna L., et al. Moisture dependence on mechanical properties of pine nuts *from Pinus pinea* L. *Journal of Food Engineering*, 2012, vol. 110, no. 2, pp. 294–297. DOI: 10.1016/j.jfoodeng.2011.04.018.
13. Wang H., Zu G., Yang L., et al. Effects of Heat and Ultraviolet Radiation on the Oxidative Stability of Pine Nut Oil Supplemented with Carnosic Acid. *Journal of Agricultural and Food Chemistry*, 2011, vol. 59, no. 24, pp. 13018–13025. DOI: 10.1021/jf203454v.
14. Lee B.M., Choi J.H., Hong S.I., et al. Enrichment of pinolenic acid from pine nut oil via lipase-catalyzed ethanolysis with an immobilized Candida antarctica lipase. *Biocatalysis and Biotransformation*, 2011, vol. 29, no. 4, pp. 155–160. DOI: 10.3109/10242422.2011.590983.
15. Lantto T.A., Dorman H.J.D., Shikov A.N., et al. Chemical composition, antioxidative activity and cell viability effects of a Siberian pine (*Pinus Sibirica Du Tour*) extract. *Food Chemistry*, 2009, vol. 112, no. 4, pp. 936–943. DOI: 10.1016/j.foodchem.2008.07.008.
16. Kolesnikova T.G., Subbotina M.A., and Shubenkina N.S. Study on the chemical composition of the protein-lipid product of pine nuts. *Food Processing: Technique and technology*, 2013, vol. 2, no. 29, pp. 22–26. (In Russian).
17. Konstantinova O.V., Rafal'son A.B., and Krishtofovich S.N. Chemical composition of cedar nut kerne and products of its processing. *Bulletin of the All-Russian Scientific Research Institute of Fats*, 2011, no. 1, pp. 16–17. (In Russian).
18. Dagvalday I.V. Issledovanie biokhimicheskogo sostava kedrovykh orekhov, proizrastayushchikh v taezhnykh zonakh respubliki Tyva [Study of the biochemical composition of pine nuts growing in the taiga zones of the Republic of Tyva]. *Materialy XIX mezhdunarodnoy konferentsii «Ekologiya Yuzhnoy Sibiri i sopredel'nykh territoriy»* [Proc. of the Intern. Sci. Conf. «Ecology of Southern Siberia and adjacent territories»]. Abakan, 2015, pp. 14–15.
19. Deyneka V.I. and Deyneka L.A. Cedar oil (*Pinus Sibirica seed oil*): HPLC analysis and falsification. *Chemistry of plant raw material*, 2006, no. 3, pp. 21–26. (In Russian).
20. Xie K.Y., Miles E.A., and Calder P.C. A review of the potential health benefits of pine nut oil and its characteristic fatty acid pinolenic acid. *Journal of Functional Foods*, 2016, vol. 23, pp. 464–473. DOI: 10.1016/j.jff.2016.03.003.
21. Asset G., Bauge E., Wolff R.L., Fruchart J.-C., and Dallongeville J. Comparison of maritime pine oil and fish oil effects on plasma lipoproteins in apolipoprotein E-deficient mice. *Prostaglandins Leukotrienes and Essential Fatty Acide*, 2000, vol. 62, no. 5, pp. 307–310. DOI: 10.1054/plef.2000.0159.
22. Wang C., Du M., Jiang L.Z., and Zhu B.W. Preparation and characterization of pine nut peptides processing optimization of the beverage made from the pine nut (*Pinus koraiensis*) peptides. *Agro food industry hi-tech*, 2012, vol. 23, no. 3, pp. 17–20.

23. Motovilov O.K., Avanesyan E.I., Motovilov K.Ya., Mironenkova O.S., and Naumenko I.V *Sposob polucheniya pastoobraznogo kontsentrata iz yadra kedrovogo orekha* [Method for production of paste-like concentrate of cedar nut kernels] Patent RF, no. 2563697, 2013.
24. Motovilov O.K., Mazalevskiy V.B., Nitsievskaya K.N., Inerbaeva A.T., and Mironenkova O.S. *Pishchevoy produkt iz yader kedrovykh orekhov* [Food product from cedar nut kernel]. Patent RF, no. 2595382, 2015.
25. Li G., Zheng H., Chang Yo., et al. Technical study on the supercritical C02 extraction of linolenic acid oil from pine nutlet. *Shipin Kexue (Beijing China)*, 2005, vol. 26, no. 9, pp. 297–299.
26. Samgurova K.S., Vrubel' M.E., Alieva M.U., Huseynov A.K., and Strugovshchik Yu.S. Effect of Siberian cedar pine fatty oil with aspirin stomach ulcer. *International Journal of Experimental Education*, 2015, no. 2, pp. 58–59. (In Russian).
27. Lee B.M., Choi J.H., Hong S.I., and Yoon S.W. Enrichment of pinolenic acid from pine nut oil via lipase-catalyzed ethanolysis with an immobilized Candida antarctica lipase. *Biocatalysis and Biotransformation*, 2011, vol. 29, no. 4, pp. 155–160. DOI: 10.3109/10242422.2011.590983.
28. Shiretorova V.G. Mineral composition of Siberian pine seeds and its products. *ESSUTM Bulletin*, 2014, no. 1, pp. 93–96. (In Russian).
29. Hughes G.M., Boyland E.J., Williams N.J., et al. The effect of Korean pine nut oil (*PinnoThin*) on food intake, feeding behaviour and appetite: A double-blind placebo-controlled trial. *Lipids in Health and Disease*, 2008, vol. 7, article number 6. DOI: 10.1186/1476-511X-7-6.
30. Lin S., Liang R., Xue P., et al. Antioxidant activity improvement of identified pine nut peptides by pulsed electric field (PEF) and the mechanism exploration. *LWT – Food Science and Technology*, 2017, vol. 75, pp. 366–372. DOI: 10.1016/j.lwt.2016.09.017.
31. Chen S.-J., Hsu C.-P., Li C.-W., Lu J.-H., and Chuang L.-T. Pinolenic acid inhibits human breast cancer MDA-MB-231 cell metastasis in vitro. *Food Chemistry*, 2011, vol. 126, no. 4, pp.1708–1715. DOI: 10.1016/j.foodchem.2010.12.064.
32. Wu Y.-P., Liang X., Liu X.-Y., et al. *Cedrus deodara* pine needle as a potential source of natural antioxidants: Bioactive constituents and antioxidant activities. *Journal of Functional Foods,* 2015, vol. 14, pp. 605–612. DOI: 10.1016/j.jff.2015.02.023.
33. Zeng W.C., Zhang Z., and Jia L.R. Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*). *Carbohydrate Polymers*, 2014, vol. 108, no. 1, pp. 58–64. DOI: 10.1016/j.carbpol.2014.03.022.
34. Zeng W.C., He Q., Sun Q., Zhong K., and Gao H. Antibacterial activity of water-soluble extract from pine needles of *Cedrus deodara*. *International Journal of Food Microbiology*, 2012, vol. 153, no. 1, pp. 78–84.
35. Bauer A.W., Kirby W.M., Sherris J.C., and Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal Clinical Pathology*, 1996, vol. 45, no. 4, pp. 493–496.
36. Kimura H., Sashihara T., Matsusaki H., Sonomoto K., and Ishizaki A. Novel bacteriocin of Pediococcus sp. ISK-1 isolated from well-aged bed of fermented rice bran. *Annals of New York Academy of Sciences*, 1998, vol. 864, pp. 345–348. DOI: 10.1111/j.1749-6632.1998.tb10336.x.
37. Shillinger U. and Lücke F.K. Antibacterial activity of Lactobacillus sake isolated from meat. *Applied and Microbiology,* 1989, vol. 55, no. 8, pp. 1901–1906.
38. Fuller R. Probiotics in human medicine. *Gut*, 1991, vol. 32, no. 4, pp. 439–442. DOI: 10.1136/gut.32.4.439.
39. Sukhikh S.A., Noskova S.Yu., Milent'eva I.S., Bulgakova O.M., Chaplygina T.V., and Poplavskaya M.S. Kedrovye orekhi i produkty ikh pererabotki kak ob"yekty issledovaniy i vizualizatsii [Cedar nuts and their derivative products as research and visualization objects]. *Materialy mezhdunarodnoy konferentsii «Vizual'naya analitika: sbornik dokladov»* [Proc. of the Intern. Conf. «Visual analytics: a collection of reports»]. Kemerovo, 2017, pp. 126–131.
40. Babich O.O., Milent'eva I.S., Ivanova S.A., et al. The potential of pine nut as a component of sport nutrition. *Foods and Raw Materials*, 2017, vol. 5, no. 2, pp. 170–177. DOI: 10.21179/2308-4057-2017-2-170-177.

ORCID IDs
Aleksandr Yu. Prosekov https://orcid.org/0000-0002-5630-3196
Lyubov' S. Dyshlyuk https://orcid.org/0000-0002-7333-8411
Irina S. Milent'eva https://orcid.org/0000-0002-3536-562X
Valery A. Pavsky http://orcid.org/0000-0001-9460-7126
Svetlana A. Ivanova http://orcid.org/0000-0002-1252-9572

Please cite this article in press as: Prosekov A.Yu., Dyshlyuk L.S., Milent'eva I.S., Pavsky V.A., Ivanova S.I., and Garmashov S.Yu. Study of the Biofunctional Properties of Cedar Pine Oil with the Use of *in Vitro* Testing Cultures. *Foods and Raw Materials*, 2018, vol. 6, no. 1, pp. 136–143. DOI: 10.21603/2308-4057-2018-1-136-143.