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Geroprotective activity of trans-cinnamic acid isolated from the Baikal skullcap (*Scutellaria baicalensis*)

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Abstract.
Trans-cinnamic acid is a phenolic compound with a wide range of bioactive properties, including antioxidant and antibacterial effects. It also has high potential in the food and cosmetic industries. We aimed to isolate trans-cinnamic acid from the Baikal skullcap (*Scutellaria baicalensis*) and study its geroprotective activity on *Caenorhabditis elegans* nematodes used as a model organism.
Our study objects included the *S. baicalensis* root culture and its extract, trans-cinnamic acid isolated from the extract, and *C. elegans* nematodes. Trans-cinnamic acid was isolated by high-performance liquid chromatography. The acid's geroprotective activity was studied by evaluating its effect at concentrations of 10, 50, 100, and 200 µmol/L on the lifespan, stress resistance, and reproductivity of *C. elegans*. For the lifespan study, the nematodes were cultivated at 20°C for 61 days. To assess their resistance to oxidative stress, 15 µL of 1M paraquat was added to each well of the plate. Thermal stress resistance was determined by raising the temperature to 33°C. For the reproductivity study, the nematodes were cultivated in the S-medium with the addition of *Escherichia coli* OP50 and trans-cinnamic acid at required concentrations for 72 h.
The maximum increase in lifespan (9.8%) was observed in the nematodes treated with 50 µmol/L of trans-cinnamic acid. Under oxidative stress, all the concentrations of trans-cinnamic acid increased the survival of nematodes, while under thermal stress, trans-cinnamic acid reduced the percentage of surviving nematodes. At a concentration of 100 µmol/L, trans-cinnamic acid increased the nematodes' reproduction by 1.48 times.
Based on our data, trans-cinnamic acid isolated from *S. baicalensis* can be recommended as a bioactive compound with geroprotective activity. However, further research is needed on other model organisms with detailed toxicity studies.

Keywords. Trans-cinnamic acid, root culture, *Scutellaria baicalensis*, *Caenorhabditis elegans*, geroprotector, life expectancy, stress resistance, reproduction

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Геропротекторная активность транс-коричной кислоты, выделенной из шлемника байкальского (*Scutellaria baicalensis*)

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Аннотация.
Транс-коричная кислота является фенольным соединением с широким спектром биологической активности, включая антиоксидантную и антибактериальную способность. Обладает высоким потенциалом для применения в пищевой и косметической промышленности. Целью работы являлось выделение транс-коричной кислоты из шлемника байкальского (*Scutellaria baicalensis*) и исследование ее геропротекторной направленности на модели нематоды *Caenorhabditis elegans*. Объектами исследования являлись корневая культура *S. baicalensis* и ее экстракт, транс-коричная кислота, полученная из экстракта корневой культуры *S. baicalensis*, и штамм *C. elegans*. Выделение транс-коричной кислоты проводили методом высокоэффективной жидкостной хроматографии. Исследование геропротекторной активности транс-коричной кислоты осуществляли путем оценки влияния на продолжительность жизни, стрессоустойчивость и репродуктивные способности нематод *C. elegans* при концентрациях 10, 50, 100 и 200 мкмоль/л. Продолжительность жизни нематоды изучали в процессе культивирования при 20 °C в течение 61 дня. Для оценки стрессоустойчивости при окислительном стрессе в каждую ячейку планшета добавляли 15 мкл 1М параквата, при температурном стрессе – повышали температуру до 33 °C. Репродуктивные способности оценивали с использованием нематод, которые культивировали в S-среде с добавлением *Escherichia coli* OP50 и транс-коричной кислоты в необходимой концентрации в течение 72 ч.
Максимальная продолжительность жизни наблюдалась у нематод, обработанных 50 мкмоль/л транс-коричной кислоты (9,8 %). При окислительном стрессе транс-коричная кислота приводила к увеличению выживаемости во всем диапазоне испытуемых концентраций, а под действием температуры снижала процент выживших нематод. При концентрации транс-коричной кислоты 100 мкмоль/л омечено увеличение репродуктивности в 1,48 раз.
Основываясь на полученных данных, транс-коричную кислоту из *S. baicalensis* можно рекомендовать как биологически активное соединение с геропротекторной активностью. Однако необходимы дополнительные исследования на других модельных организмах с подробными исследованиями токсичности.

Ключевые слова. Транс-коричная кислота, корневая культура, *Scutellaria baicalensis*, *Caenorhabditis elegans*, геропротектор, продолжительность жизни, стрессоустойчивость, репродуктивность

Финансирование. Работа выполнена в рамках государственного задания по теме «Скрининг биологически активных веществ растительного происхождения, обладающих геропротекторными свойствами, и разработка технологии получения нутрицевтиков, замедляющих старение» (проект FZSR-2020-0006). Работа выполнена с использованием оборудования ЦКП «Инструментальные методы анализа в области прикладной биотехнологии» на базе Кемеровского государственного университета (КемГУ).

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Introduction

Life expectancy in the first-world countries has significantly increased due to proper hygiene and nutrition. According to the World Health Organization, there are 125 million people aged 80+ in the world [1]. This explains a growing need for developing drugs to prevent age-related diseases.

Old age is a major risk factor for common chronic, neurodegenerative, and oncological diseases such as cancer, cardiovascular diseases, multiple sclerosis, Parkinson's disease, etc. [2]. Aging is often accompanied by genomic damage, mitochondrial dysfunction, telomere shortening, epigenetic changes, proteostasis dysregulation, disruption of intercellular communication, and other processes. Cellular aging is defined as a response to stress that gives cells an irreversible proliferative capacity, thus causing the body to age [3].

Creating anti-aging drugs is a long process with many variables and it is difficult to assess their effect in clinical trials [4, 5]. Metformin is one of the drugs with an anti-aging effect that is used to treat diabetes mellitus [6]. However, we should distinguish between drugs that are aimed at reversing the aging process and geroprotective drugs that can prevent premature aging and increase life expectancy [2, 7, 8].

Polyphenols, whose molecules contain one or more phenolic hydroxyl groups, exhibit antioxidant, antitumorous, cardioprotective, anticancerous, and antimicrobial properties [9]. There is growing evidence that phenolic acids, especially hydroxycinnamic acids, have an effect on the regulation of lipid metabolism. For example, caffeic, ferulic, and coumaric acids significantly reduce hepatic lipids in rats with high cholesterol [10].

Trans-cinnamic acid (3-phenylpropenoic acid) is the main phenolic compound in plants [11]. Many studies have reported its geroprotective activity due to antibacterial, antidiabetic, anticancerous, and antiaging properties [12, 13].

Many medicinal plants growing in the Siberian Federal Okrug are sources of geroprotective compounds [14]. For example, the Baikal skullcap (*Scutellaria baicalensis*) contains flavonoids (quercetin, rutin, catechin, luteonin, etc.), phenolic acids (caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, and cinnamic), vitamins, carotenoids, and terpenes [15–19]. In order to preserve the diversity of its species, trans-cinnamic acid should be isolated from this plant's cell cultures *in vitro* [20]. Previous studies have found a significant amount of trans-cinnamic acid in the *in vitro* root culture extract of *S. baicalensis* [21].

A number of model organisms are used to study the effect of bioactive substances of plant origin on the aging process. They include nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), yeasts (*Saccharomyces cerevisiae*), short-lived fish (*Nothobranchius furzeri*), and rodents (mice and rats) [1].

In this study, we used nematodes *C. elegans* as a preclinical experimental model mainly due to their short lifespan under normal growth conditions [22]. This feature of nematodes allows scientists to study processes that affect aging and life expectancy. In addition, using *C. elegans* is cost-effective since they feed on inexpensive microorganisms such as *Escherichia coli* bacteria. Also, growing worms can be fully automated using a flow cytometry apparatus, where they are distributed in analytical plates, as well as robots that place the experimental samples in the wells [23]. Nematodes have a transparent body and do not need to be stained at all growth stages, so their internal organs are easily visible under a microscope. At the subcellular and tissue levels, fluorescent label reporters are used to study the distribution of expressing genes and their protein products. Powerful phase-contrast microscopes enable scientists to observe the division and death of individual worm cells [24, 25].

C. elegans has a lifespan of only three weeks, which makes it a convenient model to use. With sufficient food, optimal temperatures, and population control, nematodes can reach the adult growth stage in three days. Their embryogenesis is faster compared to other model organisms. At 20–25°C, the development of each cell can be traced in just 10–12 h. After embryonic development, nematode larvae go through several stages (L1–L4) before becoming adults [26]. The studies into the lifespan of nematodes are usually carried out in Petri dishes using liquid and solid nutrient media [27]. Thus, *C. elegans* can be used as model organisms to study the geroprotective properties of various bioactive substances.

In this study, we aimed to isolate trans-cinnamic acid from the Baikal skullcap (*S. baicalensis*) and study its geroprotective activity in the *C. elegans* nematode.

Study objects and methods

Our study objects were:

– *in vitro* root culture of the Baikal skullcap (*Scutellaria baicalensis*);
– *in vitro* root culture extract of the Baikal skullcap (*S. baicalensis*);
– trans-cinnamic acid obtained from the *in vitro* root culture extract of the Baikal skullcap (*S. baicalensis*); and
– soil nematodes *Caenorhabditis elegans* (strain N2 Bristol).

Germinated sterile seeds of *S. baicalensis* were used to obtain the root culture (Botanical Garden of the Immanuel Kant Baltic Federal University, Kaliningrad). The seeds were sterilized in several stages: they were washed with detergent, placed in 95% ethanol for 30 s, and transferred to a 6% NaOCl solution for 30 min. After sterilization, the seeds were rinsed with sterile distilled water and then washed three times with it for 20 min. The seedlings grew for 14–28 days on a nutrient medium containing 50.00 mg of B_5 macrosalts,

10.00 mg of B_5 microsalts, 5.00 mL of Fe-EDTA, 10.00 mg of thiamine, 1.00 mg of pyridoxine, 1.00 mg of nicotinic acid, 30.00 g of sucrose, 100.00 mg of inositol, 0.05 mg of 6-benzylaminopurine, 1.00 mg of indoleacetic acid, and 20.00 g of agar. The seedlings were transformed with the soil agrobacteria *Agrobacterium rhizogenes* 15834 Swiss (Moscow, Russia). The *in vitro* root cultures of *S. baicalensis* were cultivated for 5 weeks. They were first grown in 100 mL flasks (40 mL of medium) and then transplanted into 300 mL flasks (100 mL of medium). The initial weight of the root culture ranged from 0.5 to 1.0 g [21].

The extract of the *S. baicalensis* root culture was obtained by water-alcohol extraction. For this, the dried and crushed plant roots were treated with 30.0 ± 0.2% ethyl alcohol (1:86) at 70.0 ± 0.1°C for 6.0 ± 0.1 h. The extraction was performed in an EKROS PE-4310 water bath (Ekroskhim, Russia) with a reflux condenser [28].

Then, trans-cinnamic acid was isolated from the obtained water-alcohol extract of the *S. baicalensis* root culture by high-performance liquid chromatography (HPLC) on a liquid chromatograph (Shimadzu LC-20 Prominence, Japan). The process of isolation and purification consisted of several stages, namely:

1. The extract of the *S. baicalensis* root culture was evaporated under vacuum at 50°C max;
2. Diethyl ether was added to the evaporated residue in three repetitions;
3. The ether fraction obtained was chromatographed on PF silica gel in an n-hexane-acetone gradient (1:0 → 0:1) to isolate hydroxycinnamic acids; and
4. Trans-cinnamic acid was isolated by subsequent rechromatography on PF silica gel in *n*-hexane-chloroform (1:0 → 0:1).

The trans-cinnamic acid isolated from the *S. baicalensis* root culture extract was at least 95% pure.

Infra-red (IR) spectroscopy was performed to analyze the chemical composition of trans-cinnamic acid on an FSM-1202 apparatus (Infraspek, St. Petersburg, Russia). IR spectra were recorded in potassium bromide disks (Fluka, Germany) in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and the number of scans being 30. Air was used as a reference sample and it was recorded before the analysis of each sample. The Fspec (4.0.0.2) and Aspec (1.1) software was used to control the apparatus and process spectral data.

Next, we assessed the effect of trans-cinnamic acid on the lifespan, stress (oxidative and thermal) resistance, and reproductive abilities of *C. elegans* nematodes. The N2 Bristol strain was provided by the Laboratory for the Development of Innovative Medicines and Agrobiotechnologies (Moscow Institute of Physics and Technology, Dolgoprudny, Russia). The nematodes were fed on *Escherichia coli* OP50 provided by the V.A. Engelhardt Institute of Molecular Biology (Moscow, Russia). We used a total of 100 nematodes for all the stages of the study. The control nematodes were not treated with solutions of trans-cinnamic acid. However, they were used in the lifespan and reproductivity tests and were subjected to oxidative and thermal stress.

To assess the effect of trans-cinnamic acid on nematodes, we used a stock solution of this acid in 10 mmol/L of dimethyl sulfoxide. Then, the acid was titrated by diluting stock solutions in sterile distilled water to concentrations of 2000, 1000, 500, and 100 μM. Each well with nematodes was filled with 15 μL of freshly prepared stock solutions, thus obtaining working concentrations of trans-cinnamic acid of 2000, 1000, 500, and 100 μmol/L, respectively. The stocks were stored at 4°C.

At the first stage, nematodes were cultivated on solid agar. For this, a daily culture of *E. coli* OP50 was obtained by inoculating one bacterial colony, which was previously grown on L-broth (15 g bacteriological agar, 10 g tryptone, 5 g yeast extract, 5 g NaCl, 1000 mL distilled water), in 5–10 mL of L-broth (10 g tryptone, 5 g yeast extract, 10 g NaCl, 800 mL distilled water). The bacteria were cultivated at 37°C for 24 h with intensive stirring. After the incubation of *E. coli* OP50, 50 μL of the overnight culture was inoculated into Petri dishes with an NGM medium (3 g NaCl, 17 g bacteriological agar, 2.5 g peptone, 975 mL distilled water) and incubated at 37°C for 24 h. Then, an NGM medium was prepared for cultivating nematodes. For this, the autoclaved NGM agar medium was cooled to 55°C in an EKROS PE-4310 water bath (Ekroskhim, Russia) for 15 min. Then, 1 mL of 1M $CaCl_2$, 1 mL of 5 mg/mL cholesterol in alcohol, 1 mL of 1M $MgSO_4$, and 25 mL of 1M KPO_4 buffer were added to the cooled agar. The nematodes were transferred to new NGM agar dishes in two ways: by a loop and by a piece of agar. The first method involved hooking a nematode with a calcined and cooled bacteriological loop and planting it on a bacterial lawn in the center of a new Petri dish with NGM agar. In the second method, a 5×0.5 cm piece of agar containing a nematode was cut out with a sterile scalpel from an NGM dish and transferred to the center of the new dish surface down. The dishes were incubated at 20°C.

At the second stage, the nematodes were synchronized. For this, 5–10 mL of sterile water was added to the Petri dish with a nematode and pipetted until its eggs were completely attached to the agar. The liquid from the dish was placed in a 50 mL centrifuge tube and centrifuged for 2 min (1200 rpm). Then, the supernatant was removed and the precipitate was washed with 10 mL of distilled water and centrifuged as described above. After repeated centrifugation, the supernatant was removed, and 5 mL of a freshly prepared mixture of 1 mL of 10 N NaOH, 2.5 mL of household bleach, and 6.5 mL of H_2O was added to the precipitate. The mixture was thoroughly mixed on a vortex (Biosan, Latvia) for 10 min with a break every 2 min to observe the hydrolysis of nematodes under an Axio Observer Z1

microscope (Karl Zeiss, Germany). After that, 5 mL of M9 medium was added to neutralize the reaction. The resulting mixture was centrifuged for 2 min (2500 rpm). The supernatant was removed, and 10 mL of sterile water was added to the precipitate, with washing and centrifugation repeated 3 times. In the fourth repetition, the precipitate was washed with 10 mL of S-medium and the test tube with nematode eggs was placed on a slow shaker for a day at room temperature so that the nematodes could enter the L1 stage.

When the nematodes reached the L1 stage, an overnight bacterial culture of *E. coli* OP50 was added to the S-medium. The culture had been previously washed from the L-broth and resuspended in the S-medium to a concentration of 0.5 mg/mL. Then, 120 µL of the suspension containing bacteria and nematodes was added to each well of a 96-well plate (TPP, Switzerland). The plate was sealed with a film and left for 48 h at 20°C. After that, 15 µL of 1.2 mM 5-fluoro-2-deoxyuredin (FUDR) was poured into each well and left for a day at 20°C to prevent the nematodes from reproduction. At the end of incubation, the worms entered the L4 stage. Then, 15 µL of a solution with trans-cinnamic acid in different concentrations was added to the wells and the plates were cultivated at 20°C on day 5.

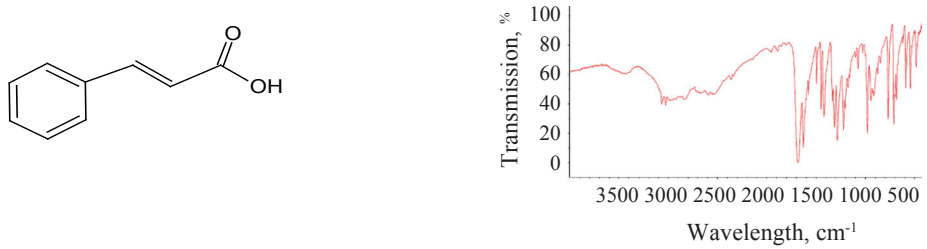
Next, we analyzed the effects of trans-cinnamic acid on the lifespan of *C. elegans* nematodes, their resistance to oxidative and thermal stress, as well as reproductive ability.

To assess the effect of trans-cinnamic acid on the lifespan of *C. elegans*, we used the acid at concentrations of 0 (control), 10, 50, 100, 200 µmol/L. The experiment was carried out in 96-well plates in the liquid S-medium for the cultivation of nematodes in 6 repetitions. The numbers of live and dead nematodes were counted every 4–7 days during 61 days of the experiment. The experiment was considered completed when all the control nematodes died.

To determine the resistance of *C. elegans* to oxidative stress, we added 15 µL of 1M paraquat to each well and continued incubation in the thermostat at 20°C. The numbers of live and dead nematodes were counted twice: after 24 and 48 h of incubation.

The resistance of *C. elegans* to thermal stress was studied by increasing the temperature to 33°C. Live and dead nematodes were counted after 24 and 48 h of incubation.

The effect of trans-cinnamic acid on the reproductive ability of *C. elegans* was analyzed as follows. The synchronized nematodes at stage L1 in the S-medium with *E. coli* OP50 were placed in 48-well 270 µL plates and 30 µL of trans-cinnamic acid at the required concentration was immediately added to them. Thus, L1 larvae developed to the sexually mature stage of L4 in the presence of trans-cinnamic acid throughout the experiment for 72 h. Trans-cinnamic acid at each concentration was added in triplicate. When the nematodes reached stage L4, each well was filled with 300 µL of a lysis solution prepared in a 2-fold concentration and containing 2 mL of 10 M NaOH and 5.0 mL of bleach in 3.0 mL ddH_2O. The plate was covered with an adhesive film, placed in a MaxMate plate shaker (USA), and vortexed for 5 min at 1800 rpm. Then, the wells were filled with repeat nematodes for each test condition of each sample. The lysed nematodes were transferred into a 2 mL Eppendorf-type tube and centrifuged for 2 min at 1100 g at room temperature in an Eppendorf 5424 centrifuge (Eppendorf, USA). The supernatants were collected and transferred into a new Eppendorf tube, with 1 mL of M9 buffer added to the egg precipitate. The mixture was vigorously stirred and centrifuged for 2 min at 1100 g at room temperature. Then the procedure was repeated. Namely, the M9 washing medium was transferred into a new Eppendorf tube, and 1 mL of distilled water was added to the egg precipitate. The mixture was intensively stirred and used later to count the number of eggs formed in the L4 stage nematodes under the action of trans-cinnamic acid. For the count, 100 µL was taken from 1 mL of the



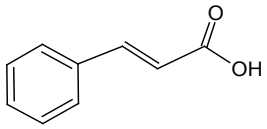
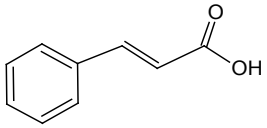
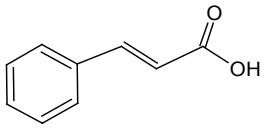
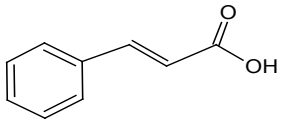
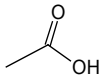
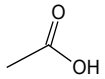
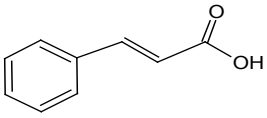
a b

Figure 1. Trans-cinnamic acid obtained from the water-alcohol extract of the *Scutellaria baicalensis* root culture *in vitro*: a) structure; b) IR-spectrum

Рисунок 1. Транс-коричная кислота, полученная из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*: а) структура; b) ИК-спектр

Table 1. Vibrational frequencies and their correlation with the structural fragments of trans-cinnamic acid obtained from the water-alcohol extract of the *Scutellaria baicalensis* root culture *in vitro*

Таблица 1. Характеристичные колебательные частоты и их соотнесение с основными структурными фрагментами образца транс-коричной кислоты, полученной из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*

Reference	Wavelength, cm^{-1}	Type of vibrations/bonds of structural fragments
	3064	Stretching vibrations of the diene fragment = C-H
	3026	Stretching vibrations of the C-H bond
	1680	C=C of the diene fragment
	1631	Stretching vibrations C=O of the carboxyl group
	1576, 1451, 1420, 1176	Planar stretching vibrations of the C-C bonds of aromatic fragments
	1332, 1313, 1221	Planar deformation vibrations of the O-H bond
	1285	Stretching vibrations of the C-O bond
	979	Planar deformation vibrations of the ring hydroxyl groups
	766	Out-of-plane deformation vibration of the C-C bond

aqueous suspension of eggs previously obtained after the nematode lysis stage and transferred to a 96-well plate. The samples were placed in duplicate for each concentration of trans-cinnamic acid. The count was performed on an Axio Observer Z1 microscope (Karl Zeiss, Germany). If the well contained more than 100 eggs, they were additionally diluted and recounted.

Results and discussion

The results of IR spectroscopy of trans-cinnamic acid obtained from the *in vitro* root culture extract of *Scutellaria baicalensis* are shown in Fig. 1 and Table 1.

As we can see in the IR spectrum of trans-cinnamic acid (phenylpropenoic acid) isolated from *S. baicalensis*, the 3064 cm^{-1} band is due to the stretching vibrations of the acid's diene fragment =C-H, while the 3026 cm^{-1} band is determined by the C-H stretching vibrations in the benzene ring. These bands can be considered as characteristic for trans-cinnamic acid.

In this case, the 1680 cm^{-1} band can also be considered as the C=C of the diene fragment. The 1631 cm^{-1} band is due to the stretching vibrations of the carboxyl fragment. The 1576, 1451, 1420, and 1176 cm^{-1} bands correlate with the stretching vibrations of the aromatic fragment's C-H bonds. The absorption band at 1451 cm^{-1} is due to the deformation vibrations of the carboxyl fragment's C-O-H. The bands at 1332, 1313, and 1221 cm^{-1} result from the O-H deformation vibrations and C-O stretching vibrations, including those of the carboxyl fragment. The 1285 cm^{-1} band is associated with the stretching vibrations of the C-O bond.

The band at 979 cm^{-1} correlates with the diene fragment in the trans-form. The monosubstituted ring

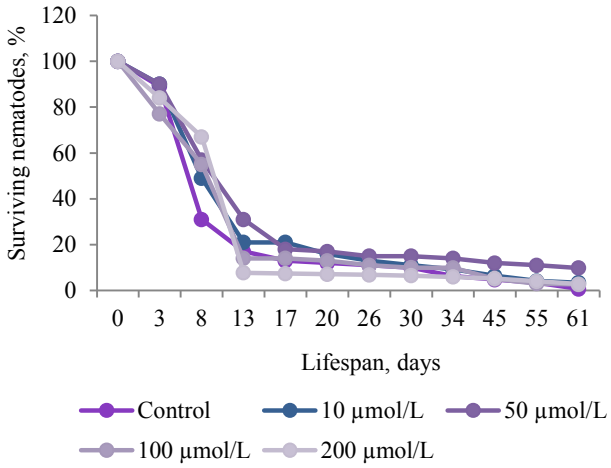


Figure 2. The effect of trans-cinnamic acid isolated from the water-alcohol extract of the *Scutellaria baicalensis in vitro* root culture on the lifespan of *Caenorhabditis elegans* nematodes

Рисунок 2. Влияние транс-коричной кислоты, выделенной из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*, на продолжительность жизни нематод *Caenorhabditis elegans*

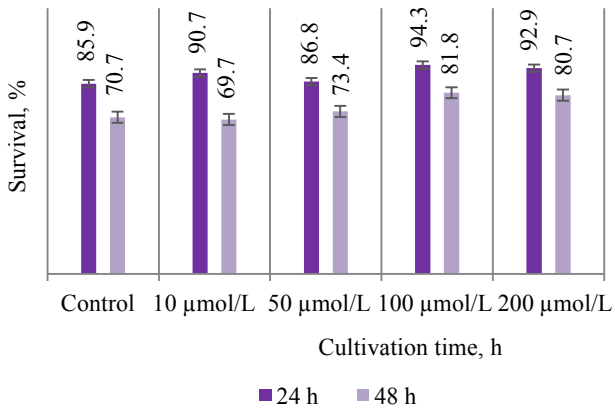


Figure 3. The effect of trans-cinnamic acid isolated from the water-alcohol extract of the *Scutellaria baicalensis in vitro* root culture on the resistance of nematodes to oxidative stress

Рисунок 3. Влияние транс-коричной кислоты, выделенной из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*, на устойчивость нематод при окислительном стрессе

is characterized by an out-of-planedeformation vibration of the C-C bond at 766 and 711 cm^{-1}.

Thus, the spectral activity corresponded to the structural features of trans-cinnamic acid.

Figure 2 shows the effect of trans-cinnamic acid (0 (control),10, 50, 100, and 200 µmol/L) isolated from the water-alcohol extract of the *S. baicalensis in vitro* root culture on the lifespan of *Caenorhabditis elegans* nematodes.

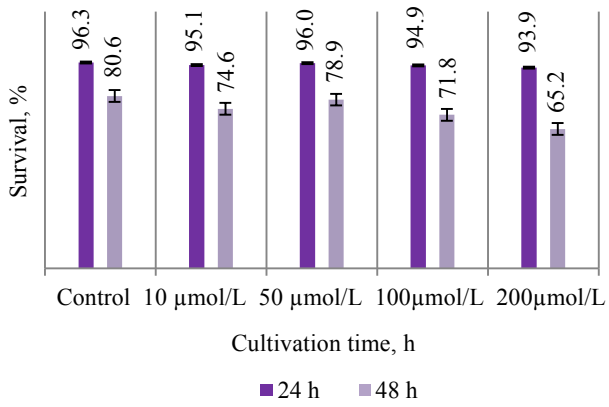


Figure 4. The effect of trans-cinnamic acid isolated from the water-alcohol extract of the *Scutellaria baicalensis in vitro* root culture on the resistance of nematodes to thermal stress

Рисунок 4. Влияние транс-коричной кислоты, выделенной из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*, на устойчивость нематод к температурному стрессу

As we can see in Fig. 2, on day 8 of the experiment, trans-cinnamic acid at all the concentrations under study (10, 50, 100, and 200 µmol/L) increased the lifespan of worms (by 18.1, 26.3, 24.1, and 36.6%, respectively). From day 13 to 34, 200 µmol/L of trans-cinnamic acid did not have a positive effect on the lifespan of nematodes, unlike the other concentrations. From day 34 to 61, all the concentrations increased the percentage of surviving nematodes. The highest increase in lifespan (9.8%) was observed in the nematodes treated with 50 µmol/L of trans-cinnamic acid.

Figures 3 and 4 show the effect of trans-cinnamic acid isolated from the water-alcohol extract of the *S. baicalensis in vitro* root culture on stress resistance.

As can be seen in Fig. 3, all the concentrations of trans-cinnamic acid (10–200 µmol/L) had a positive effect on the resistance of nematodes to oxidative stress during 24 and 48 h, i.e. increased their survival, compared to the control. We also found a gradual decrease in the percentage of surviving nematodes during 24-h oxidative stress with increased concentrations of trans-cinnamic acid.

After 5 days of nematode incubation in the presence of trans-cinnamic acid, the experimental plate was transferred to a 33°C incubator. Dead worms were counted after 24 h of nematode incubation at elevated temperature and after 48 h of incubation under prolonged thermal stress.

According to Fig. 4, trans-cinnamic acid significantly reduced the percentage of surviving nematodes under

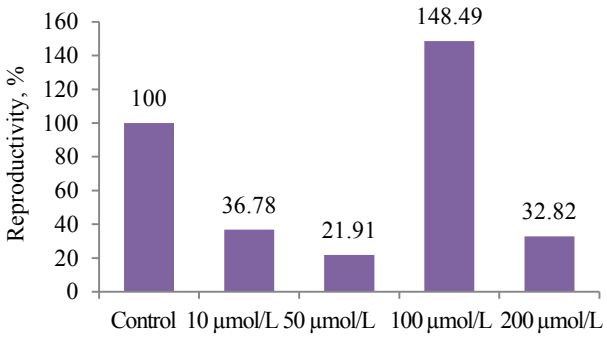


Figure 5. The effect of trans-cinnamic acid isolated from the water-alcohol extract of the *Scutellaria baicalensis in vitro* root culture on the reproductive abilities of nematodes

Рисунок 5. Влияние транс-коричной кислоты, выделенной из водно-спиртового экстракта корневой культуры *in vitro Scutellaria baicalensis*, на репродуктивные способности нематод

thermal stress with increased concentrations from 10 to 200 µmol/L.

Figure 5 shows the effect of trans-cinnamic acid isolated from the water-alcohol extract of the *S. baicalensis in vitro* root culture on the reproductive abilities of *C. elegans* nematodes.

As we can see in Fig. 5, trans-cinnamic acid at the concentrations of 10, 50, and 200 µmol/L did not significantly affect the reproductive performance of nematodes, compared to the control. The concentration of 100 µmol/L was the most effective since it produced 1.48 times more eggs, compared to the control.

Conclusion

Modern medical gerontology is looking for ways to increase life expectancy with a focus on geroprotectors – special compounds that reduce the rate of aging. Plants are the main source of geroprotectors, including *Scutellaria baicalensis*. Since this is a rare plant included in the Russian Red Data Book, we used its root culture as a source of trans-cinnamic acid.

In particular, we isolated trans-cinnamic acid from the water-alcohol extract of the *S. baicalensis in vitro* root culture b HPLC. The isolated bioactive compound was at least 95% pure. According to IR spectroscopy, the spectral activity corresponded to the structural features of trans-cinnamic acid.

To study the geroprotective activity of trans-cinnamic acid, we evaluated its effect in various concentrations on the lifespan, oxidative and thermal stress resistance, as well as reproductivity of *Caenorhabditis elegans* used as a model organism. As a result, we drew the following conclusions:

– all the studied concentrations of trans-cinnamic acid increased the lifespan of *C. elegans* worms, with the highest increase achieved by 50 µmol/L;
– all the concentrations of trans-cinnamic acid (10–200 µmol/L) had a positive effect on the resistance of nematodes to oxidative stress increasing their survival, compared to the control;
– under thermal stress, increased concentrations of trans-cinnamic acid significantly reduced the percentage of surviving nematodes; and
– trans-cinnamic acid at a concentration of 100 µmol/L increased the reproductive capacity of nematodes, producing 1.48 times more eggs, compared to the control. The remaining concentrations did not have such an effect.

Based on our data, trans-cinnamic acid can be used as a bioactive substance with geroprotective properties. However, further research is needed on other model organisms with detailed toxicity studies to determine the full potential of trans-cinnamic acid as an anti-aging agent capable of slowing down the aging process and extending life.

Contribution

All the authors equally contributed to the study concept, data processing and analysis, as well as writing the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Критерии авторства

Все авторы внесли равный вклад в создание исследования, обработку и анализ полученных результатов, а также в оформление статьи.

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

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