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Original Article

Antimicrobial and antioxidant activity of *Panax ginseng* and *Hedysarum neglectum* root crop extracts

Atividade antimicrobiana e antioxidante de extratos de raízes de *Panax ginseng* e *Hedysarum neglectum*

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Abstract

In order to ensure the timely and uninterrupted supply of medicinal plant raw materials, the methods of cultivation of plant cell cultures, namely, the production of plant root cultures, are relevant. In this paper, the geroprotective potential of Hedysarum neglectum Ledeb and Panax ginseng C. A. Mey root cultures is studied. They were cultured under in vitro conditions by transforming the rhizome (H. neglectum) and seed seedlings (P. ginseng) with Agrobacterium rhizogenes 15834 Swiss. To identify the geroprotective potential, the antimicrobial disc-diffusion method and the antioxidant activity were analyzed by titration of KMnO4 extracts of plant root cultures. The qualitative and quantitative composition was analyzed using high-performance liquid chromatography, thin-layer chromatography, and gas chromatography with mass spectrometry. In the course of the work, the presence of antimicrobial and antioxidant activity of plant root culture extracts was established. Biologically active substances contained in extracts of Hedysarum neglectum Ledeb root crops and Panax ginseng C. A. Mey are characterized by geroprotective potential, so they can act as a source of natural antioxidants in the functional nutrition of the geroprotective orientation.

Keywords: root culture, plant extracts, antimicrobial activity, antioxidant activity.

Resumo

Para garantir o abastecimento em tempo e ininterrupto de matérias-primas de plantas medicinais, são relevantes os métodos de cultivo de culturas de células vegetais, nomeadamente a produção de culturas de raízes vegetais. Neste trabalho, foi estudado o potencial geroprotetor de culturas de raízes de *Hedysarum neglectum* Ledeb e *Panax ginseng* C. A. Mey. Eles foram cultivados em condições in vitro pela transformação do rizoma (*H. neglectum*) e mudas de sementes (*P. ginseng*) com *Agrobacterium rhizogenes* 15834 Swiss. Para identificar o potencial geroprotetor, o método antimicrobiano de difusão em disco e a atividade antioxidante foram analisados por titulação de extratos de KMnO4 de raízes de plantas. A composição qualitativa e quantitativa foi analisada por cromatografia líquida de alta eficiência, cromatografia em camada delgada e cromatografia gasosa com espectrometria de massa. No decorrer do trabalho, foi constatada a presença de atividade antimicrobiana e antioxidante dos extratos de raízes de plantas. Substâncias biologicamente ativas contidas em extratos de raízes de *H. neglectum* Ledeb e *P. ginseng* C. A. Mey são caracterizadas pelo potencial geroprotetor, podendo atuar como fonte de antioxidantes naturais na nutrição funcional da orientação geroprotetora.

Palavras-chave: cultura de raízes, extratos vegetais, atividade antimicrobiana, atividade antioxidante.

1. Introduction

Aging is a natural physiological process that occurs throughout life, accompanied by a gradual decrease in the efficiency of the cellular apparatus (Chattopadhyay and Thirumurugan, 2018). Progressive aging is accompanied by changes in the psychological and physiological state of the human body, which leads to the development of neurodegenerative diseases (Alzheimer's, Parkinson's, etc.), cancer, diabetes, obesity, cardiovascular diseases, etc. (Ribeiro et al., 2013; Schlachetzki et al., 2013; Chattopadhyay and Thirumurugan, 2018; Prasanth et al., 2021). Research in the field of old age and aging is engaged in gerontology – the science that studies the processes of aging and old age, as well as the impact of the onset of old age on the human body and society as a whole (Evans, 1997). Recently, research in the field of gerontology has been actively developing. Special attention is paid to natural methods of combating old age (Morsli and Bellantuono, 2021). Phytochemicals contained in medicinal plants

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can be used as geroprotectors – a group of substances characterized by the ability to slow down the aging process and increase the body's resistance. Compared with synthetic drugs, geroprotectors have a wide range of preventive and therapeutic effects, do not have a toxic effect on the body, do not cause addiction (Morsli and Bellantuono, 2021; Bellantuono and Marengoni, 2021).

Many geroprotectors are characterized by the presence of antioxidant properties, so they are relevant preventive means of early aging, which occurs due to the presence of an excessive amount of free radicals in the body, leading to damage to macromolecules (proteins, nucleic acids, lipids) and disorders in the functioning of the body (Anisimov et al., 2006; Aralbaeva et al., 2017). Antioxidants neutralize the reactive oxygen species, thereby protecting the body from oxidative stress (Pisoschi et al., 2021). Plants are rich sources of antioxidants (Chen et al., 2019). They contain flavonoids, phenolic acids, anthocyanins, catechins, etc. (El-Hawary et al., 2020; Farag et al., 2020; Rammohan et al., 2020). In recent years, more and more attention has been paid to obtaining geroprotectors from plant sources (Argyropoulou et al., 2013; Aiello et al., 2016; Lim et al., 2017). A promising raw material is Panax ginseng C. A. Mey. and Hedysarum neglectum Ledeb. (Aiello et al., 2016; Kim et al., 2019). Panax ginseng is a slow-growing perennial plant of the genus Panax, which is often used as a component for functional products, and as a phytotherapeutic agent for the prevention and treatment of diseases (cancer, allergies, inflammatory diseases, diabetes mellitus) (Kim et al., 2019). According to scientists, P. ginseng is used as an adaptogenic agent to improve physical performance, vitality, immunity, resistance to stress, and aging (Kim, 2016). The main biologically active components of P. ginseng include:

1. Polysaccharides were isolated from the root of P. ginseng immunomodulating glycans-ginseng PA and ginseng PB, consisting of L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid, and D-glucuronic acid (Konno et al., 1984; Oshima et al., 1985).
2. Saponins, especially ginsenosides, classified as oligoglycosides of the dammaran, ocotillol, and oleanan types, are divided into protopanaxadiol and protopanaxatriol.
3. Flavonoids (kaempferol, trifolin and panasenoside).
4. Polyacetylenes (panaxinol and panaxidol).

Ginsenosides (Re, Rg1, Rd, S-Rg2, Rb2, F1, Rc, S-Rh1, Rb3, R-Rg2) are the main active ingredients of P. ginseng; they belong to the class of natural products of steroid glycosides and triterpene saponins and accumulate in the roots, berries, and leaves of the plant (Nam et al., 2019; Yoon et al., 2019).

Previous studies have shown that ginsenosides exhibit abundant pharmacological effects, such as relieving fatigue, improving immunity, slowing aging, inhibiting cancer cell metastasis, regulating blood glucose levels, and protecting liver and kidney functions (Mancuso and Santangelo, 2017). Ginsenosides have anti-allergic activity in models of bronchial asthma and atopic diseases in mice. In addition, they inhibit the development of the inflammatory process by inhibiting the activation of NF-kB and increasing immunity.

Polyphenols are natural, safe antioxidants that can prevent a number of diseases (cardiovascular, cancer, hypertension, diabetes, atherosclerosis) (Yao et al., 2019). Many natural polysaccharides are highly valuable biomaterials that are characterized by their biocompatibility, low toxicity, and biodegradable properties. Plant-derived polysaccharides have various pharmacological properties, including antioxidant, antitumor, and immunomodulatory properties (Zhao et al., 2019).

P. ginseng extract induces an improvement in the blood lipid profile mainly by reducing the level of total and LDL cholesterol (Hernández-García et al., 2019).

Hedysarum neglectum Ledeb. it is a forage plant (Sazanova et al., 2017). From this plant, xanthone glycoside - mangiferin is isolated, from which the drug "Alpizarin," which has an antiviral effect, was produced. In the work of S. A. Kubentaev, it is indicated that in addition to substances of xanthonic nature (mangiferin and isomangiferin), H. neglectum in its terrestrial part contains: sugars, vitamins, and provitamins, tannins; the underground part of the plant contains such compounds as alkaloids, isoflavonoids, saponins, tannins, etc. (Sathasivam et al., 2019).

Due to the content of such BAS, the plant has anti-inflammatory, antitumor, immunostimulating, and tonic properties.

To ensure a timely and uninterrupted supply of medicinal raw materials, it is necessary to widely introduce modern methods of plant biotechnology in parallel with traditional methods of reproduction. Their use significantly accelerates the process of obtaining environmentally friendly raw materials with increased content of biologically active substances. Such methods include the cultivation of plant cells and organs under in vitro conditions, namely, the production of plant root cultures.

The aim of the work is to study the antimicrobial and antioxidant activity of extracts of root cultures of Panax ginseng C. A. Mey and Hedysarum neglectum Ledeb for the purpose of practical use in the composition of geroprotective drugs.

2. Material and Methods

Root cultures in vitro of Hedysarum neglectum Ledeb were obtained according to the method described in the work of T. Novikova and her colleagues (Novikova et al., 2020). The rhizomes of H. Neglectum were used as the object of transformation. Sterilization of the H. Neglectum explants used was carried out in the following way: the rhizomes were washed in a soap solution from large external contaminants and placed in a 0.1% solution of mercury chloride (Reagent, Russia) for 1 min; after that, the rhizome was thoroughly washed in bidistilled sterile water three times with an exposure of 20-25 min; next, the parts of the rhizome were cut with a scalpel into explants measuring 5×5 mm. Soil Agrobacterium rhizogenes 15834 Swiss (Moscow, Russia) was used to transform the rhizomes. These bacteria were cultured on agarized YEB culture medium (5 g / l of peptone, 1 g / l of yeast extract, 5 g/l of sucrose, 0.5 g / l of MgCl2) (Vervliet et al., 1975). The essence of the technique was that for transformation,

explants were pierced perpendicularly with a sterile needle and placed on a B5 culture medium (Gamborg et al., 1968), containing a suspension of agrobacteria with a density of OD600=0.4. Before the transformation, the agrobacterium suspension culture was cultured on YEB medium for 24 hours in the dark at +23 °C in the ISS-4075 shaker incubator (Jeio Tech, Republic of Korea) with circular rotation (amplitude 5-10 cm, rotation speed 90 rpm). As a control, we used needle-pierced explants placed on a solid medium B5 without applying a suspension of the microorganism.

After 12-48 hours of exposure in bacterial suspension, the explants were washed with sterile water, dried with sterile filter paper, and placed on a solid medium B5 containing 500 mg / l of the antibiotic claforan (Patheon UK, Limited, UK). The antibiotic was used to remove the remains of agrobacteria. After 30 days of cultivation, the frequency of transformation was estimated (the number of transformed explants to their total number). Transformed explants were selected based on phenotypic features (lateral branching, absence of geotropism, ability to grow in an environment without hormones). Subsequently, two passages of the obtained roots were performed on a solid medium B5, which did not contain hormones, but contained a reduced dose of claforan (250 mg/l).

Cultivation took place in the absence of light at a temperature of +23 ° C on a rocking chair with a frequency of 100 rpm, the duration of 5 weeks. Subsequent transplantation to a fresh medium was carried out as soon as contamination with agrobacteria appeared. After 14 days, rhizogenesis was observed on individual explants.

The cultivation cycle for the in vitro root culture was five weeks, and they were first grown in 100 ml flasks (40 ml medium volume), then transplanted into 300 ml flasks with a 100 ml medium volume. The initial weight of the root culture in vitro was from 0.5 to 1.0 g.

To obtain the roots of P. ginseng, we relied on the work of Reis et al. (2019). Seed seedlings were used to obtain root cultures. The seeds were sterilized according to the method described above for H. Neglectum explants. Sterile seedlings were cut into segments of 0.5 cm (0.5–1.0 g) and immersed in the Agrobacterium rhizogenes strain for 15 minutes. After infection, the explants were dried on sterilized filter paper and cultured at +23 °C in the dark on a medium of 1/2 MS (Murashige and Skoog, 1962), containing 500 mg/l of claforan. After two days of co-cultivation, the explants were transferred to a 1/2 MS medium containing 500 mg / l of claforan to destroy Agrobacterium. The cultivation cycle was five weeks, and at first, the roots were grown in 300 ml flasks with a medium volume of 150 ml 1/2 MS with an initial inoculation of 1.0 g of bearded roots.

Ethyl alcohol (Kemerovo Pharmaceutical Factory, Russia) was used as an extractant to extract the root cultures of H. Neglectum and P. ginseng. The biomass of plant root cultures was dried in a drying cabinet SHSVL-80 (KRET, Russia) at a temperature of 58 ± 2 ° C to a residual humidity of 10-15%. The dried root biomass was crushed in the LZM-1M mill (Olis, Russia) to a particle size of no more than 1 mm.

To obtain the BAS extract, the crushed dried material in an amount of 3 g was placed in a round-bottomed flask with a 250 ml volume, after which 130 ml of the extractant was added. Extraction was carried out using a reverse refrigerator for ½ τ (Tables 1 and 2) by heating in a PE-4310 water bath (EKROSHIM, Russia). At the end of the process, the extract was filtered through a paper filter, after which another 130 ml of extractant was added to the remaining raw material, and the process was repeated. The extraction parameters are shown in Tables 1 and 2.

The resulting extracts of H. Neglectum and P. ginseng were analyzed for antioxidant and antimicrobial properties. The antioxidant activity was determined by Moharram and Youssef (2014). Bidistilled water in an amount of 8 ml, 20% sulfuric acid solution in an amount of 1 ml, and 0.05 N KMnO4 solution in an amount of 1 ml was added to the flask with a volume of 50 ml. The resulting solution was thoroughly mixed and then titrated with the studied plant extract of the root culture of H. Neglectum and P. ginseng. The completion of the titration process was characterized by the disappearance of the pink color of the solution. Titration based on quercetin: 1 ml of 0.05 N potassium permanganate solution corresponds to 0.25 mg of quercetin.

The antimicrobial activity of the extracts was determined by the discodiffusion method against 15 pathogenic and conditionally pathogenic strains (Escherichia

Table 1. Parameters of BAS extraction from *H. Neglectum* root cultures.

Extract no.	Temperature,t, ºC	Volume fraction of ethyl alcohol,C, %	Exposure,τ, hour
1	30,0	50,0	2
2	30,0	70,0	4
3	30,0	50,0	6
4	30,0	70,0	6
5	50,0	50,0	4
6	50,0	70,0	4
7	50,0	50,0	6
8	50,0	70,0	6
9	70,0	70,0	4

Table 2. Parameters of BAS extraction from *P. ginseng* root cultures.

Extract no.	Temperature,t, ºC	Volume fraction of ethyl alcohol,C, %	Exposure, τ, ч
1	30,0	50,0	2
2	30,0	60,0	4
3	30,0	70,0	6
4	50,0	30,0	2
5	50,0	50,0	2
6	50,0	70,0	2
7	50,0	70,0	4
8	50,0	70,0	6
9	30,0	50,0	5

coli, Candida albicans, Bacillus cereus, Pseudomonas aeruginosa, Enterococcus faecium, Klebsiella pneumonia, Helicobacter pylori, Streptococcus viridans, Streptococcus bovis, Porphyromonas gingivalis, Acinetobacter baumannii, Borrelia burgdorferi, Propionibacterium acnes, Aggregatibacter actinomycetemcomitans, Streptococcus intermedius (State Collection of Pathogenic Microorganisms, Russia)) (Druzhikin et al., 2020).

The suspension of test strains was prepared from the broth culture according to the turbidity standard. Antimicrobial activity was determined using a standard medium-meat-peptone agar (MPA). After inoculation, disks impregnated with the studied extracts and a disk with an antibiotic as control were applied to the surface of the culture medium. Next, the Petri dishes were placed in a thermostat and incubated at 35-37 °C for 18-24 hours. The results were taken into account by placing the cups upside down on a dark matte surface so that the light fell on them at an angle of 45°. The diameter of the growth delay zones of the test strains was measured with an accuracy of 1 mm.

The extract of the root culture of H. Neglectum was studied on the HPLC chromatograph Color Yauza -04. We used a UV / VID detector with a photodiode array with 255, 280 nm specified wavelengths. Control of the device and processing of the received data was carried out using the MulticHrom software, version 3.1.1550 (CJSC Ampersend, Russia), column: Gemini 5mkm C18 110A, size 250x4.6 mm (Phenomenex, USA), pre-column Analitical Guard (KJO -4282).

Additionally, the extract was analyzed by LC chromatography. The preparative isolation and accumulation of mangiferin were performed in the low-pressure chromatography mode using cross-linked agarose as a sorbent. Water-alcohol extracts were used in work. After evaporation, the extraction data were chromatographed on CL-4 B sepharose gel (Pharmacia, Sweden) under working conditions. Gel volume-10 ml, elution rate-0.1-0.4 ml / min. The eluent was: deionized water and 0.01 M sodium hydroxide solution. Also, it should be mentioned that the chromatography was performed on a BioLogic low-pressure chromatograph (BioRad, USA).

GC-MS was also used for extracts of H. Neglectum root cultures. The TLC-platinum chromatographic zones were cut out and further analyzed. GC-MS was performed on a column with an internal diameter of 0.25 mm and a length of 30 m. Carrier gas: helium. Carrier gas flow rate: 1.4 ml/min. Injector temperature: 2400 ° C, interface temperature 280 ° C. Column temperature: programmed from 100 to 2700 ° C at a speed of 20 0C /min. Sample volume: 3 µl. Method of administration: without dividing the flow of the carrier gas. When using the electronic shock mode at 70 eV, followed by scanning in the range from 50 to 550 m / z mass. After the study, the mass spectra taken from the tops of the chromatographic peaks were compared according to the standard method with the mass spectra of the libraries. The substance was considered identified when its mass spectrum coincided with the library spectrum by more than 95%.

The extract of the root culture of P. ginseng was analyzed on a Shimadzu LC-20 Prominence chromatograph with a Shimadzu SPD-20-MA diode-matrix detector and a RID-10A refractometric detector. We used a chromatographic column Kromasil 5 microns C18, 250×4.6 mm; a pre-column Security Guard Gartridge (C18) Phenomenex (USA), injection volume 20 µl. In addition, TLC was performed. The authenticity and qualitative characteristics of the water-alcohol extracts were analyzed according to the FS FS.2.5.0013.15 (Park et al., 2020). To do this, 20 µl of the test solution and 50 µl of the standard sample solution (CO) of panaxoside Rg1 were applied to the start line of the 10 × 15 cm Sosbfil 254 UV analytical chromatographic plate on an aluminum substrate. The application was carried out using a mechanical USP 1M applicator (IMID, Russia). The plate with the applied samples was dried and placed in a chamber previously saturated with mobile phase vapors. The mobile phase was a mixture (vol/vol) of chloroform-methanol-water (26: 14: 3). When the finish line was reached (no more than 95% of the plate height), the plate was removed from the chamber and dried until traces of solvents were removed. As a developer, a 20% alcohol solution of phosphoric-wolframic acid was used and heated at 100 °C for 3 minutes on the thermal stand of a mechanical applicator of the USP 1M brand, after which the light of densitometer lamps viewed it. We used a densitometer with a Sony photo fixing system (Handycam HDR-CX405) (IMID LLC, Russia). Photo fixation was performed at wavelengths of 254, 365 nm and in the visible radiation range after specific derivatization.

GC-MS of the sample was performed on a column with an internal diameter of 0.25 mm and a length of 30 m. Carrier gas: helium. Carrier gas flow rate: 1.4 ml/min. Injector temperature: 2400 ° C, interface temperature: 2800 ° C Column temperature: programmed from 100 to 2700 ° C at a speed of 200 S/min. Sample volume: 3 µl. Method of administration: without dividing the flow of the carrier gas. When using the electronic shock mode at 70 eV, followed by scanning in the range from 50 to 550 m / z mass. After the study, the mass spectra taken from the tops of the chromatographic peaks were compared according to the standard method with the mass spectra of the libraries. The substance was considered identified when its mass spectrum coincided with the library spectrum by more than 95%.

Statistical data analysis was carried out using the Microsoft Office Excel 2007 software product. Statistical analysis of the obtained data was carried out using the student's simultaneous pair test for each pair of interests. The differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

To determine the geroprotective potential of the extracts of *H. Neglectum* and *P. ginseng* root cultures, their antioxidant and antimicrobial activity was analyzed. The results of the determination of antioxidant activity are presented in Table 3.

According to the results shown in Table 3, it was concluded that the activity of *H. Neglectum* exceeds the

activity of the root culture extract of P. ginseng by 11.3 times when comparing the average value. The antioxidant activity of H. Neglectum ranges from 0.4100 to 0.6410 mg / g, and for P. ginseng, the range was from 0.0430 to 0.0480 mg/g. The maximum activity for both *H. Neglectum* and *P. ginseng* is shown in extracts numbered No. 9 (0.6410 mg/g and 0.0480 mg/g, respectively).

The results of determining the antimicrobial activity of extracts of root cultures of *H. Neglectum* and *P. ginseng* are presented in Tables 4 and 5. The analysis of the data showed that both extracts have antimicrobial activity against pathogenic and conditionally pathogenic microorganisms considered in this work. The maximum antimicrobial activity of *H. Neglectum* is shown in samples of alcohol extracts No. 4 and 5. Extracts numbered 7 and 9 showed the maximum antagonistic activity for P. ginseng. For the samples of *H. Neglectum* (No. 4) and *P. ginseng* (No. 9), a qualitative and quantitative analysis of the composition was performed.

Table 3. Results of determining the antioxidant properties of root crop extracts in terms of quercetin, mg / g.

Extract no.	Antioxidant activity of extracts, mg / g	
	H. Neglectum	***P. ginseng***
1	0,4840±0,0058	0,0430±0,0002
2	0,4100±0,0054	0,0490±0,0005
3	0,4360±0,0042	0,0440±0,0002
4	0,5240±0,0068	0,0460±0,0003
5	0,5590±0,0054	0,0470±0,0003
6	0,5320±0,0041	0,0470±0,0001
7	0,5550±0,0065	0,0440±0,0004
8	0,5210±0,0067	0,0450±0,0001
9	0,6410±0,0060	0,0480±0,0004

The analyzed extracts of *H. Neglectum* root cultures are characterized by the following spectral–chromatographic and component features. All samples contain mangiferin and a significant amount of tannic and tannic substances. The component composition of extracts of root cultures of the forgotten kopek, according to chromatographic analysis, is shown in Figure 1. Analyzing the composition of the extract of the root culture of *H. Neglectum* (No. 4), it was revealed that the following compounds were found in it: quercetin-rhamnopyranoside, quercetin, gallic acid, mangiferin. The maximum content of water for gallic acid is 57.02 mg / ml.

The components identified in the HPLC mode with amperometric detection in *H. Neglectum* extracts are presented in Table 6.

The presence of these components was confirmed in a different chromatographic system using a gradient elution mode and UV detection (Figure 2). The results of the qualitative and quantitative determination of BAS are presented in Table 7.

The UV spectrum of mangiferin is shown in Figure 3, where the absorption maxima are traced:

The isolated fraction of mangiferin was analyzed using the IR spectroscopy method. The IR spectrum is shown in Figure 4.

The obtained IR spectrum for the absorption bands corresponds to the mangiferin standard. As a result of a comparative analysis of the results obtained with the data from the literature, it was found that the following compounds were not previously described in the literature for H. Neglectum: mangiferin, 5-hydroxy-4-methoxy-8-prenyl-2-hydroxyisopropyl dihydrofurano-isoflavone (Anisimov et al., 2006; Aralbaeva et al., 2017; Morsli and Bellantuono, 2021; Bellantuono and Marengoni, 2021).

The HPLC chromatogram of the extracted sample from the root culture of P. ginseng is shown in Figure 5. The results of the qualitative and quantitative determination of BAS are shown in Table 8.

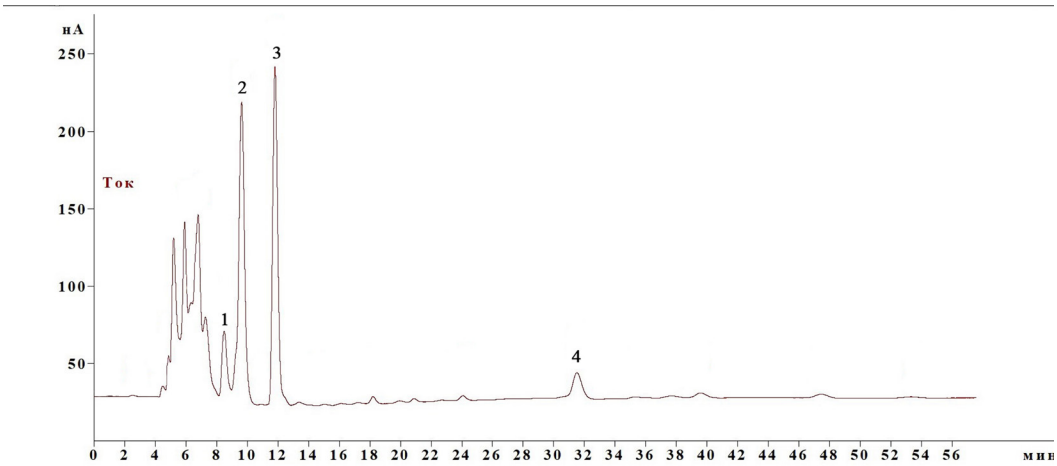

Figure 1. HPLC-chromatogram of *H. Neglectum* root crop extracts.

Table 4. Results of determination of antimicrobial activity of H. Neglectum root crop extracts.

Extract no.	1	2	3	4	5	6	7	8	9
Typical strains	Growth inhibition zones, mm								
Escherichia coli	10,90± 0,02	10,80± 0,06	11,10± 0,02	11,30±0,05	11,30±0,01	11,20± 0,03	10,80±0,08	11,00±0,07	10,90±0,03
Candida albicans	9,90± 0,02	9,80± 0,02	10,10± 0,06	10,30± 0,05	10,50±0,08	10,30± 0,08	9,90± 0,01	10,30± 0,08	10,20± 0,03
Bacillus cereus	10,10± 0,02	10,10± 0,05	10,20± 0,03	10,60±0,01	10,60± 0,06	10,40±0,05	10,10± 0,05	10,30± 0,03	10,30± 0,07
Pseudomonas aeruginosa	11,20± 0,06	11,00± 0,01	11,20± 0,02	11,50± 0,08	11,60± 0,07	11,50± 0,06	11,00± 0,03	11,30± 0,01	11,10± 0,08
Enterococcus faecium	10,20± 0,01	9,90± 0,02	10,20± 0,05	10,50± 0,06	10,70± 0,03	10,50±0,06	10,00± 0,08	10,30±0,01	10,10± 0,07
Klebsiella pneumonia	11,00± 0,06	11,00± 0,05	11,20± 0,06	11,30± 0,03	11,40± 0,01	11,30± 0,06	11,00± 0,07	11,10± 0,03	11,20± 0,01
Helicobacter pylori	9,50± 0,02	9,50± 0,06	9,60± 0,03	9,70± 0,08	9,80± 0,02	9,80± 0,05	9,40± 0,03	9,60± 0,01	9,60± 0,02
Streptococcus viridans	9,10± 0,05	9,10± 0,01	9,20± 0,02	9,60± 0,08	9,60± 0,06	9,40± 0,01	9,00± 0,06	9,40± 0,07	9,10± 0,01
Streptococcus bovis	9,40± 0,01	9,30± 0,03	9,60± 0,06	9,80± 0,05	9,90± 0,07	9,70± 0,03	9,40± 0,02	9,60± 0,07	9,50± 0,08
Porphyromonas gingivalis	9,70± 0,01	9,70± 0,02	9,90± 0,03	10,10± 0,06	10,20± 0,08	9,90± 0,02	9,60± 0,01	9,90± 0,05	9,70± 0,03
Acinetobacter baumannii	9,90± 0,05	9,90± 0,06	10,20± 0,02	10,50± 0,03	10,60±0,01	10,50± 0,07	9,90± 0,06	10,30± 0,01	10,10± 0,07
Borrelia burgdorferi	9,50± 0,03	9,50± 0,02	9,60± 0,01	9,70± 0,05	9,90± 0,08	9,70± 0,07	9,40± 0,02	9,70± 0,03	9,60± 0,07
Propionibacterium acnes	10,20± 0,05	10,20± 0,03	10,40± 0,06	10,60± 0,02	10,70± 0,07	10,50±0,01	10,10± 0,08	10,40±0,06	10,20± 0,07
Aggregatibacter actinomycetemcomitans	9,30± 0,03	9,30± 0,08	9,50± 0,02	9,70± 0,01	9,80± 0,05	9,80± 0,08	9,20± 0,07	9,50± 0,01	9,30± 0,07
Streptococcus intermedius	8,90± 0,05	9,00± 0,02	9,20± 0,06	9,30± 0,01	9,50± 0,08	9,40± 0,02	8,90± 0,03	9,20± 0,01	9,00± 0,05

Table 5. Results of determination of antimicrobial activity of *P. ginseng* root culture extracts.

Extract no.	1	2	3	4	5	6	7	8	9
Typical strains	Growth inhibition zones, mm								
Escherichia coli	10,00±0,01	9,70±0,09	10,00±0,08	9,70±0,01	10,20±0,01	10,10±0,09	10,30±0,01	9,80±0,01	10,30±0,08
Candida albicans	9,80±0,02	9,50±0,09	9,90±0,06	9,50±0,08	10,20±0,01	9,80±0,01	10,20±0,06	9,70±0,06	10,10±0,08
Bacillus cereus	9,40±0,01	9,20±0,01	9,50±0,08	9,00±0,07	9,50±0,06	9,50±0,09	9,70±0,08	9,40±0,09	9,70±0,08
Pseudomonas aeruginosa	9,80±0,09	9,60±0,01	9,90±0,06	9,60±0,08	9,80±0,06	9,90±0,08	10,20±0,09	9,60±0,08	10,00±0,01
Enterococcus faecium	9,10±0,08	8,80±0,01	9,10±0,06	8,80±0,06	9,40±0,01	9,20±0,08	9,40±0,09	8,90±0,08	9,30±0,08
Klebsiella pneumonia	9,00±0,05	8,90±0,05	9,10±0,09	8,70±0,06	9,20±0,08	9,00±0,01	9,20±0,08	8,90±0,08	9,20±0,08
Helicobacter pylori	9,70±0,08	9,50±0,09	9,80±0,06	9,60±0,08	9,80±0,08	9,90±0,09	9,90±0,01	9,70±0,08	9,90±0,08
Streptococcus viridans	8,90±0,01	8,70±0,09	8,80±0,06	8,60±0,08	8,90±0,05	8,90±0,08	9,20±0,08	8,70±0,08	9,00±0,08
Streptococcus bovis	8,60±0,02	8,40±0,06	8,60±0,08	8,30±0,08	8,80±0,08	8,70±0,08	8,80±0,08	8,40±0,09	8,80±0,08
Porphyromonas gingivalis	9,30±0,01	9,00±0,09	9,40±0,07	9,00±0,08	9,40±0,01	9,40±0,07	9,80±0,08	9,20±0,08	9,50±0,08
Acinetobacter baumannii	8,70±0,01	8,50±0,07	8,80±0,07	8,30±0,01	8,70±0,07	8,70±0,07	8,90±0,09	8,50±0,07	8,90±0,08
Borrelia burgdorferi	8,40±0,08	8,20±0,09	8,50±0,07	8,00±0,07	8,50±0,09	8,50±0,09	8,60±0,07	8,20±0,09	8,70±0,08
Propionibacterium acnes	9,60±0,01	9,50±0,09	9,80±0,01	9,40±0,09	9,80±0,07	9,90±0,03	9,90±0,07	9,60±0,07	9,90±0,09
Aggregatibacter actinomycetemcomitans	8,90±0,01	8,70±0,09	8,90±0,09	8,60±0,08	9,10±0,07	9,00±0,08	9,30±0,07	8,70±0,09	9,10±0,07
Streptococcus intermedius	8,70±0,09	8,60±0,06	8,80±0,06	8,40±0,06	8,90±0,06	8,90±0,08	9,00±0,07	8,60±0,08	8,90±0,01

Table 6. Components identified in *H. Neglectum* root crop extract samples.

Holding time, min	Component	Quantitative content, mg / ml
8,49	Quercetin-rhamnopyranoside	21,44 ± 0,88
9,63	Quercetin	43,17 ± 0,74
11,81	Gallic Acid	57,02 ± 0,76
31,52	Mangiferin	16,19 ± 0,84

Also, according to HPLC data, the content of phenolcarboxylic acids in the samples was established: lilac and p-coumaric. The content of isopropylbenzene was determined.

Thin-layer chromatography of the obtained plant extracts of P. ginseng root cultures revealed adsorption zones ranging from light pink to dark pink in the chromatogram of the solution; the dominant zone is at the level of the zone on the chromatogram of the solution with panaxoside Rg1 (Figure 6).

The separation was carried out on a Sorbfil plate, and track 1 corresponds to 20 µl of the test solution of water-

Table 7. Components identified in *H. Neglectum* root culture extract.

Peak no.	Holding time, min	Component	Quantitative content, mcg / ml
1	5,15	Gallic acid	28,34 ± 0,56
2	10,15	Quercetin	29,46 ± 0,40
3	18,33	Quercetin-rhamnopyranoside	7,23 ± 0,16
4	32,62	Mangiferin	7,32 ± 0,74

Table 8. Components of the extract from the root culture of *P. ginseng.*

Peak no.	Holding time, min.	Component Name	Quantitative content, mg / ml
1	4,42	lilac acid	11,48 ± 0,57
2	5,26	ginsenoside LC1	9,36 ± 0,47
3	5,84	panaxen	0,92 ± 0,05
4	6,30	ginsenoside Rb1	16,65 ± 0,74
5	6,50	panaxoside	6,37 ± 0,32
6	6,59	panaxoside	11,83 ± 0,73
7	6,96	panaxoside	11,44 ± 0,75
8	8,22	p-coumaric acid	1,35 ± 0,28
9	10,57	isopropylbenzene	0,87 ± 0,03
10	15,69	homisin A	1,59 ± 0,26
11	15,94	homizin B	2,31 ± 0,20

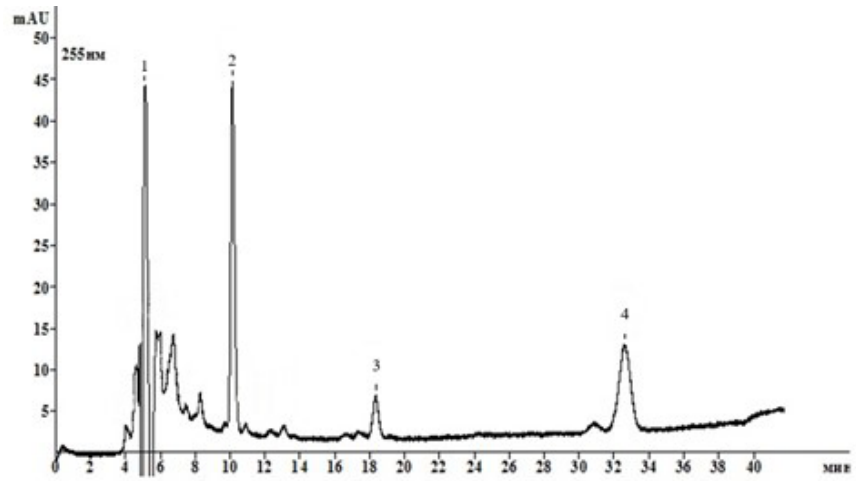

Figure 2. HPLC-chromatogram of *H. Neglectum* root crop extracts in UV detection mode, gradient elution.

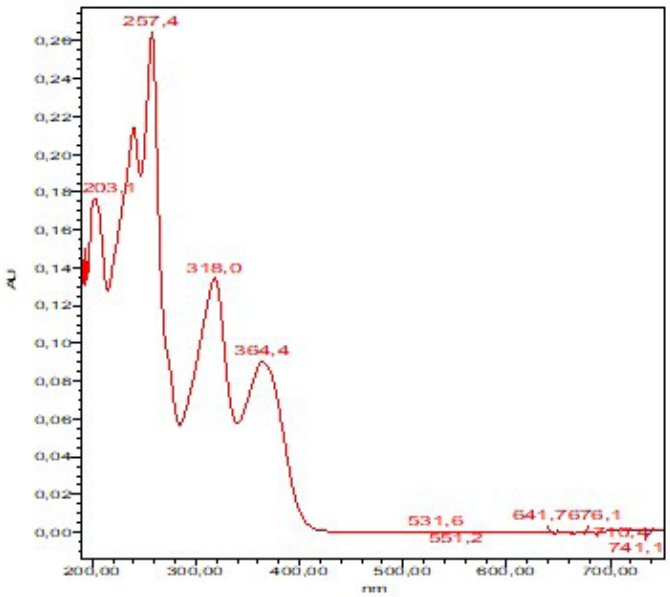

Figure 3. UV spectrum of mangiferin, isolated one.

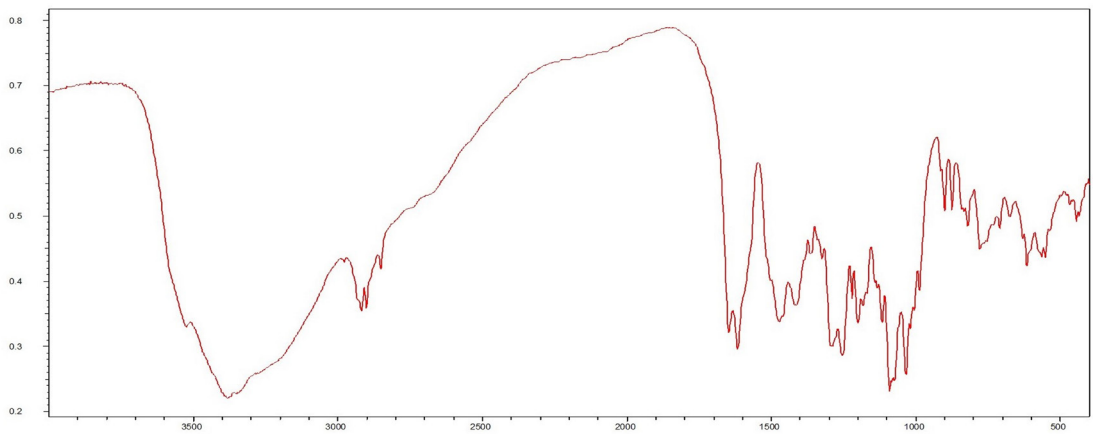

Figure 4. The IR spectrum of mangiferin.

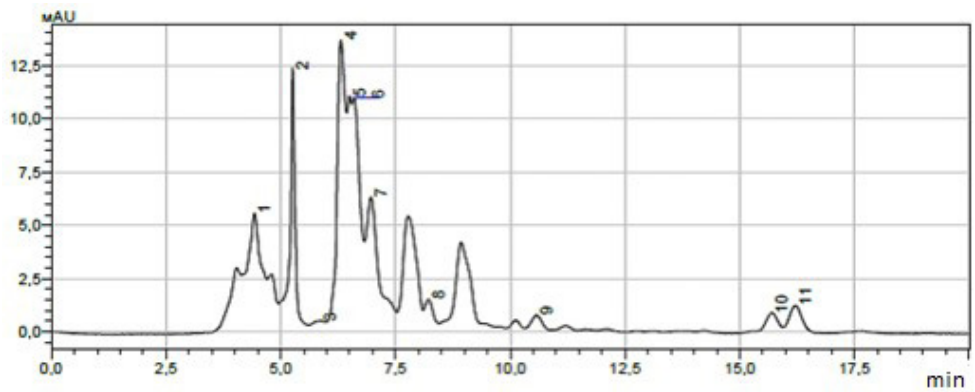

Figure 5. HPLC chromatogram of P. ginseng root culture extract: 1-lilac acid, 2-ginsenoside LC1, 3-panaxene, 4-ginsenoside Rb1, 5,6,7-panaxoside, 8-p-coumaric acid, 9-isopropylbenzene, 10-homizin A, 11-homizin B.

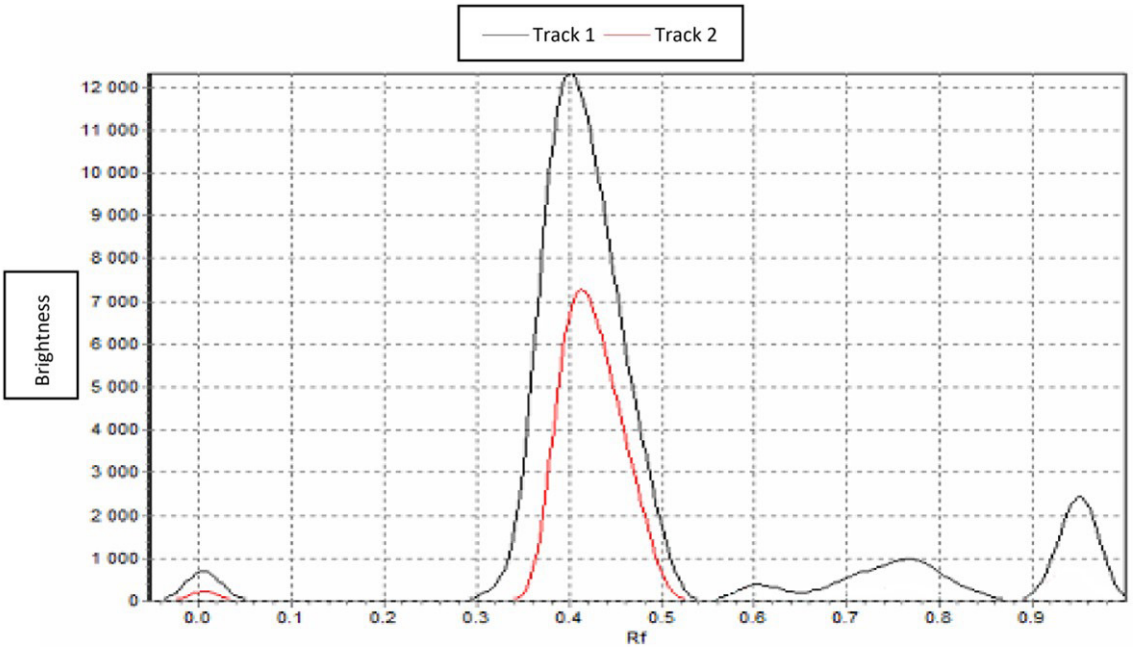

Figure 6. Densitogram of the sample extract from the root culture of *P. ginseng.*

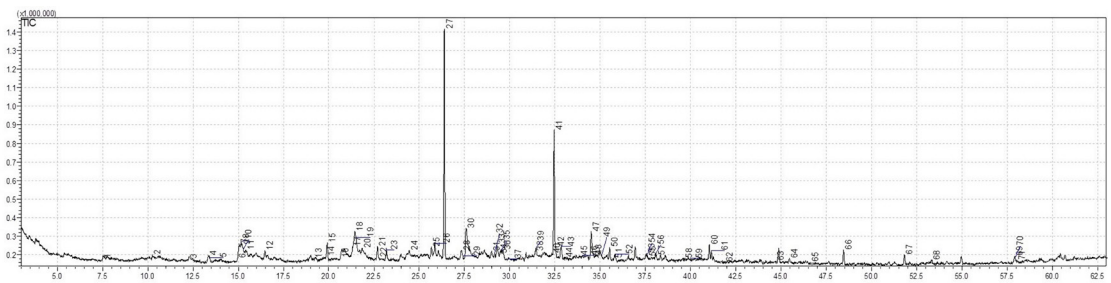

Figure 7. GC-MS chromatogram of the chloroform fraction from extracts of *P. ginseng* root cultures.

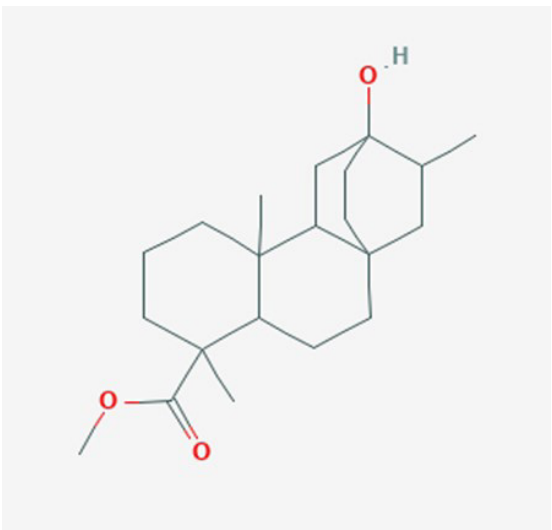

Figure 8. Structure of isosteviol methyl ether, yield time under GC-MS conditions 32.32 min.

ethanol extraction of sample No. 2; track 2 of the standard sample (CO) solution panaxoside Rg1, the application volume is 50 µl.

According to GC-MS data, the extracts of *P. ginseng* root cultures contain higher fatty acids: myristic, palmitic, stearic, oleic, as well as their ethyl and methyl esters. The chromatogram is shown in Figure 7.

The methyl ester of isosteviol, whose structural formula is shown in Figure 8, has been identified. A significant amount of tetracyclic triterpenoids belonging to the dammaran group was found in the studied samples.

4. Conclusions

In the course of scientific work, nine extracts of root cultures of *Panax ginseng* C. A. Mey and *Hedysarum neglectum* Ledeb were obtained. The extraction parameters varied according to the following parameters: temperature (30, 50, 70 ° C), the volume fraction of alcohol in the extractant (30, 50, 70%), exposure (2, 4, 5, 6 h).

The maximum antioxidant activity was established for P. ginseng extract No. 9 (0.0480 mg/g) and for H. Neglectum No. 4 (0.6410 mg/g). The analysis of antimicrobial activity showed that all the studied samples have antagonistic activity in relation to pathogenic and conditionally pathogenic strains. The maximum indicators are shown in the samples of alcohol extracts of *H. Neglectum* numbered 4 and 5, while for P. ginseng, the maximum antagonistic activity was shown by extracts numbered 7 and 9. It should be noted that the quantitative and qualitative composition of the extracts was analyzed using HPLC.

Water-alcohol extract of root cultures of *H. Neglectum* contains the following substances: quercetin-rhamnopyranoside (21.44 mg / ml), quercetin (43.17 mg/ml), gallic acid (57.02 mg/ml), mangiferin (16.19 mg/ml).

The resulting extract of P ginseng is characterized by the presence of lilac acid (11.48 mg/ml), ginsenoside LC1 (9.36 mg/ml), panaxen (0.92 mg/ml), ginsenoside Rb1 (16.65 mg/ml), panaxoside (6.37 mg/ml), panaxoside (11.83 mg/ml), panaxoside (11.44 mg/ml), p-coumaric acid (1.35 mg/ml), isopropylbenzene (0.87 mg/ml), homizin A (1.59 mg/ml), homizin B (2.31 mg/ml).

In the future, studies are planned on isolating certain biologically active substances from extracts of root cultures of Panax ginseng C. A. Mey. and Hedysarum neglectum Ledeb. to use them as functional components of gerontological nutrition.

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