Foods and Raw Materials, 2021, vol. 9, no. 1

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

https://doi.org/10.21603/2308-4057-2021-1-126-134
Available online at http:jfrm.ru/en

Geroprotective potential of *in vitro* bioactive compounds isolated from yarrow (*Achilleae millefolii* L.) cell cultures

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Received February 01, 2021; Accepted in revised form March 03, 2021; Published online April 14, 2021

Abstract:
Introduction. There is an urgent need for geroprotectors that prevent premature aging, especially antioxidants of plant origin. Due to the shortage of medicinal plant materials, scientists look for alternative sources of bioactive compounds of phenolic nature, for example, cell cultures and organs of higher plants. This paper describes a study of the geroprotective potential of *in vitro* bioactive compounds isolated from yarrow (*Achilleae millefolii* L.) cell cultures.
Study objects and methods. Callus, suspension and hairy root cultures of *A. millefolii* were obtained by *in vitro* cultivation on modified nutrient media. High performance liquid chromatography (HPLC) was used to analyze the composition of the cell cultures and ethanol extracts. The extracts' antimicrobial activity was studied by the disk diffusion method and their antioxidant activity was measured based on titration of a potassium permanganate solution.
Results and discussion. The biomass of all yarrow cell cultures contained essential oils, flavonoids, glycosides, phenolic acids, carotenoids, as well as vitamins C and E. The suspension culture had a higher content of essential oils, flavonoids and glycosides than the callus and hairy root cultures. The extracts of the *A. millefolii* suspension culture also contained geroprotectors – phenylpropanoids, flavonoids, and simple phenols, with a prevalence of caffeic acid, cynaroside, 4,5-dicofeylquinic acid, apigenin, and luteolin. In addition, HPLC revealed the presence of cumic aldehyde, umbelliferone, 3-caffeylquinic acid, and caffeic acid – the bioactive compounds previously not reported in yarrow. *In vitro* experiments with the extracts proved their antimicrobial and antioxidant activity.
Conclusion. The complex of bioactive compounds isolated from the biomass of yarrow suspension culture provides this plant with potential geroprotective properties. Thus, yarrow can be used to create nutraceuticals that prevent premature aging.

Keywords: Medicinal plant, yarrow, *Achilleae millefolii* L., bioactive compound, geroprotector, plant cell culture, extraction, antimicrobial properties, antioxidant properties

Funding: The research was performed within the state assignment entitled "Screening bioactive substances of plant origin with geroprotective properties and developing technology for obtaining nutraceuticals that slow down aging" (No. FZSR-2020-0006).

Please cite this article in press as: Asyakina LK, Fotina NV, Izgarysheva NV, Slavyanskiy AA, Neverova OA. Geroprotective potential of *in vitro* bioactive compounds isolated from yarrow (*Achilleae millefolii* L.) cell cultures. Foods and Raw Materials. 2021;9(1):126–134. https://doi.org/10.21603/2308-4057-2021-1-126-134.

INTRODUCTION

Aging is one of the global problems of humanity. In 2018, Russians aged 60+ accounted for 25.4% of the country's population. By 2050, their proportion is estimated to reach 31.2%. Aging is an inevitable process and one of the factors that can trigger the development of certain chronic diseases (cancer, type 2 diabetes mellitus, atherosclerosis, hypertension, myocardial infarction, etc.). Despite plentiful research on the biology of aging, its mechanisms are still far from being fully clear [1–3].

Premature aging is age-related changes that occur earlier than in healthy people of the same age group. According to A. Zhuravlev and Yu. Golubeva, "with premature aging, an individual's biological age is ahead of their calendar age" [4]. The causes of premature aging go beyond the negative impact of environmental

factors and lifestyle (bad habits, unhealthy diet, irregular daily routine), including genetic predisposition to such changes (Hutchinson-Guildford syndrome, Werner's syndrome) [5].

Gerontology is a science that studies aging and related processes. Some of its branches include geriatrics, gerocomia, and gerontopsychology that study prevention and treatment of old age diseases, care for older people, as well as their psychological and behavioral patterns. Gerontologists argue that some old age diseases can be prevented or delayed by certain chemicals, thus increasing life expectancy. As a result, pharmacologists are trying to create drugs capable of protecting the body from aging. These drugs include geroprotectors [6–8].

Geroprotectors are substances with a proven ability to increase the life span of animals (antioxidants, cross-linking inhibitors, enterosorbents, immunomodulators, antidiabetic agents, and adaptogens). Antioxidants play an important role in fighting against premature aging and thus preventing the negative effects of oxidative stress [9, 10].

Highly valuable are plant-based antioxidants (flavonoids, stilbenes, phenolic acids). They have a wide range of preventive and therapeutic effects and, unlike their synthetic analogues, do not have a toxic effect on the body or cause addiction [11–13].

In this regard, recent years have seen a sharply increased interest in the production of natural bioactive compounds with gerontological properties. The commercialization of such technologies could enable Russia to discontinue importing functional food ingredients, support domestic production and export bioactive compounds abroad. However, there is a shortage of medicinal raw materials due to a rapidly increasing demand for them and the unfavorable environmental situation. A new solution might be to use cell cultures (suspension, callus) and organs (hairy roots) of higher plants as an alternative source of renewable environmentally friendly materials.

Common yarrow (*Achilleae millefolii* L.) is rich in antioxidant substances and therefore has high geroprotective potential. It is a widespread plant traditionally used to heal wounds and treat inflammatory and respiratory infections since it contains bioactive compounds with anti-inflammatory, anti-ulcer, and anti-tumor effects [14]. These bioactive substances include amino acids, fatty acids, other acids (salicylic, succinic, ascorbic, folic, caffeic, chlorogenic) and flavonoids (luteolin, apigenin, quercetin) [15, 16].

In this work, we aimed to study the geroprotective potential of *in vitro* bioactive substances isolated from cell cultures (callus, suspension) and hairy roots of common yarrow (*A. millefolii* L.)

STUDY OBJECTS AND METHODS

The study objects were callus and suspension cell cultures and hairy roots obtained from the yarrow (*Achilleae millefolii* L.) seeds germinated in the Botanical Garden of Immanuel Kant Baltic Federal University (Kaliningrad).

To sterilize the seeds, we washed them with a detergent and kept first in 70% ethanol for 1 min and then in a 5% sodium hypochlorite solution for 50 min. After sterilization, the seeds were planted on agar media in 60 and 90 mm Petri dishes to grow sterile seedlings.

The nutrient medium for growing callus cultures (per 1 liter of distilled water) contained 50.00 mL MS (Murashige and Skoog) macro-salts (20×), 1.00 mL MS micro-salts, 5.00 mL Fe-EDTA, 0.10 mg thiamine; 0.50 mg pyridoxine; 0.50 mg nicotinic acid; 30.00 g sucrose; 1.00 mg kinetin; 2.00 mg β-indoleacetic acid; and 20.00 g agar [17]. The first seedlings appeared after 6–8 weeks of cultivation. To induce callus cultures, we used eight-week-old sterile seedlings with 2–4 true leaves. For this, the leaves and stems of the seedlings were cut into pieces and planted on an agar medium in 60 and 90 mm Petri dishes. Primary callus were formed on days 7–14 of cultivation. They were separated from the remains of plant explants and transferred to fresh nutrient media. The further cultivation cycle was 4–5 weeks.

To obtain cell suspensions, 300–400 mg of yarrow callus cultures was placed in 250 mL flasks with 25 mL of liquid nutrient medium and cultivated on a circular shaker at 95–100 rpm. After 18–20 days, we subcultured the cell supernatant, gradually increasing the dilution rate from 1/2 to 1/8 (inoculum to fresh nutrient medium) and shortening the subculturing cycle. Suspension cultures were grown in 250 mL flasks (30–40 mL of suspension per flask) on a shaker at 100 rpm. The nutrient medium (per 1 liter of distilled water) contained 50.00 mL MS macrosalts (20×), 1.00 mL MS microsalts, 5.00 mL Fe-EDTA, 0.10 mg thiamine, 0.50 mg pyridoxine, 0.50 mg nicotinic acid, 30.00 g sucrose, 1.00 mg kinetin, and 2.00 mg β-indoleacetic acid.

Hairy roots were obtained from the leaves of 14–28 day old yarrow seedlings. The leaves were transformed with *Agrobacterium rhizogenes* strain 15834 Swiss (Moscow, Russia) [18]. The strain was grown on the YEB agar medium (5 g/L peptone, 1 g/L yeast extract, 5 g/L sucrose, 0.5 g/L $MgCl_2$) for 24 h in the dark at 23°C on a shaker with circular rotation (5–10 cm amplitude, 90 rpm). The explants were pierced with a sterile needle and placed on Gamborg B5 medium containing a suspension (OD_{600} = 0.4) of *A. rhizogenes* [19].

After 12–48 h of incubation in the bacterial suspension, the explants were washed with sterile water, wiped with sterile filter paper until dry, and placed on solid B5 medium containing 500 mg/L of cefotaxime (Claforan, UK). The antibiotic was added to remove the agrobacterium residues. After

30 days of cultivation, we assessed the frequency of transformation (the number of transformed explants to their total number). The transformed explants were selected on the basis of their phenotypic traits (lateral branching, absence of geotropism, ability to grow in a hormone-free environment). The obtained roots were passaged two times on solid hormone-free B5 medium containing a reduced amount of cefotaxime (250 mg/L). They were cultivated in the dark at 23°C on a shaker at 100 rpm during 5 weeks. Subsequent passage to fresh medium was carried out as soon as the agrobacterium contamination appeared. After 14 days, individual explants featured rhizogenesis.

The growth of callus biomass was calculated as:

$$P_i = \frac{X_i - X_0}{X_0} \quad (1)$$

where X_i is the weight of culture on the i-th day of cultivation (standard: i = 7, 14, 21, 28 and 35 days); X_0 is the initial weight of culture (transplant weight).

Suspension cultures were analyzed for all growth parameters (dry mass, cell viability). To determine the content of wet and dry biomass in a liter of medium (M_{max_dw}, g/L), a fixed amount of suspension (not less than 15 mL, in triplicate) was filtered through a paper filter using a Buchner funnel under vacuum. The biomass was dried to constant weight in a stream of air at 30°C. The viability (v) of cell cultures was determined by counting live (unstained) and dead (stained) cultures under a microscope using phenosafranine (0.1% solution) or 0.025% Evans blue.

The growth index (I_{dw}) and the specific growth rate in the exponential phase (μ_{dw}, day^{-1}) were calculated as:

$$I_{dw} = X_{max}/X_0 \quad (2)$$

where X_{max} is the maximum content of dry biomass in a liter of medium; X_0 is the initial content of dry biomass in a liter of medium.

$$\mu_{dw} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (3)$$

where X_2 is the content of dry biomass in a liter of medium at point t_2; X_1 is the content of dry biomass in a liter of medium at point t_1.

The growth index was used to characterize the growth of root cultures *in vitro*.

The composition of yarrow callus, suspension and hairy root culture biomass, as well as the extracts, was analyzed by high performance liquid chromatography (HPLC) according to [20]. For this, we used the following eluent compositions: 1) tetrahydrofuran: acetic acid:5% H_3PO_4:water (19:20:2:59); 2) tetrahydrofuran:dioxane:MeOH:acetic acid:5% H_3PO_4:water (14.5:12.5:5:2:2:66). The substances were separated on a Shimadzu LC-20 Prominence chromatograph (Japan) with a Shimadzu SPD20M diode-matrix detector, using the columns Kromasil C18, 5 µm, 250×4.6 mm and HyperClone 5 µm, BDS 130Å, C18 250×4.6 mm.

Bioactive substances were extracted with ethyl alcohol (Kemerovo Pharmaceutical Factory, Russia). Dried plant biomass was ground in an LZM-1M mill (Olis, Russia) and sieved through a 1-mm hole sieve. 3 g of dried plant biomass was extracted in 260 mL of ethyl alcohol (30, 50, and 70 % ethanol) under static conditions in a PE-4310 water bath (EKROSKHIM, Russia) with a reflux condenser (30, 50, and 70°C). The extraction was performed twice during 2, 4, and 6 h.

To assess the geroprotective potential of the bioactive compounds isolated from the yarrow callus, suspension, and hairy root biomass, we analyzed their antimicrobial and antioxidant properties.

The antimicrobial activity of the extracts was analyzed according to Methodological Guidelines 4.2.1890-2004 by the disk diffusion method. The microorganisms used as test strains included *Escherichia 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*, and *Streptococcus intermedius*.

A suspension of microorganisms under study was prepared from a broth culture according to the turbidity standard. Antimicrobial activity was determined using a standard meat-and-peptone agar (MPA). After inoculation, extract-impregnated discs were placed onto the medium, with an antibiotic disc used as a control. Then, the Petri dishes were put in a thermostat upside down and incubated at 35–37°C for 18–24 h (depending on the microorganism). To record the results, we placed the dishes upside down on a dark matte surface so that the light fell at an angle of 45°. The diameter of the growth inhibition zones was measured with an accuracy of 1 mm using a vernier caliper.

To determine the extracts' antioxidant activity, we filled a 50 mL titration glass with 8 mL of freshly boiled and cooled distilled water, 1 mL of a 20% sulfuric acid solution, 1 mL of 0.05 N potassium permanganate solution. After stirring, the solution was titrated with

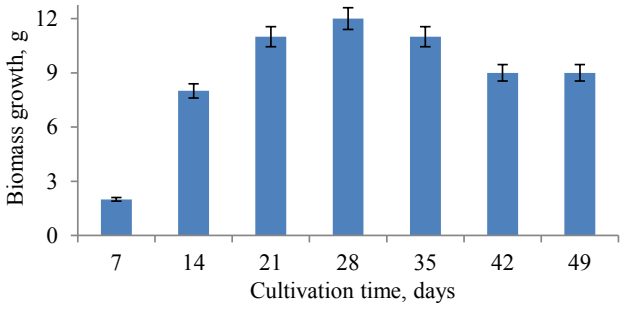


Figure 1 *In vitro* yarrow (*Achilleae millefolii* L.) hairy root culture growth index vs. cultivation time

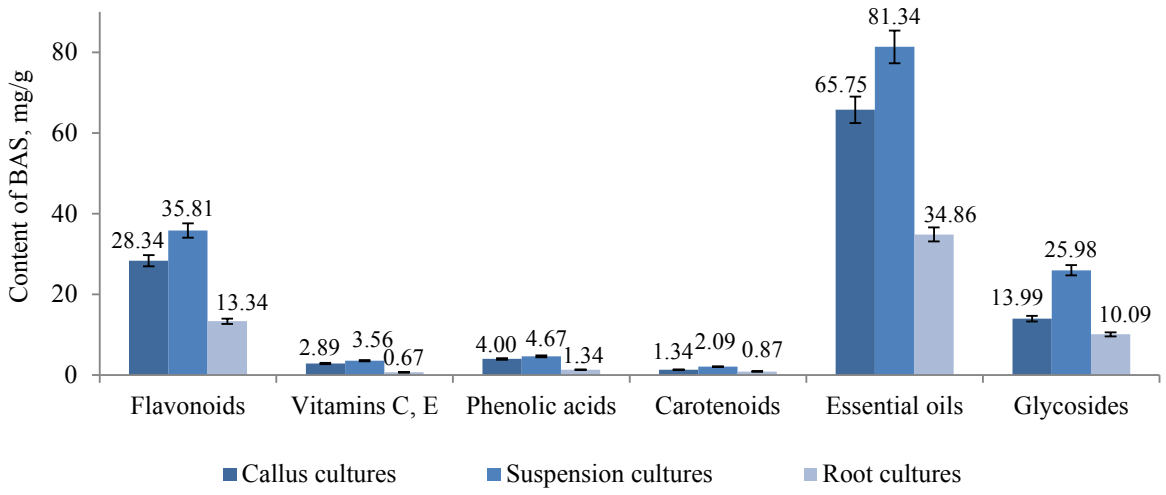


Figure 2 Bioactive substances in callus, suspension and hairy root cultures of yarrow (*Achilleae millefolii* L.)

an extract under study from a 1 mL microburette with a division value of 0.01 mL until the pink color disappeared.

Statistical data was analyzed using Microsoft Office Excel 2007 and the paired Student's test. The differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The dry biomass growth index for the callus culture of yarrow (*Achilleae millefolii* L.) was 8.7–10.6 with a subcultivation cycle of 28–32 days.

The suspension culture showed high growth characteristics, namely:

– the content of dry and wet biomass in a liter of medium M_{max_dw} = 14.53–16.12 g/l;

– the viability of cell cultures v = 82–91%;

– the specific growth rate in the exponential phase μ_{dw} = 0.16–0.19 day^{-1}; and

– the exponential growth index I_{dw} = 9.56–10.23.

We found two types of cells in the yarrow suspension culture: mainly meristemetic and a number of parenchyma-like cells. The latter tended to increase by the end of the stationary phase of subcultivation. Although the aggregates were predominantly round in shape, there was also a number of elongated aggregates in the form of chains consisting of 5–20 small cells. Noteworthily, the primary suspensions of yarrow showed extremely unstable growth and significant

Table 1 Parameters for extracting bioactive compounds from yarrow (*Achilleae millefolii* L.) suspension culture biomass

No. extract	Extraction parameters		
	Tempe-rature, °C	Ethanol, %	Extraction time, h
1	30	70	2
2	30	70	4
3	50	50	2
4	50	30	4
5	50	50	4
6	50	70	4
7	50	70	6
8	70	30	2
9	70	50	6

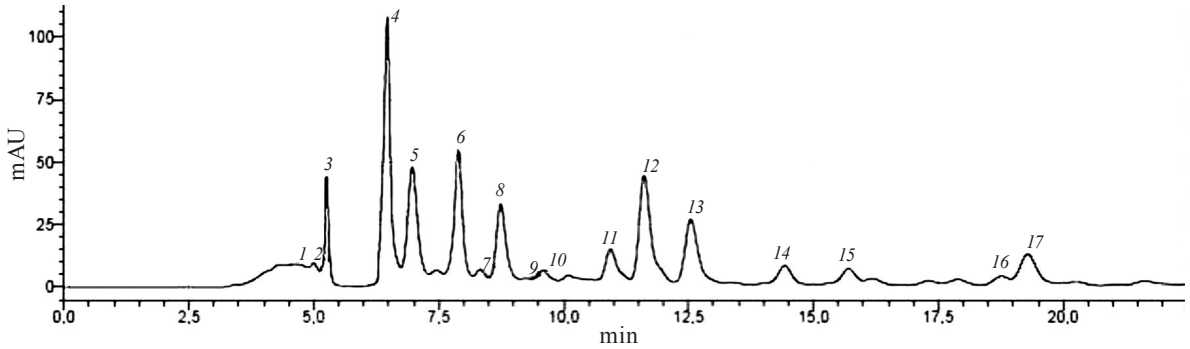


Figure 3 Chromatogram of aqueous-alcoholic extract (70% ethanol, 30°C, 2 h) of yarrow (*Achilleae millefolii* L.) suspension culture: *1* – benzyl alcohol; *2* – cumin aldehyde; *3* – 3-caffeylquinic acid; *4* – caffeic acid; *5* – 4,5-dicofeylquinic acid; *6* – apigenin; *7* – isoramnetin; *8* – casticin; *9* – 1-caffeoyl-3-feruloylquinic acid; *10* – 3,4-dicofeylquinic acid; *11* – vitsenin-2; *12* – cynaroside; *13* – luteolin; *14* – rutin; *15* – cosmosiin; *16* – esculetin; *17* – umbelliferone

Table 2 Qualitative and quantitative analyses of bioactive compounds in aqueous-alcoholic extract (70% ethanol, 30°C, 2 h) of yarrow (*Achilleae millefolii* L.) suspension culture by HPLC

No. peak	Retention time, min	Component	Content, mg/mL
1	4.39	Benzyl alcohol	0.21 ± 0.05
2	5.03	Cumin aldehyde	0.38 ± 0.05
3	5.25	3-caffeylquinic acid	3.05 ± 0.50
4	6.47	Caffeic acid	22.21 ± 0.88
5	6.97	4,5-dicofeylquinic acid	12.70 ± 0.32
6	7.89	Apigenin	11.15 ± 0.60
7	8.32	Isoramnetin	0.52 ± 0.05
8	8.74	Casticin	7.65 ± 0.50
9	9.22	1- caffeoyl-3-feruloylquinic acid	0.30 ± 0.02
10	9.58	3,4-dicofeylquinic acid	1.03 ± 0.20
11	10.93	Vitsenin-2	4.18 ± 0.27
12	11.61	Cymaroside	14.55 ± 0.32
13	12.54	Luteolin	9.27 ± 0.50
14	14.42	Rutin	2.96 ± 0.30
15	15.70	Cosmosin	1.80 ± 0.11
16	18.76	Esculetin	1.50 ± 0.20
17	19.29	Umbelliferone	5.30 ± 0.50

differences in the cell shape and size. A decrease in the inoculum amount led to an increase in the duration of biomass growth.

According to Fig. 1, the growth curve of the *in vitro* yarrow root culture is S-shaped. After a lag phase of 7–14 days, the culture entered the exponential phase on the 21st day and slowed down in growth on the 28th day, entering the stationary phase. The growth index was 26.

Figure 2 shows the qualitative and quantitative composition of bioactive substances in the callus, suspension and hairy root cultures of yarrow.

Table 4 Antioxidant properties of extracts obtained from yarrow (*Achilleae millefolii* L.) suspension culture biomass

Sample No.	Antioxidant activity, mg/g	Sample No.	Antioxidant activity, mg/g
1	0.2020 ± 0.0070	6	0.2220 ± 0.0065
2	0.2100 ± 0.0071	7	0.2270 ± 0.0078
3	0.2250 ± 0.0067	8	0.2080 ± 0.0074
4	0.2050 ± 0.0075	9	0.1900 ± 0.0075
5	0.2150 ± 0.0069		

As we can see in Fig. 2, the biomass of callus, suspension and hairy root cultures of *A. millefolii* L. had a high content of essential oils, flavonoids, and glycosides and lower concentrations of phenolic acids, carotenoids, and vitamins C and E. Compared to callus and hairy root cultures, the suspension culture was richer in essential oils (by 23.7 and 133.3%), flavonoids (by 26.4 and 168.4%), and glycosides (by 85.7 and 157.5%).

Based on the results, we decided to use a suspension culture of yarrow to extract a complex of bioactive compounds with potential geroprotective properties.

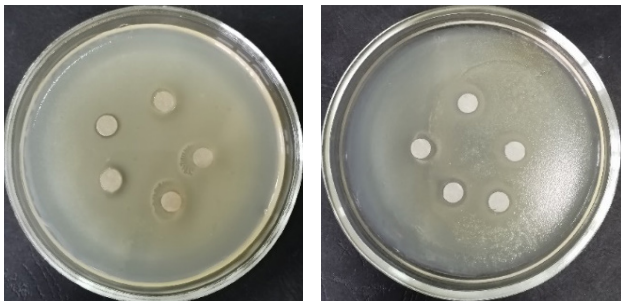
We examined nine extracts obtained from the suspension culture biomass with different extraction parameters (Table 1).

Extract No. 1 (70% ethanol, 30°C, 2 h) was qualitatively and quantitatively analyzed for bioactive compounds by HPLC (Fig. 3, Table 2).

As we can see, the aqueous-alcoholic extract of *A. millefolii* suspension culture contained phenylpropanoids, flavonoids, and simple phenols – potential geroprotectors. It also had a high content of caffeic acid, cynaroside, 4,5-dicofeylquinic acid, apigenin, and luteolin. In addition, we found cumin aldehyde, umbelliferone, 3-caffeylquinic acid, and caffeic acid – the compounds that have never been reported in yarrow before.

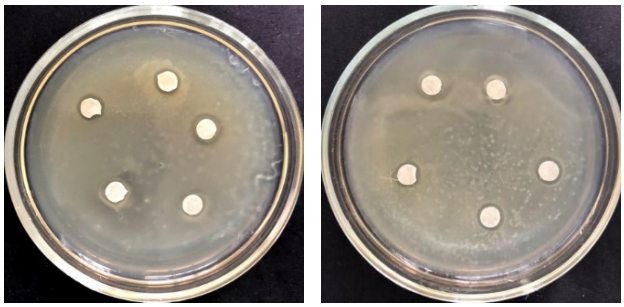
Table 3 Antimicrobial properties of extracts obtained from yarrow (*Achilleae millefolii* L.) suspension culture biomass

Sample No.	1	2	3	4	5	6	7	8	9
Test strain	Growth inhibition zones, mm								
Escherichia coli	9.4 ± 0.5	9.6 ± 0.5	9.2 ± 0.5	9.6 ± 0.5	9.8 ± 0.5	9.3 ± 0.5	9.4 ± 0.5	9.8 ± 0.5	9.5 ± 0.5
Candida albicans	9.2 ± 0.5	9.5 ± 0.5	9.1 ± 0.5	9.5 ± 0.5	9.6 ± 0.5	9.2 ± 0.5	9.4 ± 0.5	9.5 ± 0.5	9.6 ± 0.5
Bacillus cereus	8.8 ± 0.4	9.1 ± 0.5	8.6 ± 0.4	9.1 ± 0.5	9.3 ± 0.5	8.6 ± 0.4	8.9 ± 0.5	9.1 ± 0.5	9.0 ± 0.5
Pseudomonas aeruginosa	6.5 ± 0.3	6.7 ± 0.3	6.2 ± 0.3	6.8 ± 0.3	6.8 ± 0.3	6.3 ± 0.3	6.5 ± 0.3	6.9 ± 0.3	6.7 ± 0.3
Enterococcus faecium	7.9 ± 0.4	8.2 ± 0.4	7.8 ± 0.4	8.2 ± 0.4	8.2 ± 0.4	7.7 ± 0.4	7.9 ± 0.4	8.2 ± 0.4	8.1 ± 0.4
Klebsiella pneumonia	9.2 ± 0.5	9.4 ± 0.5	9.0 ± 0.5	9.5 ± 0.5	9.4 ± 0.5	9.0 ± 0.5	9.3 ± 0.5	9.4 ± 0.5	9.3 ± 0.5
Helicobacter pylori	8.8 ± 0.4	9.2 ± 0.5	8.9 ± 0.4	9.2 ± 0.5	9.5 ± 0.5	8.9 ± 0.5	8.9 ± 0.5	9.3 ± 0.5	9.2 ± 0.5
Streptococcus viridans	7.9 ± 0.4	8.1 ± 0.4	7.6 ± 0.4	8.2 ± 0.4	8.2 ± 0.4	7.7 ± 0.4	7.9 ± 0.4	8.2 ± 0.4	8.0 ± 0.4
Streptococcus bovis	7.6 ± 0.4	7.9 ± 0.4	7.5 ± 0.4	7.9 ± 0.4	8.0 ± 0.4	7.6 ± 0.4	7.8 ± 0.4	8.0 ± 0.4	7.9 ± 0.4
Porphyromonas gingivalis	9.5 ± 0.5	9.8 ± 0.5	9.4 ± 0.5	9.9 ± 0.5	10.0 ± 0.5	9.5 ± 0.5	9.7 ± 0.5	10.1 ± 0.5	9.8 ± 0.5
Acinetobacter baumannii	9.6 ± 0.5	9.9 ± 0.5	9.4 ± 0.5	9.9 ± 0.5	10.0 ± 0.5	9.4 ± 0.5	9.7 ± 0.5	9.9 ± 0.5	9.8 ± 0.5
Borrelia burgdorferi	7.9 ± 0.4	8.2 ± 0.4	7.8 ± 0.4	8.3 ± 0.4	8.4 ± 0.4	7.9 ± 0.4	7.9 ± 0.4	8.3 ± 0.4	8.1 ± 0.4
Propionibacterium acnes	9.8 ± 0.5	10.0 ± 0.5	9.5 ± 0.5	9.9 ± 0.5	10.2 ± 0.5	9.7 ± 0.5	9.8 ± 0.5	10.0 ± 0.5	9.9 ± 0.5
Aggregatibacter actinomycetemcomitans	8.3 ± 0.4	8.5 ± 0.4	8.0 ± 0.4	8.5 ± 0.4	8.6 ± 0.4	8.1 ± 0.4	8.3 ± 0.4	8.6 ± 0.4	8.4 ± 0.4
Streptococcus intermedius	7.6 ± 0.4	7.9 ± 0.4	7.5 ± 0.4	7.9 ± 0.4	8.1 ± 0.4	7.6 ± 0.4	7.8 ± 0.4	8.1 ± 0.4	7.8 ± 0.4



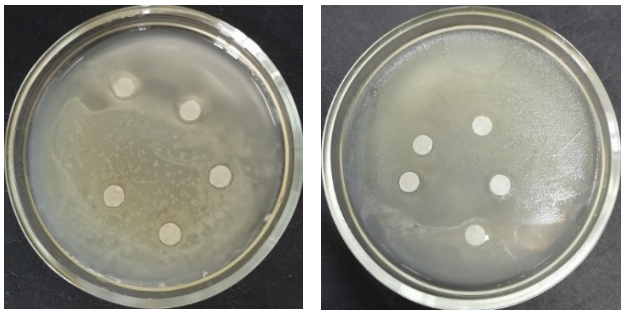
(a) (b)

Figure 4 Antimicrobial activity of yarrow suspension culture extracts against *Escherichia coli*: a) extract No. 5 (9.8 ± 0.5 mm); b) extract No. 8 (9.8 ± 0.5 mm)



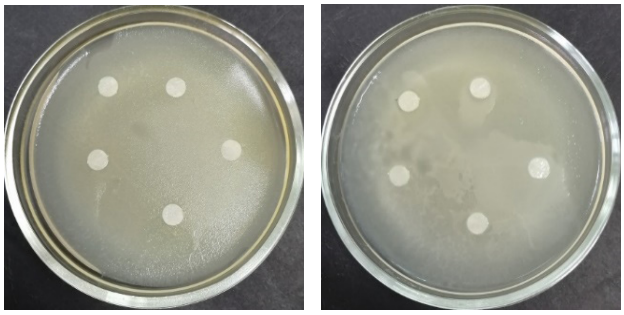
(a) (b)

Figure 5 Antimicrobial activity of yarrow suspension culture extracts against *Candida albicans*: a) extract No. 5 (9.6 ± 0.5 mm); b) extract No. 8 (9.5 ± 0.5 mm)



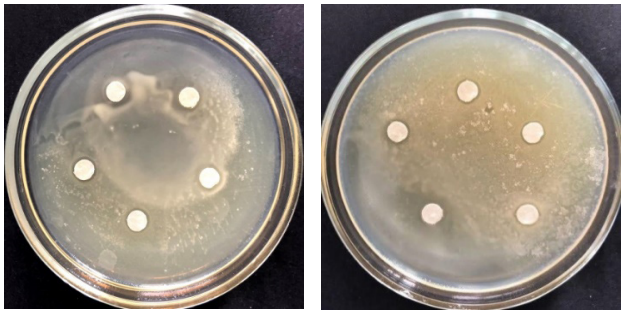
(a) (b)

Figure 6 Antimicrobial activity of yarrow suspension culture extracts against *Bacillus cereus*: a) extract No. 5 (9.3 ± 0.5 mm); b) extract No. 8 (9.1 ± 0.5 mm)



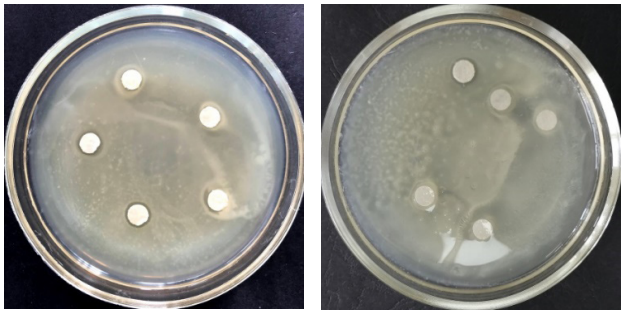
(a) (b)

Figure 7 Antimicrobial activity of yarrow suspension culture extracts against *Pseudomonas aeruginosa*: a) extract No. 5 (6.8 ± 0.3 mm); b) extract No. 8 (6.9 ± 0.3 mm)



(a) (b)

Figure 8 Antimicrobial activity of yarrow suspension culture extracts against *Enterococcus faecium*: a) extract No. 5 (8.2 ± 0.4 mm); b) extract No. 8 (8.2 ± 0.4 mm)



(a) (b)

Figure 9 Antimicrobial activity of yarrow suspension culture extracts against *Klebsiella pneumonia*: a) extract No. 5 (9.4 ± 0.5 mm); b) extract No. 8 (9.4 ± 0.5 mm)

In order to assess the geroprotective potential of the extracts, we studied their antimicrobial (Table 3) and antioxidant properties (Table 4) in *in vitro* experiments.

According to Table 3, all the samples performed antimicrobial activity against the test strains used. The maximum antimicrobial activity was observed in samples No. 5 (50% ethanol, 50°C, 4 h) and No. 8 (70% ethanol, 30°C, 2 h) (Fig. 4–18).

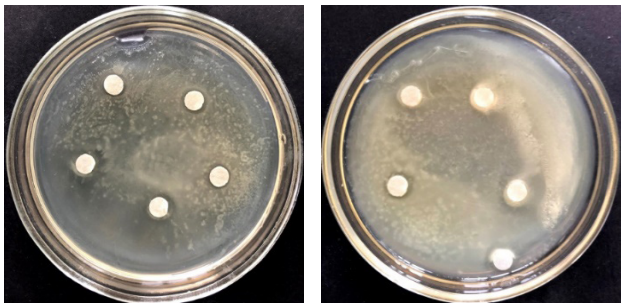
The antioxidant activity of the yarrow suspension culture extracts is shown in Table 4.

According to Table 4, the antioxidant activity of the aqueous-alcoholic extracts obtained from the yarrow suspension culture biomass varied from 0.1900 to 0.2270 mg/g.

Due to their antimicrobial and antioxidant activity and a high content of phenylpropanoids, flavonoids, and simple phenols, the extracts can be considered as having geroprotective properties and can be used to create nutraceuticals that prevent premature aging.

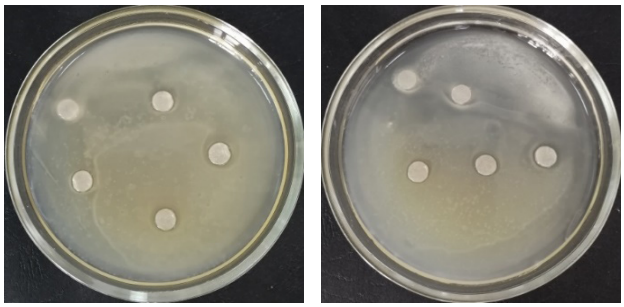
CONCLUSION

We cultivated *in vitro* cell cultures of yarrow (*Achilleae millefolii* L.), namely callus cultures, with a dry biomass growth index of 8.7–10.6; suspension



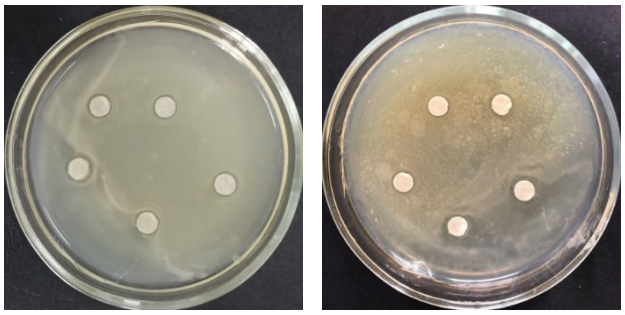
(a) (b)

Figure 10 Antimicrobial activity of yarrow suspension culture extracts against *Helicobacter pylori*: a) extract No. 5 (9.5 ± 0.5 mm); b) extract No. 8 (9.3 ± 0.5 mm)



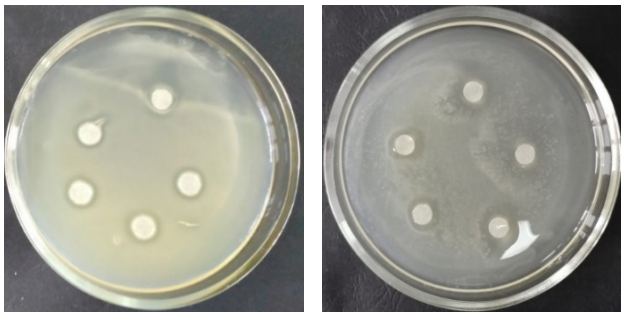
(a) (b)

Figure 11 Antimicrobial activity of yarrow suspension culture extracts against *Streptococcus viridans*: a) extract No. 5 (8.2 ± 0.4 mm); b) extract No. 8 (8.2 ± 0.4 mm)



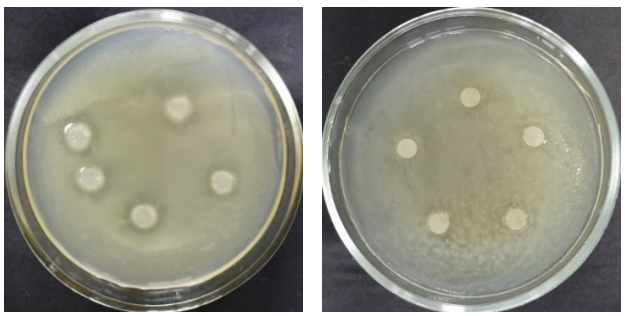
(a) (b)

Figure 12 Antimicrobial activity of yarrow suspension culture extracts against *Streptococcus bovis*: a) extract No. 5 (8.0 ± 0.4 mm); b) extract No. 8 (8.0 ± 0.4 mm)



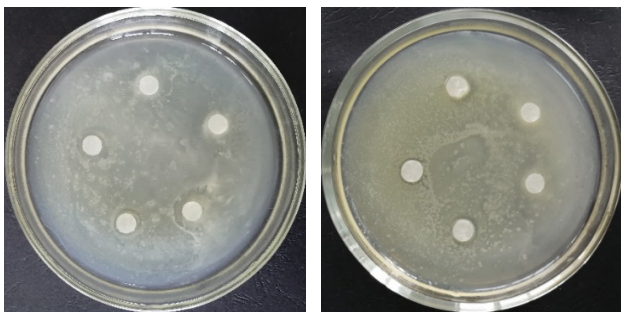
(a) (b)

Figure 13 Antimicrobial activity of yarrow suspension culture extracts against *Porphyromonas gingivalis*: a) extract No. 5 (10.0 ± 0.5 mm); b) extract No. 8 (10.1 ± 0.5 mm)



(a) (b)

Figure 14 Antimicrobial activity of yarrow suspension culture extracts against *Acinetobacter baumannii*: a) extract No. 5 (10.0 ± 0.5 mm); b) extract No. 8 (9.9 ± 0.5 mm)



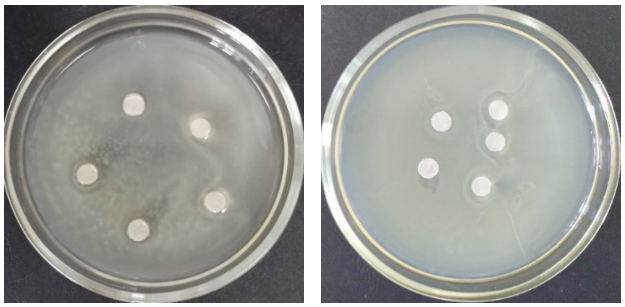
(a) (b)

Figure 15 Antimicrobial activity of yarrow suspension culture extracts against *Borrelia burgdorferi*: a) extract No. 5 (8.4 ± 0.4 mm); b) extract No. 8 (8.3 ± 0.4 mm)

cultures, with 82–91% viability and a growth index of 9.56–10.23; and hairy root cultures (hairy roots), with a growth index of 26.0.

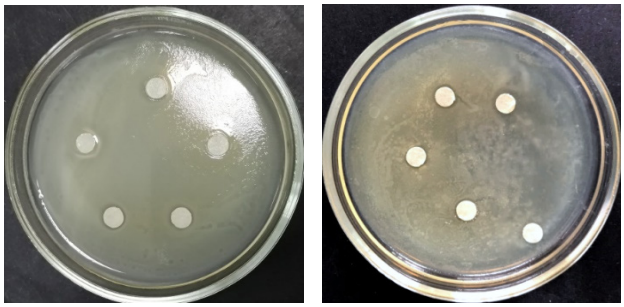
All the cell cultures contained essential oils, flavonoids, glycosides, phenolic acids, carotenoids, as well as vitamins C and E. The suspension cultures had a high content of dominant compounds – essential oils, flavonoids, and glycosides.

We obtained nine samples of ethanol extracts from the biomass of yarrow suspension culture using different extraction parameters: temperature (30, 50, and 70°C), volume of ethanol (30, 50, and 70%), and extraction time (2, 4, and 6 h). HPLC showed the presence of compounds with potential geroprotective properties: phenylpropanoids, flavonoids, and simple phenols. Quantitatively, the dominant compounds were caffeic acid, cynaroside, 4,5-dicofeylquinic acid, apigenin, and luteolin. In addition, HPLC revealed a number of compounds that have not been previously reported in yarrow, such as cumic aldehyde, umbelliferone, 3-caffeylquinic acid, and caffeic acid.



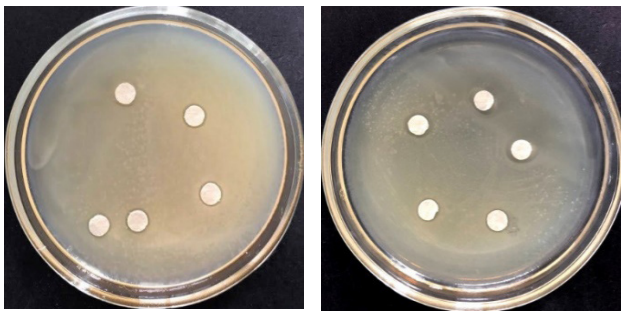
(a) (b)

Figure 16 Antimicrobial activity of yarrow suspension culture extracts against *Propionibacterium acnes*: a) extract No. 5 (10.2 ± 0.5 mm); b) extract No. 8 (10.0 ± 0.5 mm)



(a) (b)

Figure 17 Antimicrobial activity of yarrow suspension culture extracts against *Aggregatibacter actinomycetemcomitans*: a) extract No. 5 (8.6 ± 0.4 mm); b) extract No. 8 (8.6 ± 0.4 mm)



(a) (b)

Figure 18 Antimicrobial activity of yarrow suspension culture extracts against *Streptococcus intermedius*: a) extract No. 5 (8.1 ± 0.4 mm); b) extract No. 8 (8.1 ± 0.4 mm)

Our *in vitro* experiments with the extracts established the presence of antioxidant properties and antimicrobial activity against pathogenic and opportunistic strains, including *Escherichia coli*, *Candida albicans*, *Helicobacter pylori*, and *Pseudomonas aeruginosa*. Due to their geroprotective potential, yarrow cell cultures can be used to create nutraceuticals that prevent premature aging.

Further research in this area could focus on developing techniques to isolate and purify individual bioactive compounds with geroprotective potential from the extracts of yarrow suspension culture biomass. Particular attention should be drawn to those substances which were found in yarrow cell cultures for the first time, namely cumin aldehyde, umbelliferone, 3-caffeylquinic acid, and caffeic acid.

CONTRIBUTION

The authors are equally responsible for the findings and the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Shabalin VN, Shatokhina SN. Ageing as a fundamental mechanism of living matter adaptation and development. Ulyanovsk Medico-biological Journal. 2016;(4):53–61. (In Russ.).
2. Veronese N, Cereda E, Stubbs B, Solmi M, Luchini C, Manzato E, et al. Risk of cardiovascular disease morbidity and mortality in frail and pre-frail older adults: Results from a meta-analysis and exploratory meta-regression analysis. Ageing Research Reviews. 2017;35:63–73. https://doi.org/10.1016/j.arr.2017.01.003.
3. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 study. Journal of the American College of Cardiology. 2020;76(25):2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010.
4. Zhuravlev AK, Golubev YuYu. Topical issues of early display of diseases of aging, possibility of classical and traditional east medicine. Russian Journal of Humanistic Psychology. 2016;3(1)(9):47–52. (In Russ.). https://doi.org/10.18334/lp.3.1.36497.
5. Liu Y, Weng W, Gao R, Liu Y, Monacelli F. New insights for cellular and molecular mechanisms of aging and aging-related diseases: Herbal medicine as potential therapeutic approach. Oxidative Medicine and Cellular Longevity. 2019;2019. https://doi.org/10.1155/2019/4598167.
6. Aronson L. Healthy aging across the stages of old age. Clinics in Geriatric Medicine. 2020;36(4):549–558. https://doi.org/10.1016/j.cger.2020.06.001.
7. Trendelenburg AU, Scheuren AC, Potter P, Müller R, Bellantuono I. Geroprotectors: A role in the treatment of frailty. Mechanisms of Ageing and Development. 2019;180:11–20. https://doi.org/10.1016/j.mad.2019.03.002.

8. Pigarev SE, Trashkov AP, Panchenko AV, Yurova MN, Bykov VN, Fedoros EI, et al. Evaluation of the genotoxic and antigenotoxic potential of lignin-derivative BP-C2 in the comet assay in vivo. Environmental Research. 2021;192. https://doi.org/10.1016/j.envres.2020.110321.
9. Agar OT, Dikmen M, Ozturk N, Yilmaz MA, Temel H, Turkmenoglu FP. Comparative studies on phenolic composition, antioxidant, wound healing and cytotoxic activities of selected *Achillea* L. species growing in Turkey. Molecules. 2015;20(10):17976–18000. https://doi.org/10.3390/molecules201017976.
10. Sarubbo F, Moranta D, Pani G. Dietary polyphenols and neurogenesis: Molecular interactions and implication for brain ageing and cognition. Neuroscience and Biobehavioral Reviews. 2018;90:456–470. https://doi.org/10.1016/j.neubiorev.2018.05.011.
11. Serra D, Almeida LM, Dinis TCP. Polyphenols in the management of brain disorders: Modulation of the microbiota-gut-brain axis. Advances in Food and Nutrition Research. 2020;91:1–27. https://doi.org/10.1016/bs.afnr.2019.08.001.
12. Shahani S, Rostamnezhad M, Ghaffari-Rad V, Ghasemi A, Pourfallah TA, Hosseinimehr SJ. Radioprotective effect of *Achillea millefolium* L. against genotoxicity induced by ionizing radiation in human normal lymphocytes. Dose-Response. 2015;13(1). https://doi.org/10.1177/1559325815583761.
13. Wang L, Xie Y, Yang W, Yang Z, Jiang S, Zhang C, et al. Alfalfa polysaccharide prevents H_2O_2-induced oxidative damage in MEFs by activating MAPK/Nrf2 signaling pathways and suppressing NF-κB signaling pathways. Scientific reports. 2019;9(1). https://doi.org/10.1038/s41598-018-38466-7.
14. Ali SI, Gopalakrishnan B, Venkatesalu V. Pharmacognosy, phytochemistry and pharmacological properties of *Achillea millefolium* L.: A review. Phytotherapy Research. 2017;31(8):1140–1161. https://doi.org/10.1002/ptr.5840.
15. Setze WN. The phytochemistry of cherokee aromatic medicinal plants. Medicines. 2018;5(4). https://doi.org/10.3390/medicines5040121.
16. Vladic J, Jakovljevic M, Molnar M, Vidovic S, Tomic M, Drinic Z, et al. Valorization of yarrow (*Achillea millefolium* L.) by-product through application of subcritical water extraction. Molecules. 2020;25(8). https://doi.org/10.3390/molecules25081878.
17. Murashige T, Scoog F. A revised medium for rapid growth and bio assays with Tobacco tissue culture. Physiology Plantarum. 1962;15(3):473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.
18. Richaud F, Aubry C, Beyou A, Boulanger F, Estramareix C, Fleury-Guerout A-M, et al. The Agrobacterium rhizogenes root-inducing system. In: Verma DPS, Brisson N, editors. Molecular genetics of plant-microbe interactions. Dordrecht: Springer; 1987. pp. 6–10. https://doi.org/10.1007/978-94-009-4482-4_2.
19. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research. 1968;50(1):151–158. https://doi.org/10.1016/0014-4827(68)90403-5.
20. Ladygina EYa, Safronich LN, Otryashenkova VEh, Balandina IA, Grinkevich NI, Sorokina AA, et al. Khimicheskiy analiz lekarstvennykh rasteniy [Chemical analysis of medicinal plants]. Moscow: Vysshaya shkola; 1983. 176 p. (In Russ.).

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