In vitro study of the antioxidant activity of extracts from dried biomass of callus, cell suspension, and root cultures

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Abstract. The most promising sources of antioxidants are plant objects that contain natural antioxidants such as polyphenols, carotenoids, and vitamins. The paper studies the antioxidant activity of extracts from dried biomass of callus, suspension cell cultures, and root cultures in vitro. The study of the antioxidant activity of plant extracts showed that the maximum antioxidant activity (250.6 mg AA/g) is possessed by extracts from the dried biomass of in vitro hyssop root cultures. According to this indicator, extracts from dried biomass of hyssop hairy exceed extracts from dried biomass of Amur maakia root cultures by 2.23 times, and extracts from dried biomass of Siler root cultures by 5.61 times. Extracts from the dried biomass of in vitro callus, cell suspension, and root cultures of Siler do not possess high antioxidant activity, which varies from 16.56 to 44.66 mg AA/g. The maximum indicator of antioxidant activity for this medicinal plant is observed in extracts from dried biomass of in vitro root cultures. For extracts from dried biomass of in vitro callus, cell suspension and root cultures of Amur maakia, the range of antioxidant activity indices is from 85.40 mg AA/g to 112.33 mg AA/g. The maximum antioxidant activity was observed in extracts from dried biomass of in vitro root cultures. A higher accumulation of anthocyanins was also found in the callus culture of Malus sieversii f. niedzwetzkyana cultured on a nutrient medium containing both auxins and cytokinins.

1.Introduction
In recent years, research on medicinal plants has intensified in terms of searching for species whose organs contain vital biologically active substances for the treatment of diseases such as cardiovascular, oncological, diabetes, and others. The problem of cardiovascular diseases is acute. These diseases remain the leading cause of death worldwide. In 2010, they accounted for 29.6% of all deaths. According to the WHO forecast, this figure will increase. In Russia, heart diseases rank first among the causes of disability in the population of our country. Among men, 4% qualify as 1 degree of disability, 60% - as 2 degree of disability. The prevalence of the disease in children of the first 14 years of life has increased significantly - almost 2.5 times over the period 2000–2014. In 2014, this figure reached 75.3% of coronary heart disease cases registered among the entire population of the country. This group includes many diseases, but three of them - coronary heart disease, including myocardial infarction, arterial hypertension, and cerebrovascular diseases, including stroke - account for 80% of deaths [1].

Thousands of different plants grow on the earth. Among them are a large number of medicinal ones. They are found in mountains, forests, steppes, deserts, and swamps. Due to their wide distribution, availability, and valuable properties, medicinal plants have been used since ancient times.
Today, as never before there is an acute problem of creating products with a therapeutic and prophylactic effect in the food industry and public catering. This problem can be solved by developing technologies for combined food products using medicinal wild-growing food and cultural raw materials.

Currently, one of the strategic directions in the development of state policy in the field of healthy nutrition is the creation of safe food products containing amino acids, antioxidants, vitamins, and minerals. This is due to the fact that, according to the World Health Association, diet and lifestyle have a great impact on human health. It is scientifically substantiated that not only the energy value of the absorbed product is important in nutrition, but also its provision with micronutrients [2].

Over the past few years, interest in non-traditional crops of fruit and berry plants has significantly increased, which, on the one hand, are characterized by a high content of natural antioxidants and biologically active substances, and on the other hand, by attractive decorative characteristics.

Wild plants are an additional reserve for food. They allow, on the one hand, to diversify the diet, and on the other hand, to enrich it with the necessary biologically active substances. Researchers studying nature and its flora note that knowledge of plant resources not only allows a person to be provided with food but also guarantees optimal psychophysiological adaptation to harsh environmental conditions. Moreover, it is necessary to use those medicinal plants in which the chemical composition and pharmacological properties are well studied [3].

The complex chemical composition and multi-vector positive properties of each component of a medicinal plant in the human body, with a reasonable combination, allow us to develop a wide range of enriched products for healthy nutrition.

Much attention is paid to the study of medicinal plants in Russia. Their research is conducted from a variety of perspectives, starting with massive field chemical analyzes for the content of biologically active substances - alkaloids, glycosides, saponins, tannins, essential oils, etc. and ending with clinical trials of pharmacological preparations; study the distribution of medicinal plants and stocks of their raw materials; carry out work on the introduction into the culture of new medicinal plants, which have justified themselves as a source of raw materials for obtaining medicinal products, but do not have a sufficiently secure raw material base in nature. The results of these studies are constantly being introduced into healthcare practice. More and more new plants and preparations based on them are allowed for use. In this regard, the need for medicinal plant raw materials in domestic health care is steadily growing [4].

Among the Siberian medicinal plants, the following are promising: golden root, Maral root, purple milk-vetch, sweetvetch, lesser butterfly-orchid, common melilot, skullcaps, and many others. Solving the problem of treating patients is aimed at finding corrective drugs that can help eliminate the undesirable consequences of anticancer chemotherapy [2–4]. They reduce the toxic effect of cytostatics, do not block their therapeutic activity, and protect the genetic structures of cells and tissues. Relatively recently, the anticancer drug Paclitaxel has appeared in clinical practice. It is used in the treatment of ovarian cancer, breast cancer, non-small cell lung cancer, squamous cell carcinoma of the head and neck, transitional cell carcinoma of the bladder, esophageal cancer, leukemia, Kaposi's sarcoma in AIDS patients. Currently, Paclitaxel is one of the most active second-line chemotherapy drugs in patients with disease progression [5–7]. The mechanism of its cytotoxic antimitotic action is due to the ability to bind to β-tubulin, which activates the assembly of microtubules and stabilizes them [8]. The mechanism of action of the drug has also shown its apoptosis-inducing effect [9]. Biotechnological preparations are not inferior in quality to analogs of natural origin; therefore, their use in medicine is promising. For example, the industrial method of growing isolated cultures of Scutellaria baicalensis Georgi [10] under controlled conditions allows obtaining a significant amount of valuable medicinal raw material in a short time (30–45 days). There are enough similar examples of the successful use of medicinal plants in biotechnology to obtain valuable biologically active substances (BAS). For example, to obtain secondary metabolites in the hairy roots culture of Hedysarum theinum Krasnob [11] and other species.

In biological systems, reactive oxygen and/or nitrogen damage DNA molecules and lead to the oxidation of lipids and proteins in cells. Exposure to smoking, alcohol, radiation, or environmental toxins, as well as aging, increase oxidation processes, which leads to the development of certain chronic
and degenerative diseases. One solution to this problem is the use of antioxidants as dietary supplements. At the same time, recent studies show that the use of synthetic antioxidants, as a rule, does not give a positive effect, and in some cases even harmful [12]. While the components of plant antioxidants (including polyphenols) help reduce free radical processes and are also involved in a number of other metabolic processes that ensure the normal functioning of the cell. It has been proven that the introduction into the daily diet of foods, especially of plant origin, rich in compounds with antioxidant properties, significantly reduces the risk of developing cardiovascular, neurodegenerative, and oncological diseases [13].

Also, many antioxidants that reduce the intensity of free radical processes also perform a number of other metabolic functions. This is especially true for the group of non-enzymatic antioxidants. Numerous biochemical reactions with their participation are known that are not directly related to the antioxidant properties of these compounds [14].

The most promising sources of antioxidants are plant objects containing such natural antioxidants as polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamins E and C) [6, 7]. Typically, these natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and antitumor [15].

2. Research objects and methods

The objects of the study were extracts from dried biomass of in vitro callus, cell suspension, and root cultures of medicinal plants: hyssop (H1, H2, H3), Siler (S1, S2, S3), and Amur maakia (M1, M2, M3).

Cultivation of callus cell cultures was carried out under sterile conditions, in the dark at 24 ± 1 °C (Binder BD 53 incubator) and relative air humidity 60–70%. The subcultivation cycle for callus cultures was 28–35 days. During reinoculation, callus was divided into 2–3 parts, depending on the growth, and transplanted onto a medium of identical composition.

Tissues and organs of 30–35 days were used as explants to obtain the callus of Amur maakia: hypocotyl, cotyledons, young leaves, roots, and shoots, which were cut with a scalpel into 10–20 mm segments, placed on agar medium in flasks and Petri dishes with a 9 cm diameter. Initially, seeds placed on media of different compositions in Petri dishes and flasks were used as explants for hyssop and Siler, but due to low germination, bacterial and fungal contamination, it was decided not to obtain callus by this method. Subsequently, young seedlings (14 days of age) were used for hyssop as a starting material for experiments, while young seedlings and cotyledonous leaves were used for Siler.

To select the best lines of primary suspension cultures of medicinal plant cells, they were grown on nutrient media similar to those used for growing callus cultures for 30 days in the dark to determine the viability of the suspension.

The antioxidant activity of plant extracts was determined by their ability to reduce the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH, C10H12N5O6, M = 394.33). The reaction of the interaction of antioxidants with DPPH-radical proceeds according to the scheme:

\[ \text{DPPH}^* + \text{AH} \rightarrow \text{DPPH-H} + \text{A}^* \]

As a result of the reduction of the DPPH radical by the antioxidant, the purple-blue color of DPPH in ethanol decreases, and the reaction is monitored by the change in optical density by conventional spectrophotometric methods.

For the analysis, plant extracts were mixed with 2.85 ml of a freshly prepared 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl. The mixture was incubated in the dark at room temperature for 30 min. The decrease in optical density compared to the control (70% methanol solution) was recorded at 517 nm (UV-3600 spectrophotometer, Shimadzu, Japan) [15]. As standard solutions, we used solutions of ascorbic acid (AA) of known concentration. The results of the analyzes were expressed in mg AA equivalent per gram of dry weight of the plant culture or individual compound (mg AA/g). The analysis of the antioxidant activity of the samples was carried out in three replicates.
3. Results and discussion

The results of determining the viability of primary suspension cultures are presented in Table 1.

| Medicinal plants | Abbreviated name of the medium | MS-1 | MS-2 | MS-3 | B5-1 | B5-2 | B5-3 |
|------------------|-------------------------------|------|------|------|------|------|------|
| Amur maackia     |                               | –    | –    | –    | 33.9 | –    | 43.0 |
| Hyssop           |                               | –    | 48.3 | –    | –    | 51.2 | –    |
| Siler            |                               | –    | –    | –    | 30.5 | –    | –    |

* not viable

Figure 1 shows the primary suspension cell cultures of the studied plants.
Figure 1. Obtaining a primary suspension cell culture – transfer of loose callus cultures into a liquid medium: a – Amur maakia (B5-1 medium), b – Amur maakia (B5-3 medium), c – hyssop (MS-2 medium), d – hyssop (B5-2 medium), e – Siler (B5-1 medium).

The analysis of table 1 indicates that all primary suspension cultures of medicinal plant cells are characterized by low viability from 30.5% to 51.2%, which is primarily due to the low growth rate of calli used for introduction into culture.

The results of studying the antioxidant activity of extracts from dried biomass of in vitro callus, cell suspension, and root cultures of medicinal plants are presented in Table 2.

Table 2. The results of studying the antioxidant activity of extracts from dried biomass of in vitro callus, cell suspension and root cultures of medicinal plants.

| Medicinal plant / Sample code | Antioxidant activity, mg AA/g |
|------------------------------|-------------------------------|
| Hyssop / H1                  | 73.12±3.66                   |
| Hyssop / H2                  | 75.09±3.75                   |
| Hyssop / H3                  | 250.60±11.3                  |
| Siler / S1                   | 16.56±2.26                   |
| Siler / S2                   | 21.89±1.09                   |
| Siler / S3                   | 44.66±2.23                   |
| Amur maakia / M1             | 86.94±3.45                   |
| Amur maakia / M2             | 85.40±4.27                   |
| Amur maakia / M3             | 112.33±6.52                  |

The study of the antioxidant activity of plant extracts showed that the maximum antioxidant activity (250.6 mg AA/g) is possessed by extracts from the dried biomass of in vitro hyssop root cultures. According to this indicator, extracts from dried biomass of hyssop hairy exceed extracts from dried biomass of Amur maakia root cultures by 2.23 times, and extracts from dried biomass of Siler root cultures by 5.61 times. Earlier Chrpova et al. (2010) showed that aqueous extracts of intact hyssop plants were characterized by the antioxidant activity of 43.5 mg AA/g [6]. Our results indicate that the use of hairy root cultures as a source of substances with antioxidant properties is promising.

Extracts from the dried biomass of in vitro callus, cell suspension, and root cultures of Siler do not possess high antioxidant activity, which varies from 16.56 to 44.66 mg AA/g. The maximum indicator of antioxidant activity for this medicinal plant is observed in extracts from dried biomass of in vitro root
cultures. Earlier, in a number of research works it was found that the antioxidant properties of Siler extracts are associated with the presence of polysaccharides, chromones, phenolic compounds in them [15].

For extracts from dried biomass of in vitro callus, cell suspension and root cultures of Amur maakia, the range of antioxidant activity indices is from 85.40 mg AA/g to 112.33 mg AA/g. The maximum antioxidant activity was observed in extracts from dried biomass of in vitro root cultures. It is known from the literature sources that an important role in the regulation of the secondary metabolism of cultivated plants is played not only by the use of a specific phytoregulator, but also by the ratio of the used auxins and cytokinins. For example, in the study by Duangporn, Siripong (2009) on the effect of hormones on the biosynthesis of phyllanthusol A in the callus culture of Phyllanthus acidus, it was found that the Murasage-Skoog medium with the addition (2 mg/L) and BAP (0.5 mg/L) was found to be optimal [10]. A higher accumulation of anthocyanins was also found in the callus culture of Malus sieversii f. niedzwetzkyana cultured on a nutrient medium containing both auxins and cytokinins in the form of BAP [11]. Earlier in a number of papers on Amur maakia, it was shown that the antioxidant properties of its extracts are due to the presence of polyphenols of various structures, in particular isoflavonoids, stilbenes, and chalcones, both for callus cultures and intact plants [1].

Thus, as a result, the antioxidant activity of extracts from dried biomass of in vitro callus, cell suspension, and root cultures was studied.

4. Conclusion
The antioxidant activity of extracts from dried biomass of callus, cell suspension, and root cultures have been studied in vitro. The extracts from the dried biomass of in vitro hyssop root cultures have the maximum antioxidant activity (250.6 mg AA/g). In terms of antioxidant activity, the extracts from dried biomass of hyssop hairy exceed extracts from dried biomass of Amur maakia root cultures by 2.23 times, and extracts from dried biomass of Siler root cultures by 5.61 times. Extracts from the dried biomass of in vitro callus, cell suspension, and root cultures of Siler do not possess high antioxidant activity, which varies from 16.56 to 44.66 mg AA/g. For extracts from dried biomass of in vitro callus, cell suspension and root cultures of Amur maakia, the range of antioxidant activity indices is from 85.40 mg AA/g to 112.33 mg AA/g. A higher accumulation of anthocyanins was also found in the callus culture of Malus sieversii f. niedzwetzkyana cultured on a nutrient medium containing both auxins and cytokinins.

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