Determination of Biologically Active Substances in the Suspension Culture of Oxycoccus Palustris Pers

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Abstract. Due to changes in environmental conditions in the negative direction, a reduction in the areas of growth of medicinal plants was noted. For this reason, there is a reduction in the volume of medicinal plant raw materials. Marsh cranberries were no exception. It is it that is considered a valuable source of various biologically active compounds. The purpose of this work is to obtain a suspension culture of *Oxycoccus palustris* Pers in laboratory conditions and to carry out biochemical studies of the grown biotechnological raw materials. According to the results of the study, it was found that biotechnological raw materials are rich in antioxidants – 89.9 ± 0.46%. The quantitative content of arbutin in the studied raw materials is 1.2 ± 0.05%, and the proportion of anthocyanins is 7.65 ± 0.02%. In suspension culture of *Oxycoccus palustris* Pers. ascorbic acid and tannins are present, the content of which is: 1.13 ± 0.01% and 2.70 ± 0.05%, respectively.

1 Introduction

Oxycoccus palustris Pers. is considered a very valuable plant, as the fruits have nutritional and pharmaceutical properties. It is in high demand both in the Russian and in the world market [1].

Marsh cranberry is an evergreen creeping shrub. Aboveground shoots are lignified and flexible, the color of which varies from dark brown to red. Annual branches are short and fluffy. The length of vegetative shoots can reach 80 cm. They have short, erect or rising flowering branches [2].

The biochemical composition of the berries of *Oxycoccus palustris* Pers. includes various compounds. For example, the content of mineral elements in the total composition can reach 0.19–0.28%. The seeds contain the largest amount of mineral components (10.3%), in the skin of the berry their amount decreases to 1.26%, and the lowest indicator in the pulp is 0.2%.

The fruits of the marsh cranberry are in great demand due to their positive effect on human health [3]. The composition of berries includes: more than 80% water and 10% carbohydrates, various vitamins (vitamin C, provitamin A, nicotinic acid (PP), riboflavin (B

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2), thiamine (B 1)), organic acids (malic, quinic and citric acids, benzoic and glucuronic acids are present in small quantities), various phenolic groups and mineral salts [4, 5].

Three classes of flavonoids (flavonols, anthocyanins and proanthocyanidins), catechins and phenolic acids are present in the phenolic compounds of *Oxycoccus palustris* Pers., of which hydroxycoric acid is considered the main one [3, 6, 7].

The main anthocyanins of *Oxycoccus palustris* Pers. are presented in the form of galactosides and arabinosides (cyanidin and peonidin) [8]. The quantitative content of anthocyanins ranges from 25 to 91 mg per 100 g of mature fruits [9].

Suspension culture is an alternative to herbal medicinal raw materials. Biotechnology methods make it possible to obtain economically valuable pharmacologically active compounds from biotechnological raw materials. This is due to the high growth rate; the possibility of studying the influence of exogenous factors on the metabolism and growth of biomass; with a simple subcultivation procedure, and carrying out technological processes using bioreactors for large-scale production [10]. To obtain a suspension of cells of pharmaceutical raw materials, callus cultures are most often used. At the first stage of obtaining a suspension culture, 2-3 g of fresh loose mass of callus cells are introduced into 60-100 milliliters of liquid nutrient medium. The suspension is cultured in laboratory shaking flasks. Under these conditions, the suspension cells are enriched with oxygen, that is, the aeration process takes place, and the growing biomass breaks up into separate aggregates. The increase in the required volume of biomass lasts on average 15-70 days [11, 12].

In large-scale productions, open or closed systems are used in flow or periodic modes in order to obtain a suspension culture of cells or tissues [13, 14].

To date, a large number of nutrient media with different compositions are known for cultivation and microclonal reproduction. The most common and frequently used media are: Murashige and Skoog medium, Gamborg, Woody plant medium, White, Anderson and many others. [11, 13].

The presence of carbohydrates (sucrose, glucose, fructose) in the nutrient medium is due to the fact that many cells of callus cultures do not contain chlorophyll and lack the ability to autotrophic nutrition.

The presence of phytohormones in the environment also plays an important role. Auxins (2,4–D, IAA, and NAA) can cause cell dedifferentiation, and cytokinins (kinetin, 6–BAP, and zaetin) induce explant cell division [15].

2 Materials and methods

The object of the study was a suspension culture of *Oxycoccus palustris* Pers., grown on the basis of the Resource Sharing Center "Ecology, biotechnology and processes for obtaining environmentally friendly energy carriers" of the Volga State Technological University, Yoshkar-Ola.

To obtain the primary callus, and then a suspension culture, marsh cranberry seeds were used.

Cultivation of seeds, primary callus and suspension culture was carried out on a Murashige and Skoog basal medium composition (MS).

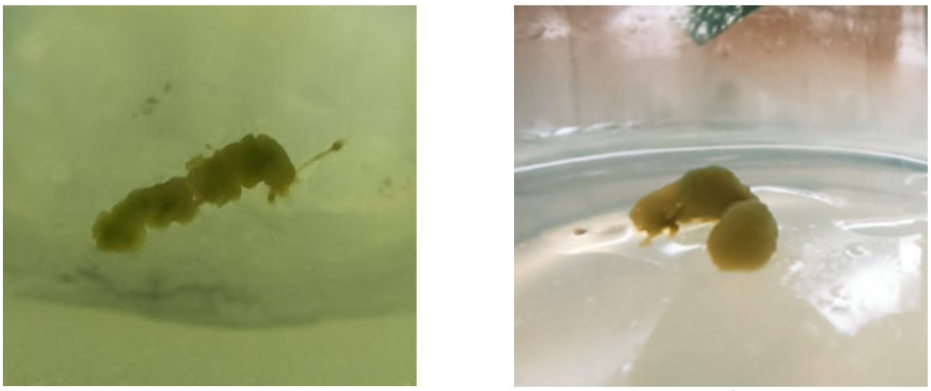

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Fig. 1. Cultivation of callus. 1 – primary callus; 2 – callus culture.

Before introduction into the culture in vitro, the seeds of the test plant were washed in running water using detergent, then the sample was rinsed in distilled water. In the future, stepwise sterilization was used: ethyl alcohol (70%), the exposure time was 30 seconds, after which the seeds were placed in a 5% solution of the detergent "Lysoformin–3000", the sterilization time was 7 minutes. At the end of the sterilization stage, the plant explants were washed three times with sterile distilled water. The sterilized seeds were planted on a hormone-free dense nutrient medium of Murashige and Skoog. A temperature of 22-25 °C and a photoperiod of 16 hours were maintained in the culture room.

The germination period of the seeds was 21 days. In the future, the sprouts were used to obtain primary callus. They were transplanted to a fresh nutrient medium, which included phytohormone 2,4–D (2 mg/l). After 10-14 days of cultivation, the appearance of mass callus formation began (Figure 1.1). The maximum volume of biomass was observed on day 28 (Figure 1.2).

The suspension consists of single cells and aggregates that grow in a liquid nutrient medium of a certain composition under sterile conditions. For deep suspension cultivation, it is recommended to use media that do not contain Ca ions. It is important to exclude cytokinins from the nutrient medium or reduce their concentration, and increase the concentration of auxins.

Suspension culture was grown in laboratory conditions from a piece of loose callus in a sterile flask filled with liquid nutrient medium. Cultivation was carried out in laboratory shaking flask in the dark, at a rotation speed of 115-125 rpm. This is necessary to ensure aeration of the tissue and the growth of cell mass. The temperature range in the culture room was 22-25 °C.

The growth of the suspension biomass lasted three weeks, after which a passage was carried out on a freshly brewed medium with a similar composition and continued cultivation for another 3 weeks.

Antioxidant activity (AOA) in biotechnological raw materials was determined according to the DPPH method [16]. The evaluation of AOA consists in the reaction of 2,2–diphenyl-1-picrylhydrazyl dissolved in methanol.

The method is based on the extraction of the test sample with the addition of 80% methanol, at a temperature of 80 °C for 15 minutes. Then the residuum was isolated by centrifugation (12,000 revolutions per minute) for 5 minutes. 2 ml of a reaction mixture consisting of DPPH and methanol was added to the selected supernatant (20 µl). The test extracts were kept for 30 minutes, then readings were taken on a Bio-Rad SmartSpec Plus spectrophotometer at a wavelength of 515 nm. Antioxidant activity is expressed as a percentage.

Biochemical analysis of the quantitative content of vitamin C in terms of absolutely dry raw materials was carried out by titration with 0.001 M solution of 2,6-dichlorophenolindophenolate sodium in the presence of hydrochloric acid.

Determination of anthocyanins in terms of cyanidin-3-o-glucoside was carried out by spectrophotometric method at a wavelength of 534 nm in a cell with a wall thickness of 10 mm.

Maceration was carried out with 96% containing 1% concentrated hydrochloric acid for 2 hours with constant stirring.

Biochemical analysis of tannins was carried out titrimetrically in terms of tannin, in the presence of a solution of 0.02 M potassium permanganate and indigosulfonic acid. The extraction was carried out with prepared water during boiling for 30 minutes with a return refrigerator with periodic stirring.

The content of hydroquinone derivatives in terms of arbutin was determined by spectrophotometric method. Extraction was carried out with 70% ethyl alcohol during boiling for 45 minutes with a return refrigerator with periodic shaking.

At the end of the experiment, all the results of the study were subjected to statistical processing.

3 Results and discussion

Initiated callus *Oxycoccus palustris* Pers. depending on the duration of the experiment, changed its color and consistency. On the 14th day of cultivation, the callus had a loose structure and a green color. On day 28, the color changed to dark green with brown fragments. The callus culture became more dense due to the increase in the cultivation time.

After 6 weeks of cultivation, a suspension culture of marsh cranberries was used for biochemical analysis. To study the biochemical composition of biotechnological raw materials, cellular biomass was used, which was separated from the liquid medium by centrifugation.

For the relevance of the conclusions of the experiment, a three-fold repetition of the measurement of the trait was used.

The data of the study of the biochemical composition in the suspension culture of *Oxycoccus palustris* Pers. are presented in Table 1.

Table 1. Data from the study of biologically active substances in various raw materials of marsh cranberries

Studied raw materials	**Biologically active compounds, %**				
	Vitamin C	**Arbutin**	**Tannins**	**Anthocyanins**	**Antioxidant activity**
Suspension culture of *Oxycoccus palustris* Pers.	1.13±0.01	1.2±0.05	2.70±0.05	7.65±0.02	89.9±0.46
Intact plant *Oxycoccus palustris* Pers.	1.55±0.05	-	-	0.15±0.09	25.19

During the study of the biochemical composition of biotechnological raw materials, a biochemical analysis of intact plant raw materials (berries) of *Oxycoccus palustris* Pers. collected in the Republic of Mari El in the Kilemarsky district was carried out in parallel.

According to the data, it can be concluded that the average vitamin C content varies in the tested samples from 1.13 to 1.55%. There is no significant difference in this feature between the two samples.

Also, it was found that the antioxidant activity of an intact plant is 25.19%, which is 3.5 times less than the indicators of the suspension.

The quantitative content of arbutin in the studied biotechnological raw materials is 1.2 ± 0.05%, and the proportion of anthocyanins is 7.65 ± 0.02%. Indicators of anthocyanins contained in marsh cranberries – 0.15%. Indicator of tannins in the suspension culture was 2.7%.

4 Conclusions

In the course of the work, a suspension culture was obtained. Suspension culture of *Oxycoccus palustris*.

Pers. contains all the studied secondary metabolites. Using the methods of biochemical analysis, the quantitative content of ascorbic acid (vitamin C) and tannins in the suspension culture was established. The quantitative content of arbutin, anthocyanins and antioxidant activity (AOA) was studied by instrumental analysis using spectrophotometry.

During the study of the biochemical composition of the suspension culture, an intact plant was studied as a control. And comparing the data obtained, it can be concluded that biotechnological raw materials are not inferior to wild plants in terms of the content of biologically active substances.

According to the data obtained, it is possible to recommend the suspension culture of *Oxycoccus palustris* Pers as an alternative biotechnological raw material for obtaining valuable secondary metabolites.

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