Optimization of extraction parameters of biologically active substances from dried biomass of callus, suspension cells and root cultures in vitro

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Abstract. Objects of research are the parameters of the extraction of biologically active substances from the dried biomass of callus, suspension cells and root cultures in vitro. The goal of the work was to optimize the parameters of the extraction of biologically active substances from the dried biomass of callus, suspension cells and root cultures in vitro. Organic solvents, a water module, the duration and temperature of the extraction of biologically active substances from the dried biomass of callus, suspension cells and root cultures in vitro were studied. It was found that the optimal parameters for the extraction of biologically active substances from dried biomass of callus cultures of Rhaponticum carthamoides are the following: methanol as a solvent, water module 1:10, extraction temperature 60 °C; for Rhodiola rosea: isopropanol as a solvent, water module 1:10, extraction temperature 60 °C; for Scutellaria baicalensis: acetone as a solvent, water module 1:10, extraction temperature 50 °C; for white cinquefoil: ethanol as a solvent, water module 1:20, extraction temperature 40 °C; for ginseng: acetone as a solvent, water module 1:5, extraction temperature 50 °C. The extraction time for all medicinal plants was 60 minutes. The novelty of this work is to optimize existing modes of extraction of biologically active substances from dried biomass of callus, suspension cells and root cultures in vitro for more complete extraction and use.

1. Introduction
An urgent problem in medicine and biology is the creation of new drugs based on plants, as well as the use of plant objects as industrial producers of target metabolites [1]. It is known [2] that up to 70% of medicinal antitumor drugs are either entirely of plant origin or contain components of plant origin. However, due to the unfavorable environmental situation, as well as the rapidly growing level of demand for medicinal raw materials, its deficit arises [3]. A new solution is the use of higher plants as cells and organs (hairy roots) as an alternative source of renewable environmentally friendly raw materials [4].

Somatic cells or cell culture is a unique biological system created experimentally. Fundamental studies in this area have established that cells grown in vitro often differ from whole plants [5–7]. And,
biologically active substances accumulate in them in different ways. Suspension cells grown in vitro grow intensively and accumulate a large amount of biomass [8–11]. Vital products (metabolites) secreted by in vitro cells differ from the metabolites of plants grown in the usual way [12–15]. The content of biologically active substances in cells obtained in vitro is often many times higher than their content in traditionally grown plants [16]. It was also established based on the experimental data, that it is necessary to carry out a lot of work to find the producer of the given biologically active substances, i.e. it is necessary to search for fundamental and applied methods of using suspension cells in vitro in order to isolate as many biologically active compounds from them as possible [17].

In Russia, cultures of higher plants, producers of biologically active compounds in vitro, are still not given due attention. Only a few scientific groups throughout Russia carry out fundamental and applied research, using, in particular, callus cultures as model objects [18]. Moreover, commercial projects successfully implemented in this area are practically absent in Russia. To date, there is only one example of obtaining a suspension of a culture cell suspension based on the biomass of the parapharmaceutical drug Vitagmal, which was created by the Institute of Plant Physiology Institute of the Russian Academy of Sciences (Department of Cellular Biology) (biotechnology under the direction of A.M. Nosov) and the commercial company “BIOFARMOS” (St. Petersburg, General Director O.A. Kotin). The drug has a pronounced anti-teratogenic effect. The Institute of Plant Physiology of the Russian Academy of Sciences has created collections of cell cultures that produce biologically active substances [14, 15]. The drug has a pronounced anti-teratogenic effect. The Institute of Plant Physiology of the Russian Academy of Sciences has created collections of cell cultures producing biologically active substances (VRKK RF) and in vitro cultivated roots obtained from more than 40 plant species belonging to 20 families [19–22].

Thus, the use of cell, tissue and organ cultures (hair roots) instead of intact plants will radically solve the problem of the lack of plant materials of rare species that do not grow in Russia (it will solve the problem of import substitution - independence from foreign supplies, patents and technologies). The availability of high-quality renewable plant materials will allow the creation of efficient and affordable nutraceuticals of wide consumption [23, 24].

2. Materials and methods
The process of extracting biologically active substances (BAS) was carried out as follows. 3.0 g of an in vitro sample of dry biomass of cells was precisely weighed, placed in a 50 ml tube, 40 ml of a solvent individual for each plant species was added, and shaken in a shaker for 60 min. The resulting suspension was filtered, the filtrate was separated by centrifugation at 3900 rpm until sedimentation of solid particles [13].

The solvent added in the previous step was evaporated from the obtained extract in vacuo in a 100 ml flask. The amount of extract was determined by weighing method. The remaining substance was refilled with solvent and the composition of the extract fractions was studied in vitro and determined by thin layer chromatography. The obtained TLC results for each plant were analyzed for the presence of witness substances, for example, quercetin, mangiferin, luteolin, rutin, quercetin-2-D-glucoside, caffeic acid, cinnamic acid, ferulic acid, synapinic acid and malvidin.

TLC was performed in accordance with the following procedure (according to OFS.1.2.1.2.0003.15). The dry residue after evaporation of the solvent from the entire extract was dissolved in 1 ml of a suitable extractant (methanol, methylene chloride, acetone) and a spot was applied to the plate for thin layer chromatography using a glass capillary [16].

The tablet was placed in a TLC chamber, after which the corresponding eluent was added. When using TLC with a silica gel plate, the extract was chromatographically gradient chromatographed in the CH2Cl2: MeOH system with a methanol concentration interval of 0–10%; the step was observed at 1%. In the case of reverse phase chromatography, the eluent system H2O: MeCN was used with an interval of acetonitrile of 0–20%, step 2%, trifluoroacetic acid was used as a modifier. This acid was poured in amount of 0.1% [23].
3. Results and discussion
The average yield of solids in total extracts was obtained as a result of the study of the effectiveness of various extraction systems. The results of determining the yield of the total extract depending on the nature of the solvent are presented in table 1.

Table 1. Efficiency of extraction of biologically active substances from callus cultures with test solvents.

| Plant                | Total extract yield % | Plant                | Total extract yield % |
|----------------------|-----------------------|----------------------|-----------------------|
|                      | methanol              |                      | ethyl acetate         |
|                      |                       | acetone              | isopropanol          |
| Rhaponticum carthamoides | 5.71±0.57            | 1.78±0.18            | 0.23±0.02            |
| Rhodiola rosea       | 2.88±0.29             | 1.64±0.16            | 0.75±0.08            |
| Scutellaria baicalensis | 3.12±0.31           | 1.53±0.15            | 4.62±0.46            |
| White cinquefoil     | 3.61±0.36             | 1.27±0.13            | 0.31±0.03            |
| Ginseng              | 2.32±0.23             | 1.50±0.15            | 5.24±0.52            |

According to the research results presented in table 1, organic solvents were selected to obtain suspension extracts using a Soxhlet apparatus. So, for example, acetone as an organic solvent is selected to obtain total extracts of ginseng and Scutellaria baicalensis. Methanol was selected to obtain extracts of the main biologically active substances from the dried biomass of cells in vitro Rhaponticum carthamoides. Using isopropanol, a complex of biologically active substances was obtained from callus cultures of Rhodiola rosea biomass. An aqueous solution of ethyl alcohol with a mass fraction of 70% was used to obtain the main biologically active substances from the biomass of dried callus cultures of white cinquefoil cells.

Tables 2 and 3 present the results of selecting the optimal parameters for the extraction of the biologically active substances complex from the dried biomass of callus cultures of Rhaponticum carthamoides.

Table 2. The yield (%) of dry extract of the biologically active substance complex from the dried biomass of callus cultures of Rhaponticum carthamoides at various extraction times.

| №, V solvent/m culture | The yield of extract, %, with different duration of the process, min |
|------------------------|------------------------------------------------------------------|
|                        | 10      | 30     | 60     | 120    | 180    | 360    |
| 1                      | 1:1     | 0.50±0.05 | 0.81±0.08 | 1.22±0.12 | 1.29±0.13 | 1.38±0.14 | 1.38±0.14 |
| 2                      | 1:2     | 0.80±0.08 | 0.94±0.09 | 1.35±0.14 | 1.58±0.16 | 1.67±0.17 | 1.71±0.17 |
| 3                      | 1:5     | 1.20±0.12 | 1.80±0.18 | 2.78±0.28 | 4.12±0.41 | 4.45±0.44 | 4.81±0.48 |
| 4                      | 1:10    | 1.40±0.14 | 1.98±0.20 | 5.96±0.60 | 5.94±0.59 | 5.97±0.60 | 6.04±0.60 |
| 5                      | 1:20    | 1.40±0.14 | 2.01±0.20 | 3.01±0.30 | 5.95±0.60 | 6.01±0.60 | 6.07±0.61 |

Table 3. Optimum temperature parameters for the extraction of biologically active substances from the dried biomass of callus cultures of Rhaponticum carthamoides.

| №, Temperature, °C | The yield of extract, %, with different duration of the process, min |
|--------------------|------------------------------------------------------------------|
|                    | 10      | 30     | 60     | 120    | 180    | 360    |
| 1                  | 25      | 1.20±0.12 | 1.80±0.18 | 2.78±0.28 | 5.88±0.59 | 5.95±0.60 | 5.81±0.58 |
| 2                  | 40      | 1.55±0.16 | 1.98±0.20 | 3.92±0.39 | 6.21±0.62 | 6.18±0.62 | 6.24±0.62 |
| 3                  | 60      | 1.79±0.18 | 2.35±0.24 | 7.38±0.74 | 7.05±0.71 | 7.01±0.70 | 7.12±0.71 |
According to tables 2 and 3, the maximum yield of the extract of the biologically active substance complex from the dried biomass of callus cultures of the cells of the medicinal plant of Rhaponticum carthamoides is achieved when the ratio of the volume of solvent to the mass of the feedstock is 1:10, the duration of the extraction process is at least 60 minutes and the process temperature is 60°C.

Similar data were obtained by optimizing the extraction parameters of the biologically active substance complex from the dried biomass of callus cultures of all studied medicinal plants: Rhodiola rosea, Scutellaria baicalensis, white cinquefoil and ginseng. The optimal extraction parameters of the BAS complex from the dried biomass of callus cultures of medicinal plant cells are presented in Table 4.

**Table 4.** Optimal parameters for the extraction of a biologically active substance complex from dried biomass of callus cultures of medicinal plant cells.

| Plant                        | Organic solvent | Water module | Duration, min | Temperature, °C |
|------------------------------|-----------------|--------------|---------------|-----------------|
| Rhaponticum carthamoides     | Methanol        | 1:10         | 60            | 60              |
| Rhodiola rosea               | Isopropanol     | 1:10         | 60            | 60              |
| Scutellaria baicalensis      | Acetone         | 1:10         | 60            | 50              |
| White cinquefoil             | 70% Ethanol     | 1:20         | 60            | 40              |
| Ginseng                      | Acetone         | 1:5          | 60            | 50              |

It follows from the tabular data that the optimal parameters for the extraction of the biologically active substances complex from the dried biomass of callus cultures of Rhaponticum carthamoides are: methanol as a solvent, water module 1:10, extraction temperature 60°C; for Rhodiola rosea: isopropanol as a solvent, water module 1:10, extraction temperature 60°C; for Scutellaria baicalensis: acetone as a solvent, water module 1:10, extraction temperature 50°C; for white cinquefoil: ethanol as a solvent, water module 1:20, extraction temperature 40°C; for ginseng: acetone as a solvent, water module 1:5, extraction temperature 50°C. The extraction time for all medicinal plants was 60 minutes.

4. Conclusion

Thus, the extraction parameters (duration, temperature, organic solvent, water module) of the biologically active substances complex from the dried biomass of callus cultures of medicinal plant cells were optimized in the process of research.

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