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RESEARCH PAPERS

# Growth and Biosynthetic Characteristics of *Phlojodicarpus sibiricus* Cell Suspension Cultures

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**Abstract**—Features of the growth and qualitative composition of secondary metabolites in two lines of suspension cell cultures of *Phlojodicarpus sibiricus* (Steph. ex Spreng.) K.-Pol., an endangered endemic species of Eastern Siberia, have been studied. A suspension cell culture of leaf origin demonstrated the best growth characteristics, which included growth indices for different criteria (dry and crude biomass and cell concentration): $I = 10–14$; specific growth rate $\mu = 0.3–0.4$ day$^{-1}$; the maximum dry biomass accumulation $M = 9.6$ g/L and economic coefficient $Y = 0.29$. A plant cell culture derived from a hypocotyl was characterized by lower growth parameters: $I = 3.6–4.9$, $\mu = 0.12–0.18$ day$^{-1}$, $M = 6.6$ g/L, $Y = 0.16$. Differences in the growth of the studied cultures correlated with the cell aggregation level: the "leaf" culture consisted mainly of small-size aggregates (10–30 cells), whereas the "hypocotyl" culture was presented by large aggregations (50 cells or more). The instrumental cultivation of the small-aggregated suspension cell culture of leaf origin was carried out using two types of laboratory bioreactors (bubble column and stirred tank). Cultivation in a bubble column reactor improved the basic growth characteristics of the cell culture: the growth index for the dry biomass $I = 12.7$; dry biomass productivity $P = 0.78$ g/L day, $\mu = 0.18$ day$^{-1}$, $M = 15.8$ g/L, $Y = 0.49$. In the case of a stirring tank reactor, all growth parameters were decreased, which was probably connected with the cell damage with stirring devices. Additionally, a phytochemical analysis of the secondary metabolite composition in the studied cell cultures was carried out in comparison with the root cells of intact *Ph. sibiricus* plants. Significant differences in the composition of phenolic compounds were revealed between in vitro cell cultures and plant roots. In the case of cell cultures, polar (hydrophilic) compounds belonging to phenolic derivatives (coumarin glycosides and benzofurans) prevailed. In roots, the main components were more hydrophobic (khellactone ethers). The obtained results confirmed the earlier developed concept of differences in the secondary metabolism of in vitro and in vivo plant cells.

**Keywords:** *Phlojodicarpus sibiricus*, suspension cell culture, bioreactors, coumarins

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## INTRODUCTION

Study of secondary metabolism is one of the most relevant tasks in current plant physiology and biochemistry. The use of cell cultures of higher plants provides fundamentally new approaches for the investigation of this process. Along with the possibility to study different factors influencing on the formation of secondary metabolites (growth regulators, elicitors, stress impacts, etc.), the use of in vitro cell cultures makes it possible to study the formation of specialized metabolic compounds in dedifferentiated proliferating cells devoid of the control systems of the organism. A comparison of the secondary metabolism specificity in intact plants (where it is usually shared between different plant organs and occurs in specialized cells) and in vitro cells allows a researcher not only to determine the mechanisms and regulating systems of this process but also to clarify the functions of secondary metabolites in plants' vital functions [1].

A study into the secondary metabolism in plant cell cultures also has an applied aspect, namely, biotechnological use of cell cultures as a renewable high-qual-

*Abbreviations:* UHPLC-ESI-MS—ultra-high-performance liquid chromatography with mass spectrometry detection and electrospray ionization.

ity raw material to obtain biologically active compounds of plant origin, which can be used to produce new safe drugs for the therapy of various diseases [1]. A study of rare and endemic plant species that are used in folk medicine and whose natural resources are limited or unavailable represents a promising direction in this field of science. From this point of view, Siberian flora is of a doubtless interest with a special accent on the natural populations of medicinal plants of Yakutia. In this region, many valuable plant species have a very limited habitat, and their populations are being rapidly reduced. Among such species, there is *Phlojodicarpus sibiricus* (Steph. ex Spreng.) K.-Pol. (family Umbelliferae) listed in the Red Books of Yakutia, Amur region, and Transbaikalia [2–4].

Roots of *Ph. sibiricus* are used for the prevention and therapy of cardiovascular diseases, some neurological disorders, and stomach and lung dysfunction. The main curative effects of *Ph. sibiricus* are determined by the presence of phenolic compounds, namely, coumarins (pyrano- and hydroxycoumarins). It is known that some coumarins possess antispasmodic, anticoagulant, hypolipidemic, sedative, neuroprotective, antitumor, bacteriostatic, and antioxidant activities [5].

The most specific secondary metabolites of *Ph. sibiricus* are prenylated coumarins represented mainly by pyranocoumarins (khellactone, visnadin, dihydrosamidin, suksdorfin, pteryxin, etc.) and, to a lesser degree, furanocoumarins (peucenidin). A composition of rhizomes and roots of *Ph. sibiricus* also includes "simple" coumarins (various derivatives of hydroxycoumarin, such as umbelliferone and scopoletin) [5–9].

Being a pharmaceutical plant, *Ph. sibiricus* was used as a raw material for production of anticonvulsive drug Floverin (dihydrosamidin/visnadin mix) and complex preparation Safinor possessing cardiovascular activity. Today, the manufacturing of these preparations is suspended due to reduction of the natural raw material [10].

In view of the growing interest in *Ph. sibiricus* as a basis for development of pharmaceutical preparations and biologically active food additives and due to the impossibility of the use of its natural populations, a study into the specificity of its secondary metabolism using in vitro cell cultures of this species is of not only fundamental but also applied significance.

Thus, the aim of this study was the investigation of growth and biosynthetic (secondary metabolite formation) characteristics of *Ph. sibiricus* suspension cell cultures grown in flasks and different bioreactor types.

## MATERIALS AND METHODS

Suspension cell cultures of *Ph. sibiricus* derived in 2019 from callus cultures of a leaf and hypocotyl origin [1] were used as the objects of this study. The cultures were grown in a Murashige–Skoog medium (MS) prepared according to [12] and supplemented with inositol (0.1 g/L), 3% sucrose, and growth regulators (2,4-dichlorophenoxyacetic acid (2,4-D, 1.0 mg/L) and 6-benzylaminopurine (BAP, 0.5 mg/L, Merck, Germany)).

Suspension cell cultures were grown on a rotary shaker in 0.25-L flasks containing 35–40 mL of cell suspension. Cultivation was conducted for 21 days in the dark at 25 ± 1°C and 100 rpm. The inoculum : fresh medium ratio used for reinoculation was 1 : 4 ("hypocotyl" cell culture) or 1 : 10 ("leaf" cell culture).

For cultivation in bioreactors, two types of fermenters were used: (1) a MF-107 stirred tank reactor (New Brunswick, United States) with a total volume of 7.5 L equipped with a fluoroplastic 4-blade marine screw propeller (original construction designed at the Department of Cultivated Cell Biology of the Timiryazev Institute of Plant Physiology; 60 mm in diameter, 100 rpm) and (2) a 21-L bubble column reactor (designed at the Department of Cultivated Cell Biology of Timiryazev Institute of Plant Physiology) equipped with a bubbler with one 6-mm hole.

Both devices used the same type of a pipe aerator with microholes providing an atomized air supply into a nutrient medium. The air supply rate varied from 0.17 to 0.25 L per liter of the medium per minute (the rate was gradually increased because of the reduction of a suspension volume after the sampling; in the case of a bubble column reactor, these values represented the total air used for both bubbling and aeration).

A 2-week cell culture grown in flasks was used to inoculate bioreactors. The initial concentration of dry biomass was ~1 g/L. The cultivation was carried out in the dark at 26 ± 0.1°C.

The viability of cell cultures was determined by a vital staining with 0.1% phenosafranin (Merck, Germany) followed by the counting of live (unstained) and dead (stained) cultivated units under a microscope. The cell concentration was determined by cell count in a Fuchs-Rosenthal hemocytometer after a 25–30-min incubation of a suspension in 20% chromic acid at 60°C. To determine the amount of crude and dry biomass in 1 L of cultivation medium, a fixed suspension volume (no less than 10 mL in two biological replications) was filtered under a vacuum through a paper filter using a Buchner funnel [13]. The biomass was then dried in a drying cabinet for 24 h at 50°C.

The photomicrographies of cells were obtained using a 0.3-Mp ToupCam SCMOS digital camera (China).

Based on the obtained results, the growth parameters of suspension cell cultures were calculated, including the growth index ($I$), specific growth rate ($\mu$), doubling time ($\tau$), maximum dry biomass accumulation ($M_{max}$), economical coefficient ($Y$), and biomass productivity ($P$).

The calculations were performed using the following formulas [12]:

$I = X_{max}/X_0$,where $X_{max}$ and $X_0$ are the maximum and initial values of the growth index, respectively (dry biomass, fresh biomass, and cell concentration).

$\mu$ (day$^{-1}$) = $(\ln X_2 - \ln X_1)/(t_2 - t_1)$, where $X_2$ and $X_1$ represent the growth index values (cell concentration, crude/dry biomass content per 1 L of the medium) at the time points $t_2$ and $t_1$, respectively, calculated for the exponential growth phase.

$\tau$ (days) = $\ln 2/\mu$.

$Y = (X_{max} - X_0)/S_0$, where $X_{max}$ and $X_0$ are the maximum and initial dry biomass content (g/L), respectively, and $S_0$ is the initial substrate (sucrose) content in the medium (g/L).

$P$ (g/L days) = $(X_i - X_0)/(t_i - t_0)$, where $X_0$ and $X_i$ represent the dry biomass amount in the beginning of cultivation and at the $t_i$ time point, respectively.

The qualitative analysis of secondary metabolites contained in the biomass of suspension cell cultures was carried out by ultra-high-performance liquid chromatography with the mass spectrometry detection and electrospray ionization (UHPLC-ESI-MS). The roots of intact *Ph. sibiricus* plants collected in 2017 in the Olyokminsky district of Yakutia and kindly provided by Yu.A. Argylov were used as a reference sample.

Air-dried plant material (40–100 mg) was ground and triple extracted with 70% (v/v) ethanol within 30 min in a UZV-12 ultrasonic bath (Sapfir, Russia) and then centrifuged for 10 min at 10000 rpm using a MCF microcentrifuge (Sistemy Analiza, Russia). The resulting supernatant was transferred to a pear-shaped flask. Combined ethanol extracts were evaporated under a vacuum at 45°C. The resulting extract was suspended in 5% (v/v) acetic acid solution in distilled water and applied onto a Supelclean ENVI-18 cartridge for solid-phase extraction (Supelco, United States). The cartridge was sequentially washed with 5% (v/v) acetic acid solution in distilled water and 70% ethanol. The resulting solution was evaporated under a vacuum at 45°C. Prior to analysis, extracts were dissolved in 1 mL of the acetonitrile : water (1 : 1) mix. The injection volume was 1–2 µL.

The UHPLC-ESI-MS analysis was performed using a Waters Acquity UPLC chromatograph (Waters, United States) equipped with a XEVO QTOF hybrid quadrupole time-of-flight mass-spectrometer (Waters, United States). The study was performed using both positive and negative ion detection modes (*m/z* range: 100–2000). The ionization source temperature was 120°C, desolvatation temperature was 250°C, capillar voltage was 3.0 kV, the voltage on a sampling cone was 30 V, and the nitrogen feeding rate was 600 L/h.

A chromatographic separation was performed using an ACQUITY UPLC BEH Phenyl column (50 × 2.1 mm, 1.7 µm; Waters, Ireland). The flow rate was 0.4 mL/min at 40°C. The mobile phase consisted of the following components: 0.1% (v/v) water solution of formic acid (solvent A) and 0.1% (v/v) formic acid solution in acetonitrile (solvent B). Two different gradient elution modes were used. In the course of the analysis, the mobile phase composition was changed in the following way (solvent B, % of volume): gradient mode A—15% (0–1 min), 15 → 30% (1–5 min), 30 → 38% (5–15 min), 38 → 45% (15–15.5 min), 45% (15.5–23 min), 45 → 95% (23–23.5 min); gradient mode B—5 → 30% (0–15 min), 30 → 95% (15–17 min), 95% (17–20 min). The obtained data were treated using a MassLynx program (Waters, United States).

The growth and physiological characteristics (fresh and dry biomass content, cell viability and concentration) were determined in two biological replications. The pictures represent mean values and their standard errors.

## RESULTS

We evaluated the growth and physiological characteristics of *Ph. sibiricus* suspension cell cultures grown in flasks using such parameters as the cell concentration and viability and fresh/dry biomass accumulation. The obtained results are shown in Fig. 1 and Tables 1 and 2. The analysis of growth curves revealed a 2-day lag phase for the "leaf" cell culture and the lack of a lag phase in the case of the "hypocotyl" cell culture. The viability of cells during the growing cycle was 70–80% decreasing to 50–60% after 19–21 days of cultivation. These results show the suspension cell culture of a leaf origin can be considered as a well-growing culture. Growth indices (fresh and dry biomass, cell concentration) exceeded ten, the specific growth rate $\mu$ varied within the range of 0.3–0.4 day$^{-1}$, and the maximum dry biomass accumulation $M$ reached approximately 10 g/L. The economical coefficient $Y$ was equal to 0.29, i.e., almost 30% of sucrose was consumed for a cell biomass accumulation. At the same time, the "hypocotyl" cell culture demonstrated much worse growth parameters under the same cultivation conditions. These parameters were 2–3 times lower than those of the "leaf" culture ($I$ = 3.6–4.9, $\mu$ = 0.12–0.18 day$^{-1}$, $M$ = 6.6 g/L, $Y$ = 0.16).

A study of the growth processes in both cell cultures also included evaluation of their aggregation level. The cell culture derived from leaf tissues consisted of mainly small aggregates, which included 10–30 meristem-like cells and a large amount of single meristem-like, parenchyma-like, elongated, and anomalous cells. In the case of the "hypocotyl" cell culture, a large amount of large dense cell aggregates consisting of at least 30–50 meristem-like and parenchyma-like cells was observed. The photomicrographies of cell suspensions are shown in Fig. 2.

Well-growing small-aggregated suspension cell culture of the leaf origin was used for cultivation in bioreactors. Two types of reactors were used: a 7.5-L stirred tank bioreactor and a 21-L bubble column reactor. The growth characteristics obtained for the cell culture

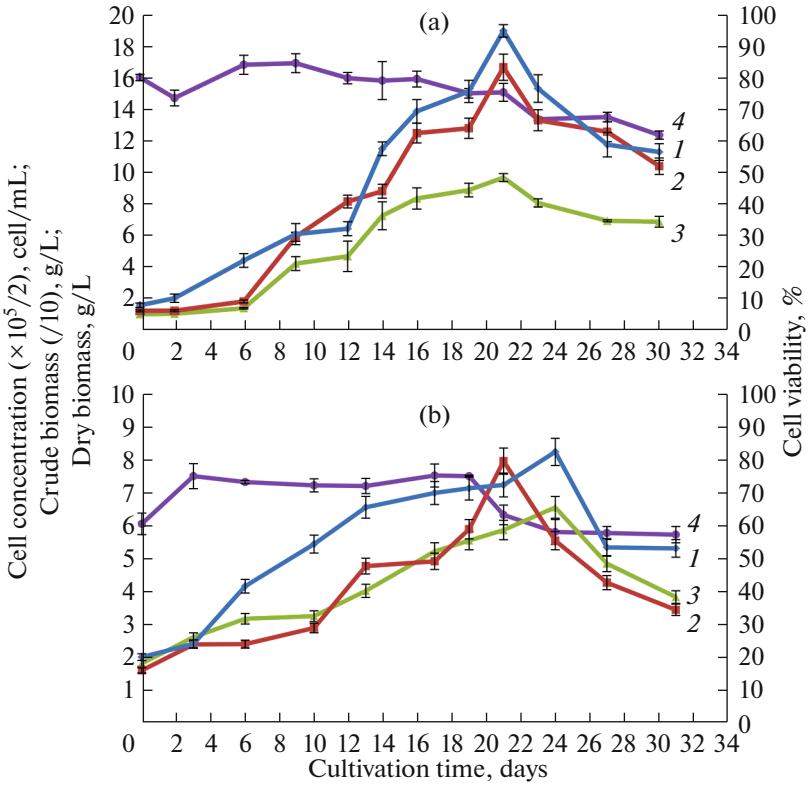


**Fig. 1.** Growth curves for *Ph. sibiricus* suspension cell cultures of (a) leaf and (b) hypocotyl origin. (*1*) Cell concentration ($\times 10^5/2$), cell/mL; (*2*) crude biomass (/10), g/L; (*3*) dry biomass, g/L; (*4*) cell viability.

growing in different bioreactor types are shown in Fig. 2 and Tables 3 and 4. According to the obtained data, cell cultivation in the bubble column reactor provided a stable culture growth with the lag phase duration of approximately 3 days and resulted in a significant improvement of the basic growth parameters (growth index for the dry biomass $I = 12.7$, the maximum dry biomass accumulation $M = 15.8$ g/L, dry biomass productivity $P = 0.78$ g/(L day), and economical coefficient $Y = 0.49$). On the contrary, the use of the stirred tank reactor worsened growth parameters (growth index for the dry biomass $I = 7.6$, the specific growth rate for dry biomass 0.13 day$^{-1}$, the maximum dry biomass accumulation $M = 8.9$ g/L). The observed worsening of growth characteristics was determined by the cell damaging by mechanical stirring devices of this bioreactor.

A qualitative phytochemical analysis of secondary metabolites in the studied cell lines and *Ph. sibiricus* roots was performed by UHPLC-ESI-MS. The chromatographic study of suspension cell cultures was divided into two stages. The first stage included the search and identification of acylated derivatives of khellactone (the basic biologically active compounds of *Ph. sibiricus*) in alcohol extracts from the cell biomass; the similar extract from *Ph. sibiricus* roots was used as a reference sample. The second stage of this phytochemical study included a preliminary identification of the revealed secondary metabolites of other structural types.

At the first stage, the UHPLC-ESI-MS analysis of extracts from the roots and biomass of two *Ph. sibiricus* suspension cell cultures was performed in a gradient mode A. This mode of separation allows a user to ana-

**Table 1.** Growth characteristics of a *Ph. sibiricus* cell suspension culture of leaf origin

| Growth parameter | Derived growth parameters | | | | | |
|---|---|---|---|---|---|---|
| | $I$ | μ, day$^{-1}$ | τ, days | $M_{max}$, g/L | $Y$ | $P$, g/(L days) |
| Cell concentration | 12.4 | 0.30 | 2.35 | 9.6 | 0.29 | 0.46 |
| Fresh biomass | 14.2 | 0.40 | 1.73 | | | |
| Dry biomass | 10.0 | 0.38 | 1.82 | | | |

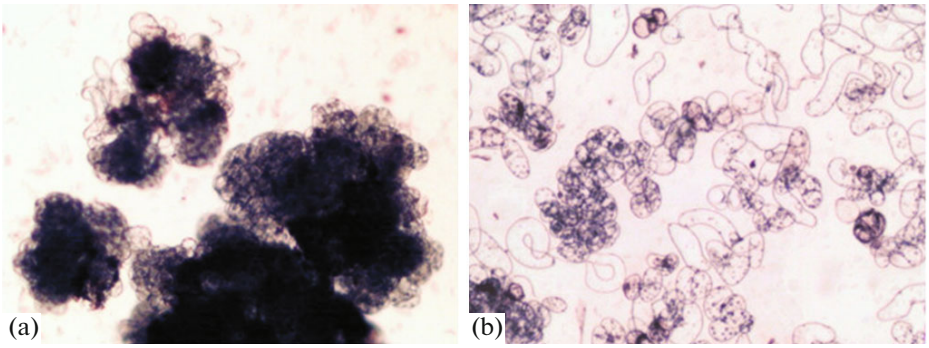


**Fig. 2.** Microphotographs of *Ph. sibiricus* suspension cell cultures derived from the callus of hypocotyl (a) and leaf (b) origin (magnification ×360).

lyze compounds of a wide polarity range [14]. The results of the study are shown in Fig. 3.

Profiles of secondary metabolites revealed in cell cultures and intact roots of *Ph. sibiricus* significantly differed from each other. Suspension cell cultures were characterized by the prevalence of more polar compounds (elution time within 1–8 min). The main components in the root extracts represented more hydrophobic compounds (elution time within 11–20 min). A comparison of mass spectrometry results obtained for the compounds from root extract with the data of other researchers [5] made it possible to conclude that all of them represented ethers of khellactone, an angular pyranocoumarin, and various organic acids. Two basic metabolites in the extract from *Ph. sibiricus* roots (compounds 2 and 3 with the elution times 13.1 and 13.5 min, respectively) were identified as visnadin and dihydrosamidin. These compounds were not detected in the extracts of suspension cell cultures.

To identify polar metabolites (elution time within 1–8 min in the gradient mode A) revealed in suspension cell cultures, the UHPLC-ESI-MS analysis of the corresponding extracts was performed in the elution program B. Chromatograms (full ion current) of extracts from the biomass of two suspension cell cultures were registered in a negative ion detection mode [15]. The corresponding results are shown in Fig. 4.

**Table 2.** Growth characteristics of a *Ph. sibiricus* cell suspension culture of a hypocotyl origin

| Growth parameter | Derived growth parameters | | | | | |
|---|---|---|---|---|---|---|
| | $I$ | $\mu$, day$^{-1}$ | $\tau$, days | $M_{max}$, g/L | $Y$ | $P$, g/(L days) |
| Cell concentration | 4.08 | 0.18 | 3.78 | 6.6 | 0.16 | 0.27 |
| Crude biomass | 4.93 | 0.17 | 4.16 | | | |
| Dry biomass | 3.60 | 0.12 | 5.62 | | | |

**Table 3.** Growth characteristics of a *Ph. sibiricus* cell suspension culture of leaf origin cultivated in a bubble column bioreactor

| Growth parameter | Derived growth parameters | | | | | |
|---|---|---|---|---|---|---|
| | $I$ | $\mu$, day$^{-1}$ | $\tau$, days | $M_{max}$, g/L | $Y$ | $P$, g/(L days) |
| Fresh biomass | 14.7 | 0.20 | 3.51 | 15.8 | 0.49 | 0.78 |
| Dry biomass | 12.7 | 0.18 | 4.10 | | | |

**Table 4.** Growth characteristics of a *Ph. sibiricus* cell suspension culture of leaf origin cultivated in a stirred tank reactor

| Growth parameter | Derived growth parameters | | | | | |
|---|---|---|---|---|---|---|
| | $I$ | $\mu$, day$^{-1}$ | $\tau$, days | $M_{max}$, g/L | $Y$ | $P$, g/(L days) |
| Fresh biomass | 6.9 | 0.13 | 5.5 | 8.9 | 0.26 | 0.33 |
| Dry biomass | 7.6 | 0.13 | 5.2 | | | |

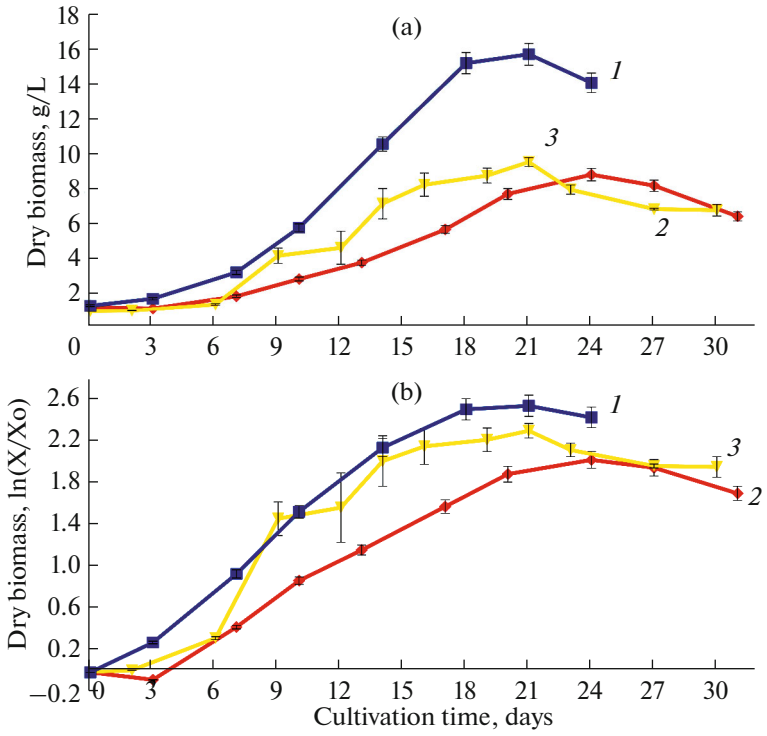


**Fig. 3.** Accumulation of a dry biomass of the *Ph. sibiricus* suspension cell culture of leaf origin. The curves are built in normal (a) and semilog (b) coordinates for different cultivation systems: (*1*) bubble column reactor; (*2*) stirred tank reactor; (*3*) flasks.

According to the signal intensity of the corresponding chromatographic peaks, six basic compounds with the elution time within 4.8–15.2 min were common for all samples. These compounds are designated with the numbers from 7 to 12 according to the increase of their hydrophobicity (Fig. 5). A composition of polar secondary metabolites of *Ph. sibiricus* suspension cultures was characterized by a high similarity degree.

A preliminary structural identification of the revealed compounds was performed by a comparison of their mass spectrometric and relative chromatographic behavior with the published data [5, 16–18]. Results from the identification of the components 7–12 are shown in Table 5. All identified components represented phenolic derivatives of different structural types. Components 7, 11, and 12 belonged to coumarins. The component seven was khellactone hexoside (probably, glucoside) [5], while components 11 and 12 were derivatives of osthenol, a simple prenylated coumarin, or 7-demethylsuberosin (isomeric coumarins, which are hardly distinguished among themselves based only on the mass spectrometry data [19]). Components 8 and 10 most likely belong to the group of benzofuran glycosides [16]. The component nine was identified as a glycosylated derivative of a matairesinol isomer, a lignan of a dibenzylbutyrolactone type [18]. Among the identified compounds, only khellactone glycoside (component seven) was considered typical for intact *Ph. sibiricus* plants [5]. However, judging by the signal intensity of the corresponding chromatographic peak, the compound seven is rather "minor" (in a quantitative respect) phenolic secondary metabolite in *Ph. sibiricus* cells cultivated in vitro. Note that identification of compounds performed in this study was almost completely based on the mass spectrometry results. Therefore, more detailed description of the revealed compounds requires further studies.

## DISCUSSION

A study of factors determining the growth and biosynthetic properties of higher plants' cell cultures is of both fundamental and applied significance. It is traditionally considered that epigenetic features of an explant (use of various plant organs to initiate cell cultures) plays a less important role for these properties than genetic features (plant species) or cultivation conditions. The results of this study do not completely agree with this point of view. The analysis of growth characteristics of two *Ph. sibiricus* cell cultures derived from callus obtained from different explants (leaf or hypocotyl of sterile plants) showed a significant difference between them. Growth parameters of "leaf" cell cultures exceeded those of the "hypocotyl" cell culture by two to three times. Interestingly, the callus lines used to derive suspension cell cultures showed the opposite characteristics: the callus culture of a leaf origin was characterized by the worst growth [11].

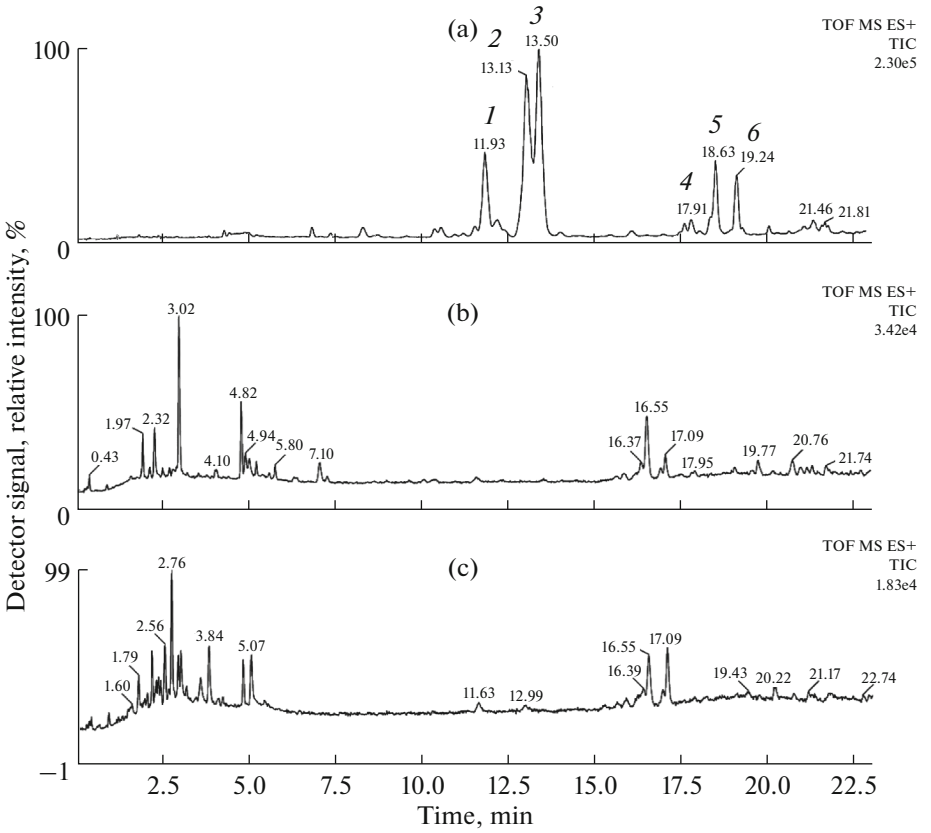


**Fig. 4.** UHPLC-ESI-MS chromatograms (total ion current, registration of positive ions; gradient elution program "A") of alcohol extracts from (a) roots and *Ph. sibiricus* suspension cell cultures of (b) hypocotyl and (c) leaf origin. (*1*) Pteryxin; (*2*) visnadin; (*3*) dihydrosamidin; (*4*) khellactone 3-isovaleroyl/2-methylbutyroyl-4-isobuturoyl ester; (*5*) khellactone 3-isovaleroyl/2-methylbutyroyl-4-senecioyl/angeloyl ester; (*6*) khellactone 3,4-diisovaleroyl/2-methylbutyroyl ester/khellactone isovaleroyl-2-methylbutyroyl ester.

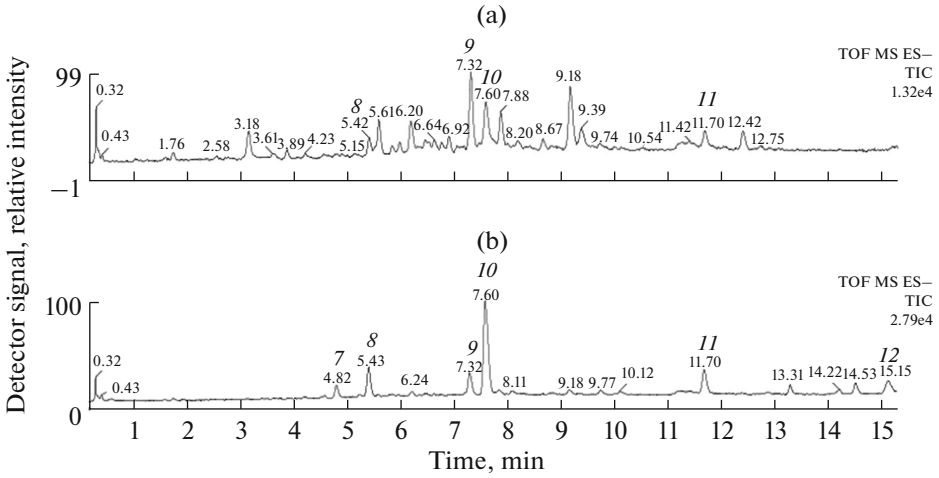


**Fig. 5.** UHPLC-ESI-MS chromatograms (total ion current, registration of negative ions; gradient elution program "B") of alcohol extracts from *Ph. sibiricus* suspension cell cultures of (a) leaf and (b) hypocotyl origin; (*7*–*12*) chromatographic peaks of identified secondary metabolites (Table 4).

**Table 5.** Results of the UHPLC-ESI-MS analysis (negative ion registration, gradient mode "B") of the extract from the biomass of the *Ph. sibiricus* suspension cell culture of hypocotyl origin

| Peak number* | $t_R$, min** | Mass spectra, *m/z*** | | Identification results |
|---|---|---|---|---|
| | | $[M-H]^-$ | Other ions | |
| 7 | 4.8 | 423.11 | 847.25 $[2M-H]^-$<br>491.10 $[M-H+HCOONa]^-$<br>486.10 $[M-H+HCOONH_4]^-$ | Khellactone-Hex |
| 8 | 5.4 | 397.10 | 795.22 $[2M-H]^-$<br>465.09 $[M-H+HCOONa]^-$<br>460.12 $[M-H+HCOONH_4]^-$ | 6-Carboxyethyl-7-methoxy-5-hydroxybenzofuran 5-Hex isomer |
| 9 | 7.3 | 605.17 | 673.16 $[M-H+HCOONa]^-$<br>561.19 $[M-H-CO_2]^-$<br>357.12 $[M-H-Hex-Mal]^-$<br>339.12 $[M-H-Hex-Mal-18]^-$ | Matairesinol-Hex-Mal isomer |
| 10 | 7.6 | 483.10 | 967.21 $[2M-H]^-$<br>469.12 $[M-H-14]^-$<br>439.09 $[M-H-CO_2]^-$ | 6-Carboxyethyl-7-methoxy-5-hydroxybenzofuran 5-Hex-Mal isomer |
| 11 | 11.8 | – | 955.26 $[2M-H]^-$<br>433.15 $[M-H-CO_2]^-$<br>229.07 $[M-H-Hex-Mal]^-$<br>545.12 $[M-H+HCOONa]^-$<br>540.13 $[M-H+HCOONH_4]^-$ | 7-demethylsuberosin/Osthenol-Hex-Mal |
| 12 | 15.1 | 229.07 | – | 7-demethylsuberosin/Osthenol |

* Peak numbering corresponds to that on Fig. 5; ** retention time on a chromatographic column, min (gradient mode "B"); *** data of mass spectra (the *m/z* values for revealed ions are shown); Hex, hexose residue; Mal, malonic acid residue.

A comparison of basic physiological characteristics of the studied suspension cell cultures of *Ph. sibiricus* allows one to suppose that the observed changes in their growth parameters are determined by differences in their aggregation level: the "leaf" cell culture consisted of small cell aggregations, while the "hypocotyl" cell culture included mainly large cell aggregations. Since both cell cultures were derived simultaneously and under identical conditions, the observed differences in their aggregation level may be provided by the characteristics (including epigenetic ones) of the initial callus cultures.

Characterization of lines obtained from different organs of the same plant is an important criterion used for selection of strains promising for biotechnological application. Growth of a suspension cell culture derived from a *Ph. sibiricus* leaf in a bubble column reactor resulted in improved growth parameters compared to the cell culture cultivation in flasks. On the contrary, cultivation of the cell culture in a stirred tank reactor resulted in reduced growth parameters. Similar results were obtained for suspension cell cultures of other plant species, such as yam (*Dioscorea deltoidea* Wall.), ginseng (*Panax japonicus* var. *repens*), and hairless tape vine (*Stephania glabra* (Roxb.) Miers) [20, 21]. An improvement of growth characteristics associated with the cell culture cultivation in a bubble column reactor is probably caused by a higher intensity of cell aeration, while growth reduction observed in the case of a stirred tank reactor may result from cell damage by stirring devices. Thus, as expected, a bubble bioreactor was shown to be more preferable system for cultivation of *Ph. sibiricus* suspension cell cultures.

In the course of the study of a phytochemical composition of suspension cell cultures, significant differences in the composition of phenolic compounds were observed between in vitro cell cultures and root cells of intact plants. The first ones were characterized by the prevalence of polar (hydrophilic) compounds, while the main components in root cells were more hydrophobic. Visnadin and dihydrosamidin representing the basic metabolites of root cells were not detected in suspension cell cultures.

All identified polar components revealed in suspension cell cultures belonged to phenolic derivatives. At the same time, the majority of compounds revealed in these cultures (excepting khellactone [22]) were not typical for intact *Ph. sibiricus* plants.

Some of the revealed compounds, such as the osthenol/7-demethylsuberosin (prenylated coumarin) hexoside ether and malonyl derivatives of coumarin glycosides, can be related to very rare phenolic derivatives of plant origin. Some other identified compounds (benzofuran glycosides) are common among different plant taxa [16, 22–26], though no data on their presence in *Phlojodicarpus* spp. were found in the available literature.

Interestingly, prenylated coumarins (visnadin, dihydrosamidin, and other khellactone ethers) typical for intact *Ph. sibiricus* plants were identified in the initial callus cultures of *Ph. sibiricus* [11] but not in suspension cell cultures cultivated for a long time.

The results of the study quite agree with the known data about changes in the secondary metabolism of in vitro cell cultures compared to intact plants. Similar tendencies were described for the formation of steroid glycosides in cell cultures of yam (*Dioscorea deltoidea* Wall.) and puncture vine (*Tribulus terrestris* L.). Both cultures accumulated only furostanol glycosides promoting cell proliferation, whereas intact plants produced mainly spirostanol forms of steroid glycosides [1, 27].

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests*. The authors declare that they have no conflicts of interest.

*Statement on the welfare of humans or animals*. This article does not contain any studies involving animals performed by any of the authors.

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