Tissue culture of *Rhodiola Kirilowii* (Regel.) Maxim — contents of biologically active compounds at different stages of growth

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Summary

The callus tissue obtained from *Rhodiola Kirilowii* (Regel.) Maxim was tested for growth dynamics and contents of active compounds. Biologically active compounds in the callus tissue were identified as salidroside, tyrosol, gallic acid, chlorogenic acid, caffeic acid and tannins. The lotaustralin was not detected either in the callus, or roots of *R. Kirilowii*.

Key words: *Rhodiola Kirilowii* (Regel.) Maxim, salidroside, p-tyrosol, lotaustraln, phenolic acids, tannins, callus tissue

*Rhodiola Kirilowii* (Regel.) Maxim (the *Crassulaceae* family) is a species of Asian origin, growing in the Northern Asia and China in mountainous regions at 2000-5000 metres above the see level. The plant does not grow in Europe, unlike *Rhodiola rosea* L., another species of the genus *Rhodiola* sp.

The active compounds isolated from roots of *R. Kirilowii* were identified as salidroside, tyrosol, daucosterol and cyanogenic glucoside – lotaustralin, beta-sitosterol [1, 2, 3, 4, 5], and rhodiocyanoside A, arbutin, epigalokatechin gallate, fructopyranos-(1-4)-glucopyranose [6] from the plants cultivated in Poland (Botanical Garden of Research Institute of Medicinal Plants). There are few studies on biological activity of *R. Kirilowii*. Moreover, the papers are published in Chinese. Only abstracts are available in English. The results of the Chinese studies show that oral administration of *R. Kirilowii* can significantly reduce pathologic damages of rat’s organs and efficiently protect the animals from cardiopulmonary disorders due to hypoxic environment caused by changing the altitude [7].
R. Kirilowii is supposed to be a plant which enhances possibilities of adaptation to changing environmental conditions and can alleviate the mountain sickness symptoms [8]. The beneficial activities of R. Kirilowii have not been confirmed efficiently in the pharmacological and clinical studies yet.

Roots of R. Kirilowii are considered to be a promising raw material supplying several interesting chemical compounds possessing adaptogenic and protective activities. It is difficult to obtain raw material containing all the spectrum of biologically active compounds in the climatic conditions in Poland. The biotechnological methods could ensure a sufficient amount of valuable raw material rich in the active substances.

Biotechnological studies on R. Kirilowii have been carried out in China [9], but in Europe only in Poland [10, 11, 12, 13, 6]. This research was an attempt to find and determine the correlation between the phase of growth and the biosynthesis of the active compounds in the callus tissue of R. Kirilowii.

MATERIAL AND METHODS

Plant material

The roots of the intact plant and the seeds were obtained from Botanical Garden of Research Institute of Medicinal Plants in Poznań. The callus tissue of R. Kirilowii were initiated from cotyledons of the sterilised seedlings.

Biotechnological methods

Seeds of R. Kirilowii were sterilised by soaking in 70% ethyl alcohol with addition of several drops of Tween (2 min.), 4% sodium hypochlorite (10–15 min.) and washed with sterile water five times. The seeds were placed in Petri dishes with wet paper and kept in breeding chamber under light.

The callus tissue started from cotyledons on MS [14] media supplemented with BA (2.0 mg/ml), adenine chloride (1.0 mg/ml), NAA (2.0 mg/ml) and subcultured every four week. The callus was incubated under a 16-hour photoperiod (2500 lux) at the temperature of 23°C. Collected samples for the chemical investigations were dried at the temperature of 25°C.

Growth rate determination

Studies of growth dynamics were performed every seven days. The size of the weekly sample was established as the 10 cultures (10 jars). The fresh and dried weights were determined by means of a moisture analyser (HR73 Metler Toledo, drying temperature – 105°C). The growth rate was calculated as the difference between the weight on the day of measurement and the weight on the day of the inoculation. The determination of growth parameters was conducted in triplicate.
Chromatographic procedures

In this study HPTLC, HPLC and spectrophotometric methods were used for chemical analyses. Samples for TLC and HPLC analyses were extracted with 70% methanol at the temperature of 60–70°C. HPLC analysis was performed with an Agilent 1100 HPLC system, equipped with a photodiode array detector. For all the separations a Lichrospher 100 RP18 column (250x4 mm, 5 μm, Merck) was used. The mobile phase consisted of 0.2% phosphoric acid in water (A) and acetonitrile (B), applied in the following gradient elution: from 95A/5B for 30 minutes to 80A/20B isocratic elution for 5 minutes, then from 80A/20B for 5 minutes to 20A/80B and an isocratic elution for 20 minutes till the end of the test. Each run was followed by a 10-minute equilibration period. The flow rate was 1ml/min, the detection wavelengths set to DAD at λ=205 nm, 254 nm, 330 nm, and 20 μL of samples was injected. All the separations were performed at the temperature of 25°C. Peaks were assigned by spiking the samples with standard compounds and comparison of the UV-spectra and retention times.

Spectrophotometric analyses of tannins expressed as pyrogallol were performed according to the methods of European Pharmacopoeia with a UV-Visible Spectrometer Cintra 20 GBC 9. The statistic data were expressed as the average value, standard deviation and standard error.

RESULTS

The seeds of *R. Kirilowii* germinated within 10 days. The callus tissue was obtained from cotyledons of sterilised seedlings on solid media MS with addition of BA, NAA and adenine chloride. The callus tissue grew at a moderately slow pace, doubling the fresh weight within 14 days and continuing to increase in size. The growth dynamics of callus tissue is presented in Fig. 1-4.

![Figure 1. Fresh weights of callus tissue of Rhodiola Kirilowii.](image-url)
Figure 2. Dried weights of callus tissue of Rhodiola Kirilowii

Figure 3. Dry weights of callus tissue of Rhodiola Kirilowii (in per cent).
Figure 4. Growth rate of dried weights of callus tissue of *Rhodiola Kirilowii*.

The growth dynamics

The fresh weights of callus reached maximum values between days 35 and 42 of culture and approximated 14–21 g per culture (Fig. 1). The fresh weights increased 5–8 times during the period of culture. Then the fresh weights decreased, starting from the beginning of day 35. The maximum value of dried weights was attained between days 21 and 35 (Fig. 2). The percentage value of dried weights oscillated between 3% and 7% (Fig. 3). The maximum occurred one week after the inoculation and then decreased in value to 3% on day 49. The peak of the growth rate of dried weights occurred between days 7 and 21 of the culture period (Fig. 4).

Biochemical compounds – preliminary investigations

Biologically active compounds in the callus tissue were identified as salidroside, tyrosol, gallic acid, chlorogenic acid, caffeic acid and tannins (Fig. 5–7). Lotaustralin was not detected either in the callus, or roots of *R. Kirilowii*. 
Figure 5. The content of salidroside and p-tyrosol in callus of *Rhodiola Kirilowii*.

Figure 6. The content of tannin in callus tissue of *Rhodiola Kirilowii*.

Figure 7. The content phenolic acids in callus of *Rhodiola Kirilowii*. 
The content of tannins increased from 0.2% (day 7) to the maximum of 0.73% (day 42; Fig. 6). The callus tissue reached the highest content of salidroside during the first two weeks after inoculation (0.99–1.56 mg/100g). The content of salidroside decreased rapidly from the beginning of the fifth week. The content of tyrosol was relatively steady and low (0.09–0.17 mg/100g). The content of caffeic acid (Fig. 7) was the highest of all the phenolic acids and ranged from 20.17 (on the day of inoculation) to 38.18 mg/100g (day 42). The contents of chlorogenic acid and gallic acid were low and inclined to increase slowly during the period of culture.

DISCUSSION

Salidroside, tyrosol, daucosterol, lotaustralin, sucrose and beta-sitosterol were previously isolated from roots of *R. Kirilowii* [1, 2, 3, 4, 5]. The new compounds, namely rhodiocyanoside A, arbutin, epigallocatechin gallate and fructopyrano-(1-4)-glucopyranose have been found recently by Wiedenfeld [6] in the root extract from the plants cultivated in Poland. According to available chemical reports, only salidroside have been detected in the callus tissue so far [9]. Caffeic acid, chlorogenic acid, gallic acids and tannins were found for the first time in callus of *R. Kirilowii*. The absence of cyanogenic glucoside (lotaustralin) in the callus tissue and in the roots create possibility of attaining safe raw material.

The callus tissue was able to synthesize the same biological compounds as plants *in vivo* but the contents of them were much lower. The contents of tannins, salidroside and caffeic acid in the callus were correlated with the phase of the growth of the tissue. The amount of caffeic acid was the highest of all the phenolic acids (the maximum content was 38.18 mg/100g), which is a relatively high value and comparable to the content of caffeic acid in the roots of *R. Kirilowii* (45 mg/100g on the average). The tyrosol, chlorogenic acid, gallic acid showed weak correlation with the stage of the growth.

Fresh and dried weights of the callus grew until day 28 of the culture so the suitable time for subculture is the fourth week.

The presence of main biologically active compounds in the callus tissue is very promising, but *R. Kirilowii* still needs more phytochemical investigations, particularly as regards variability of chemical composition, depending on the harvest time, place of origin and kind of explant.

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REFERENCES


KULTURY TKANKOWE RHODIOLA KIRILOWII (REGEL.) MAXIM – ZAWARTOŚĆ ZWIĄZKÓW BIOLOGICZNIE CZYNNYCH W RÓŻNYCH STADJACH WZROSTU

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Szerzenie

Badano dynamicę wzrostu tkanki kalusowej *Rhodiola Kirilowii* (Regel.) Maxim oraz zawartość związków biologicznie czynnych w poszczególnych etapach wzrostu kalusa. W tkance kalusowej stwierdzono obecność salidrozydu, p-tyrozolu, kwasów galusowego, kawowego, chlorogenowego oraz garbników. W kalusie i w korzeniach *R. Kirilowii* nie stwierdzono obecności lotautaliny, cyjanogennego glikozydu.

Słowa kluczowe: *Rhodiola Kirilowii* (Regel.) Maxim, salidrozyd, p-tyrozol, lotautralina, fenolokwasy, garbniki, tkanka kalusowa