

EXPERIMENTAL PAPER

Intrapopulation variability of flavonoid content in roots of Baikal skullcap (*Scutellaria baicalensis* Georgi)

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Summary

Introduction: Baikal skullcap (Scutellaria baicalensis Georgi) is an important medicinal plant, indigenous to Asia. Due to a wide range of pharmacological activities, its roots has been used for ages in Traditional Chinese Medicine. Recently, the species has become an object of interest of Western medicine, as well. Objective: The aim of the study was to determine the variability of Baikal skullcap population originated from Mongolia and cultivated in Poland, in terms of content and composition of flavonoids in the roots. Methods: The objects of the study were 15 individual plants, selected within examined population and cloned in order to obtain a sufficient amount of raw material. The total content of flavonoids in roots was determined according to Polish Pharmacopeia 6th. The qualitative analysis of flavonoids was carried out using HPLC, Shimadzu chromatograph. **Results:** The dry mass of roots ranged from 25.88 to 56.14 g \times plant⁻¹. The total content of flavonoids (expressed as a quercetin equivalent) varied between 0.17 and 0.52% dry matter (DM). Nine compounds were detected within the group, with oroxylin A 7-Oglucuronide (346.90–1063.00 mg \times 100 g⁻¹ DM) as a dominant, which differentiated investigated clones at the highest degree (CV=0.27). Baicalin (391.40–942.00 mg \times 100 $g^{-1}DM$, wogonoside (324.00–641.10 mg \times 100 $g^{-1}DM$) and hesperetine 7-O-glucoside $(163.00-346.32 \text{ mg} \times 100 \text{ g}^{-1}DM)$ were also present in a considerable amounts. Clone 7 was distinguished by the highest content of all investigated compounds, except wogonin and oroxylin A 7-O-glucuronide. Conclusions: Results obtained in present study show a high variability within Baical skullcap investigated population in respect of flavonoid

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compounds detected in roots. Thus, the results may be used in future investigations concerning the selection and breeding of this species.

Key words: Baikal skullcap, variability, roots, flavonoids, baicalin

INTRODUCTION

One of the most important causes of civilization diseases seems to be a decrease in natural resistance against harmful environmental factors. Stress also negatively affects human health and well-being. The idea of using plant tonic remedies, classified as adaptogens, to maintain homeostasis of organism has been known for ages in Far East Medicine. Various pharmacologically active compounds found in herbal adaptogens stimulate human body *via* multiple metabolic pathways. They act by increasing resistance of organism, regulating response to physical, environmental and emotional stress and modulating interconnected immune, endocrine and nervous systems [1].

In the group of plants indicating adaptogenic activity, species belonging to Scutellaria L. genus (Lamiaceae family) are considered to be one of the most important. Here, the best known species is Baikal skullcap (S. baicalenis Georgi). This plant, indigenous to Far East Asia, occurs on natural sites in Southeast Siberia, Russian Far East, Northern Mongolia, North and Northeastern China, Korea and Japan [2]. It is a perennial up to 60 cm tall, with quadrangular stem, opposite simple leaves and terminal inflorescence distinguished by purple-blue flowers with scutellumlike upper lip. Roots of Baikal skullcap are defined as herbal raw material in the Chinese, Japan and Korean Pharmacopoeias [3]. Recently, it has become an object of interest in Western medicine as well and, consequently, the monograph of Scutellariae baicalensis radix has been adopted in the European Pharmacopoeia [4]. This raw material reveals various pharmacological activities, associated with the presence of specific flavonoids, namely: baicalein, wogonin, oroxylin A and their glycosides [3, 5, 6]. Crude extracts of Baikal skullcap's root as well as flavonoids isolated from these extracts exhibit diverse pharmacological activity, i.a. antitumor [7, 8], antimutagenic [9, 10], antiangiogenesis [11, 12], antioxidant [13-15], anti-inflammatory [16], antiviral and antibacterial [17-21], antiallergic and neuroprotective [22, 23].

Baikal skullcap occurs naturally on steppes, grasslands, sunny slopes and forest habitats in a cold-dry climate of Far East Asia [24]. Over last several decades, wild resources of this species has declined significantly due to over-exploitation. In China, Baikal skullcap is cultivated in a traditional way, by seeds which are directly collected from local wild growing populations. The field cultivation not only protects natural resources but also helps to improve the safety and quality of final herbal product [25-28]. However, cultivation of wild growing plants usually produces genetic bottlenecks and may results in loss of genetic diversity [28].

Thus, it is important to know the range of natural diversity of this species derived both from intraspecific and intrapopulation variability.

The aim of the study was to determine the variability within Baikal skullcap population originating from Mongolia, in terms of the content and composition of flavonoids in the roots.

MATERIAL AND METHODS

Plant material

15 individual 3-year-old plants were selected from Baikal skullcap population cultivated on experimental field of Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW (Poland). Seeds of the population were collected from plants growing wild on natural site in Mongolia (N 49°16.400`, E 10°05.370`, 1580 m a.s.l), in 2009. Individual plants were cloned in order to obtain a sufficient amount of raw material. In 2013, at the end of May, 40 stem cuttings were taken from each single maternal plant and placed into the peat:substrate in the greenhouse. Well rooted cuttings were planted out in July (8 cuttings on a single plot), in the randomized block design (3 replications), in spacing 60×40 cm. Roots (8 plants within one replication) were collected in the second year of plant vegetation, in October. Collected roots were weighed, dried at 40°C and subjected to chemical analysis, which were performed in triplicate. The sample designed to single chemical analyze originated from one single plot. The presented results are mean values from three replications.

Voucher specimen was deposited at herbarium of Department of Vegetable and Medicinal Plants WULS – SGGW.

Spectrophotometric analysis

The total content of flavonoids was carried out by aluminium chloride colorimetric method according to Polish Pharmacopoeia 6th [29]. 0.5 g of air-dry, powdered roots was extracted with 20 ml acetone, 2 ml 25% HCl, 1 ml 0.5% metenamine and kept boiling for 30 min. Obtained hydrolyzate was filtered through a cotton wool, then a residue was extracted two times with 20 ml acetone, kept boiling for 10 min each time. Obtained mixture was completed up to 100 ml with acetone. 20 ml of mixture was subsequently extracted in the separatory funnel with 20 ml of distilled water and 15 ml of ethyl acetate by shaking for 3 minutes. Separated water-acetone phases were extracted with mentioned method three more times with 10 ml of ethyl acetate only each time. Cumulated ethyl acetate extract was washed two times with 40 ml of water and completed up to 50 ml. 10 ml of basic solution was mixed with 2 ml of 2% AlCl₃ and then completed to

25 ml with methanol: acetic acid solution (19:1). Comparison solution for each sample was prepared by completing 10 ml of basic solution to 25 ml with methanol: acetic acid solution. The absorbance was measured at 425 nm. The total flavonoid content was expressed as a quercetin equivalent (% of dry matter of raw material).

HPLC analysis

1g of homogenized, air-dry raw material was extracted with 100 ml of methanol in Büchi Extraction System B-811. Soxhlet hot extraction with twenty-five extraction cycles, flushing and drying was used. After evaporation of solvent, the residue was dissolved in 10 ml of methanol. The obtained extracts were filtered with Supelco Iso-Disc[™] Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.20 μ m and subjected to HPLC. The analyses were performed using a Shimadzu chromatograph equipped with auto sampler SIL-20, photodiode array detector SPD-M10A VP DAD and Class VP 7.3 chromatography software. C-18 reversed-phase column (Phenomenex Kinetex[®] 2.6 μ m, C18, 100A, 100×4.60 mm i.d.) was used as stationary phase. Binary gradient of mobile phase A (deionised water adjusted to pH 3 with phosphoric acid) and B (ACN) was used. Separation conditions and validation and were carried out according to Kosakowska et al. [30]. The peak table and spectra library (190-450 nm) of individual compounds were created. Detection wavelength applied: 236 nm (scutellarin, scutellarein), 266 nm (chrysin 7-O-glucuronide), 270 nm (oroxylin A 7-O-glucuronide), 275 nm (baicalin, baicalein, wogonine, wogonoside), 285 nm (hesperetine 7-O-glucoside). The content of the determined compounds was calculated in mg \times 100 g⁻¹ dry matter (*DM*).

Statistical analysis

Data were subjected to statistical analysis using Statistica[®] software. The mean values were compared by using the one way analysis of variance (ANOVA) followed by Tukey's range test. The differences between means were deemed to be significant at p<0.05. The coefficient of variation (CV) was determined, as well.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Baikal skullcap, known as "Huang Quin", has been used for ages in Traditional Chinese Medicine and Japanese Kampo Medicine. Nowadays, dry roots as well as extracts and fractions isolated from this raw material are common elements of drugs and diet supplements used in natural medicine all over the world. As mentioned before, flavonoids are the most important group of compounds. determining multidirectional biological activities of this raw material. There is a lot of detailed information on chemical composition and pharmacological activities of Baikal skullcap's root. However, the data on the variability of this species is scarce and concern rather genetic than chemical diversity [2, 28, 31]. In the present study, variability within population of *S*. *baicalensis* was investigated, in respect of content and composition of flavonoids in roots. Examined clones differed as to the mass of roots, the total content of flavonoids and the content of identified flavonoid compounds in the raw material. The fresh mass of roots ranged from 66.88 to 137.50 g \times plant¹ (CV=0.35). The dry mass of this raw material was at the level of 25.88 and 56.14 g \times plant⁻¹ (CV=0.23), respectively (tab. 1). In present work, total content of flavonoids varied from 0.17 to 0.52%DM (CV=0.24). Nine compounds were identified within this group, namely: baicalein, baicalin, wogonin, wogonoside, scutellarein, scutellarin, chrysin 7-0glucuronide, hesperetine 7-O-glucoside and oroxylin A 7-O-glucuronide (tab. 2, fig. 1). Oroxylin A 7-O-glucuronide and baicalin were the dominants. Investigated clones differ in respect of their content: from 346.90 to 1063.00 mg imes100 g⁻¹ and from 391.40 to 942.00 mg \times 100 g⁻¹ DM, respectively. Wogonoside and hesperetine 7-O-glucoside were also present in a considerable amounts, as following: from 324.00 to 641.10 mg \times 100 g⁻¹ and from 163.00 to 346.32 mg \times 100 g^{-1} DM. The content of identified flavonoid aglycones (baicalein, wogonin, scutellarein) was clearly lower in comparison with their glycosides. The highest differences between clones concerned the content of oroxylin A 7-O-glucuronide (CV=0.27) and hesperetine 7-O-glucoside (CV=0.25). Clone 7 was distinguished by the highest content of all investigated compounds, except wogonin and oroxylin A 7-O-glucuronide (tab. 2). The presence of compounds identified in this study was determined previously by other authors [32-38]. The domination of baicalin followed by high amounts of wogonoside were shown earlier, as well [36, 37]. However, the results of the present work indicate on significantly lower content of baicalin in comparison to results obtained by other authors. Taking into account the pharmacopeial requirements, the content of this compound in Baikal skullcap root should not be less than 8.0 [39] or 9.0% [40, 41]. According to Woźniak et al. [9], the content of baicalin in this raw material is at a level of 11.16%, Makino et al. [37] show the range 3.52–12.10%, while Bai et al. [2] 8.39–26.48%. However, results obtained by Islam et al. [36] as well as Zgórka and Hainos [38] indicate on a lower values (2.53 and 1.93%, respectively), what corresponds with presented results. In the case of wogonoside, values obtained in our work are lower when compared to results of other authors. Here, Islam et al. [36] show the level of 1.26%, while Makino et al. [37] 5.07%. Such differences may be associated with the fact that the content of biologically active compounds in plants can be affected by various factors e.g. genetic, edaphic or climatic [42].

Table 1.

| Clones | Fresh mass | Dry mass |
|--------|------------|----------|
| 1. | 117.55 ab | 42.96 b |
| 2. | 137.50 a | 56.14 a |
| 3. | 111.00 ab | 47.04 ab |
| 4. | 120.56 ab | 46.12 ab |
| 5. | 136.07 a | 50.80 a |
| 6. | 105.00 b | 40.92 b |
| 7. | 96.87 bc | 34.54 bc |
| 8. | 100.53 b | 37.26 bc |
| 9. | 127.68 a | 55.40 a |
| 10. | 127.60 a | 45.74 ab |
| 11. | 84.94 c | 30.40 c |
| 12. | 127.50 a | 47.80 ab |
| 13. | 66.88 d | 25.88 c |
| 14. | 91.56 bc | 35.58 bc |
| 15. | 68.40 d | 27.82 c |
| Mean | 107.84 | 41.62 |
| CV | 0.35 | 0.23 |

The mass of *Scutellaria baicalensis* roots ($g \times plant^{-1}$)

Values in columns marked with different letters differ at p < 0.05, Tukey's test; CV – coefficient of variation; presented values are means of three replications, whereas one replication is represented by a sample obtained from eight plants cultivated on a single plot



Figure 1.

Chemical structure of main flavonoids identified in the Baikal skullcap roots: a – oroxylin A 7-O-glucuronide, b – baicalin, c – wogonoside, d – hesperetine 7-O-glucoside

| | | | | | Flavonoid c | spunoduo | | | | |
|------------------|---|---------------------------------------|---------------------------|---|---|-----------------------------|--------------------|---------------------------------|------------------------------|------------------------------------|
| Clones | Total content of flavonoids | Baicalein | Baicalin | Wogonin | Wogonoside | Scutellarein | Scutellarin | Chrysin 7-0-glucuro- nide | Hesperetine 7-0-glucoside | Oroxylin A 7-0-glucuro- nide |
| 1. | 0.42 b | 98.33 ab | 687.00 b | 41.37 a | 517.20 b | 11.51 c | 53.00 ab | 23.24 d | 274.40 b | 823.60 b |
| 2. | 0.35 c | 102.90 a | 708.70 b | 41.26 a | 524.80 b | 13.28 b | 55.67 ab | 30.47 a | 303.40 ab | 1063.00 a |
| 3. | 0.29 cd | 85.36 bc | 623.00 bc | 33.82 c | 440.70 c | 10.09 d | 49.98 b | 24.78 c | 222.67 bc | 821.80 b |
| 4. | 0.52 a | 88.79 bc | 501.10 cd | 33.44 c | 389.70 d | 10.51 d | 49.48 b | 27.48 b | 181.36 c | 587.10 de |
| 5. | 0.37 c | 90.45 b | 715.60 b | 35.17 bc | 486.90 bc | 10.90 d | 49.09 b | 28.57 b | 182.90 c | 704.00 c |
| 6. | 0.34 c | 91.17 b | 581.30 с | 33.30 c | 464.20 bc | 12.01 c | 48.68 bc | 23.85 d | 235.62 bc | 721.70 с |
| 7. | 0.31 cd | 107.70 a | 942.00 a | 42.31 a | 641.10 a | 17.47 a | 59.46 a | 30.98 a | 346.32 a | 850.30 b |
| 8. | 0.34 c | 81.32 c | 391.40 d | 33.07 c | 324.00 e | 9.78 d | 45.53 bc | 24.31 c | 165.77 d | 346.90 e |
| 9. | 0.38 c | 95.24 ab | 542.10 c | 37.93 b | 401.90 d | 10.62 d | 47.46 bc | 25.46 c | 163.00 d | 386.00 e |
| 10. | 0.28 cd | 87.53 bc | 506.40 c | 36.03 b | 427.80 c | 10.41 d | 48.99 b | 27.50 b | 164.65 d | 582.70 de |
| 11. | 0.26 cd | 85.27 bc | 721.90 b | 36.43 b | 514.40 b | 10.66 d | 53.29 ab | 25.13 c | 213.70 bc | 719.80 c |
| 12. | 0.17 e | 89.03 b | 491.10 cd | 33.65 c | 402.10 d | 10.62 d | 49.73 b | 25.32 c | 187.37 c | 568.00 de |
| 13. | 0.28 cd | 90.22 b | 560.80 с | 34.64 bc | 415.40 d | 12.06 c | 49.59 b | 26.33 bc | 189.67 c | 629.40 d |
| 14. | 0.35 c | 106.10 a | 611.00 bc | 42.11 a | 468.50 bc | 11.78 c | 52.43 ab | 28.70 b | 213.57 bc | 542.30 d |
| 15. | 0.28 cd | 107.00 a | 617.60 bc | 43.19 a | 446.40 bc | 12.48 c | 50.84 b | 28.88 b | 209.30 bc | 663.90 d |
| | | | | | | | | | | |
| Mean | 0.33 | 93.76 | 613.40 | 37.18 | 457.67 | 11.61 | 50.88 | 26.73 | 216.91 | 667.37 |
| CV | 0.24 | 0.09 | 0.21 | 0.10 | 0.16 | 0.16 | 0.07 | 0.0 | 0.25 | 0.27 |
| Values one re | in columns marko plication is repres | ed with different sented by a samı | t letters differ at p^* | <0.05, Tukey's tes eight plants cultiv | st; <i>DM</i> – dry matter vated on a single p | ; CV – coefficient (lot | of variation; pres | ented values are n | neans of three repli | cations, whereas |

Table 2.

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Both baicalin, wogonoside and their aglycones (baicalein, wogonin) are characterized by a triple hydroxylation of the A ring. Due to such specific chemical structure they are considered to be the most active flavonoids in Baikal skullcap's root, revealing, i.a.: antitumor, antimicrobial, antioxidant, anti-inflammatory and anxiolytic activities [7-9, 12, 13, 15-17, 20, 23]. However, other flavonoids are also responsible for pharmacological activity of *Scutellariae radix*. Oroxylin A and its sugar derivatives, detected in a considerable amounts in the present work, show e.g. antibacterial, anti-inflammatory and anti-angiogenic properties [43, 44]. When regards hesperetine, authors indicate on its antiallergic, antidepressant and memory improving effect [45, 46]. In turn, scutellarin reveals antihypertensive and antivirus [47, 48], while chrysin – antitumor, anxiolytic and anticonvulsant activities [49-52]. Since all listed flavonoids reveal a strong pharmacological activities, they finally may be engaged in the adaptogenic effect of *Scutellariae radix*.

Obtained results show a high variability within investigated Baikal skullcap population in respect of flavonoid compounds detected in roots. Thus, the results may be used in future investigations concerning selection and breeding of Baikal skullcap, providing a high-quality raw material characterized by specific pharmacological activity.

CONCLUSIONS

A high intrapopulation variability concerning the content and composition of flavonoids in roots was found.

Oroxylin A 7-O-glucuronide was the dominant compound and differentiated examined clones at the highest degree.

Clone 7 was distinguished by the highest content of all investigated flavonoid compounds, except wogonin and oroxylin A 7-O-glucuronide.

The content of identified flavonoid aglycones (baicalein, wogonin, scutellarein) was clearly lower in comparison to their glycosides.

Conflict of interest: Authors declare no conflict of interest.

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WEWNĄTRZPOPULACYJNE ZRÓŻNICOWANIE ZAWARTOŚCI FLAWONOIDÓW W KORZENIACH TARCZYCY BAJKALSKIEJ (*SCUTELLARIA BAICALENSIS* GEORGI)

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Streszczenie

Wstęp: Tarczyca bajkalska (*Scutellaria baicalensis* Georgi) jest ważną rośliną leczniczą, naturalnie występującą w Azji. Ze względu na wielokierunkową aktywność farmakologiczną, korzenie tej rośliny wykorzystywane były od wieków w Tradycyjnej Medycynie Chińskiej. Ostatnio, gatunek ten stał się obiektem zainteresowania także medycyny zachodniej. **Cel:** Celem pracy było określenie zakresu zróżnicowania populacji tarczycy bajkalskiej, pochodzącej z Mongolii i uprawianej w Polsce, pod względem zawartości i składu flawonoidów w korzeniach. **Metody:** Obiektem badań było 15 pojedynków wyselekcjonowanych z badanej populacji i rozmnożonych wegetatywnie w celu pozyskania odpowiedniej ilości surowca. Ogólną zawartość flawonoidów oznaczono według Farmakopei Polskiej 6. Analizę HPLC flawonoidów przeprowadzono przy użyciu chromatografu firmy Shimadzu. **Wyniki:** Sucha masa korzeni wahała się od 25,88 do 56,14 g × roślina⁻¹. Ogólna zawartość flawonoidów (w przeliczeniu na kwercetynę) wynosiła od 0,17 do 0,52% suchej masy (s.m.). Wśród 9 zidentyfikowanych związków flawonoidowych w największej ilości występował 7-O-glukuronid oroksyliny A (346,90–1063,00 mg × 100 g⁻¹ s.m.), który ponadto różnicował badane klony w najwyższym stopniu (CV=0,27). Bajkalina (391,40–942,00 mg × 100 g⁻¹ s.m.), wogonozyd (324,00–641,10 mg × 100 g⁻¹ s.m.) i 7-O-glukozyd hesperetyny (163,00–346,32 mg × 100 g⁻¹ s.m.) także występowały w surowcu w znaczących ilościach. Klon 7 wyróżniał się najwyższą zawartością wszystkich zidentyfikowanych flawonoidów, z wyjątkiem wogoniny i 7-O-glukuronidu oroksyliny A. **Wnioski**: Przeprowadzone badania wskazują na wysokie zróżnicowanie omawianej populacji tarczycy bajkalskiej, pod względem zawartości związków flawonoidowych zidentyfikowanych w korzeniach. Uzyskane wyniki mogą być wykorzystane w przyszłych pracach dotyczących selekcji i hodowli tego gatunku.

Słowa kluczowe: tarczyca bajkalska, zróżnicowanie, korzenie, flawonoidy, bajkalina

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