Biotechnology for the Production of *Scutellaria baicalensis* Georgi. Plant Material and its Pharmacognostic Analysis

L I Tikhomirova and R C Lisenko

Altai State University, 61, Lenina str., Barnaul, 656049, Russia

Email: l-tichomirova@yandex.ru

**Abstract.** The paper describes a technique for micropropagation of *S. baicalensis*. It implies the use of seeds as primary explants, reproduction of an actively proliferating culture on an MS medium containing 1.0-0.5 μM kinetin and 0.25 μM IBA (indole-3-butyric acid) and 0.05 μM GA (gibberellic acid), rooting of shoots on a medium containing 1 μM IBA. A microscopic analysis of Baikal skullcap (*Scutellaria baicalensis*) grown in a tissue culture made it possible to establish the following diagnostic signs: for the leaf – diacytic stomatal complex, simple warty hairs and capitate hairs; essential oil glands with 4.6 and 8 excretory cells; for the stem – simple warty and capitate hairs, prosenchyme cells in the epidermis. Biologically active substances of 70% ethanol extracts from progeny plants were screened. Tannins, xanthones, flavonoids and other phenolic compounds were found to be present. A quantitative analysis of some biologically active substances made it possible to obtain the following data: the content of extractives amounted to 12.50±1.03%, with the tannins to make up 8.25±0.62% and a total of flavonoids to be in the range of 5.8±0.2%. For the first time, the content of xanthones was determined in progeny plants of *S. baicalensis* in terms of mangiferin to be 48±0.01% per ADM.

1. Introduction

A complex formulation of oriental medicine has shown that *Scutellaria baicalensis* is included in the ‘elite’ group of the most commonly used plants. Baikal skullcap (*Scutellaria baicalensis* Georgi) is a plant in the family Lamiaceae. It has long been used in traditional medicine of the East: China, Mongolia, and Tibet. The extract from *S. baicalensis* roots was established to have antibacterial, anti-allergic, anti-sclerotic, anti-inflammatory, and anti-thrombotic effects. The pharmacological properties of the plant are due to the high content of the flavonoid complex in a shoot/root system: in the roots – 21.2-22.8%, while the leaves contain up to 10% of flavonoids. Due to the fact that *S. baicalensis* roots are medicinal material, scientific literature mainly covers phytochemical studies for these plant organs. Despite being the place where biologically active substances (BAS) and their predecessors start to be synthesized, the aerial part of *Scutellaria baicalensis* has not been properly studied yet [1-4].

There are several ways to capture valuable and scarce medicinal plant material, namely: introduction and creation of artificial plantations; medicinal tissue and cell culture methods. Industrial crops, in comparison with resource stock, guarantee the compactness in the allocation of areas, production of standard quality medicinal plants, a significant increase in plant biomass coupled with a high concentration of biologically active substances (BAS). *In vitro* technology allows for environmentally friendly stock plant materials all year round, regardless of climatic conditions and difficulties in harvesting, increased yields of biologically active substances by regulating their
accumulation in a culture. Given the characteristics of these pathways for the production of biologically active substances, it is of interest to study in vitro the phytochemical characteristics of *S. baicalensis* [5, 6].

The study was aimed at the technique for clonal micropropagation of *S. baicalensis* and pharmacognostic analysis of multiplied stock plant material.

2. Material and methods

Tissue culture

*Scutellaria baicalensis* progeny plants were produced at the Department of Biotechnology of the South-Siberian Botanical Garden (SSBG), Altai State University. To ensure clonal micropropagation, culture media were prepared according to MS prescription [7], containing 30 g/l of sucrose, mesoinositol, vitamins: nicotinic acid, pyridoxine, thiamine hydrochloride. The media were poured into plastic containers (30 ml each) or into culture bottles (10 ml each). The prepared media were autoclaved for 20 minutes at 1200 °C. The explants were cultured under a photoperiod of 16/8 light/dark hours at 24 – 26°C.

The explants used were the seeds from the SSBG collection and shoots of intact plants produced from the seeds. The intact plants were cultivated in soil conditions on a layer-type installation (Fig. 1 a).

![Figure 1. a) intact plants of Scutellaria baicalensis, b) clonal micropropagation](image)

The slides with *Scutellaria baicalensis* were prepared in accordance with general pharmacopoeial monograph of State Pharmacopoeia of Russian Federation “Method of microscopic and microchemical studies of medicinal plants and medicinal plant preparations” (GPM.1.5.3.0003.15). The analysis involved the whole leaves or tissue of a leaf blade with a margin and vein, and stem tips. They were placed in a 5% sodium hydroxide solution on a glass slide, covered with a cover slip and carefully heated over the burner flame until completely clear. After cooling the slide, a small piece of filter paper was placed on the left side of the cover slip, and a 33% glycerol solution was pipetted to obtain a preparation with a colorless inclusion liquid. The resulting slide was examined under a microscope [8].

Phytochemical analysis

The resulting extracts were analyzed in accordance with the methodological recommendations of R.A. Muzychkina, et al. (2011) [9].
Determination of certain biologically active contents. A technique was used to quantify the content of flavonoids in the extracts, based on their ability to form a colored complex with AlCl$_3$ solution [10]. The tannins were quantified by potassium permanganate oxidation in the presence of indigo carmine [11]. The amount of xanthones was quantified based on the method by T.A. Kukushkina, et al. (2011) [12]. The content of extractive substances in the extracts [13] was evaluated taking into account the humidity. It was determined on an MX-50 moisture analyzer at 105 °C. The extractives were evaporated in a porcelain cup (previously adjusted to constant weight).

All measurements were repeated at least three times. The measurements were statistically processed using Microsoft Excel 2003. All calculations for the content of various biologically active substances were based on absolutely dry matter.

3. Results and discussion

Clonal micropropagation

As explants, the ripened *Scutellaria baicalensis* seeds were used from the collection of the South-Siberian Botanical Garden. Prior to sterilization, the seeds were rinsed under running water for 15-25 minutes. They were subsequently sterilized in a laminar flow cabinet with a 1% sulfochlorantin solution for 10 minutes, and finally rinsed three times with sterilized distilled water. This sterilization method made it possible to have 70% of the explants sterile and viable. Culture media for the stage of introduction into the tissue culture were prepared based on Murashige and Skoog (MS) medium avoiding phytohormones.

After 20-30 days, following the formation of multiple shoots they were transferred onto MS media with 1.0-0.5 μM kinetin and 0.25 μM IBA (indole-3-butyric acid) and 0.05 μM GA (gibberelllic acid) to backup micropropagation. The resulting conglomerates of the shoots were easily divided into single members and transferred onto fresh media; some shoots were drafted. To obtain an actively proliferating culture for a long time, it is necessary to use a scheme of alternating media with high and low BAP contents through one passage. This approach allows for a sterile culture *Scutellaria baicalensis* with a stable reproduction rate of 5.3±0.4. The number of shoots per explant for one passage ranged from 2 to 15 pieces. The shoots were rooted in Murashige and Skoog medium supplemented with 1.0 μM IBA (Fig. 1 b).

Microscopic analysis of *Scutellaria baicalensis*

The epidermis of stems consists of elongated cells with straight walls (prosenchymatous cells). Simple thin-walled warty and capitate hairs, consisting of 2-3 cells, are found to be present on the surface of the epidermis (Fig. 2a, b, c).

![Figure 2. Micrograph of a *Scutellaria baicalensis* stem: a) (at 100x magnification); b), c) simple thin-walled warty and capitate hairs (at 1000x magnification)](image)
A microscopic analysis of the *Scutellaria baicalensis* progeny leaves identified the following anatomy features. The epidermal cells of the upper and lower sides of the leaves had an average diameter of 126 μm, with curved walls. The cuticle was even, well defined. There were multiple stomata each surrounded by two guard cells, a common wall of which was perpendicular to the stomata (diacytic stomatal complex). By the type of stomata arrangement, the leaf was amphistomatic.

On the edge of the leaves, along the veins, there were simple warty hairs, capitate hairs with thin walls (2-3 cellular), and tenacious hairs. At the base of the hairs there were several epidermal cells that formed a rosette (Fig. 3 a, b). On both sides of the leaves there were numerous round sessile glands on a petiole. They contained 4, 6 or 8 excretory cells located radially. In the center there was a round petiole. The glands were surrounded by epidermal cells forming a rosette.

![Figure 3](image_url)

**Figure 3.** Microscopic analysis of *Scutellaria baicalensis* progeny leaves: a) at 100x magnification, b) at 1000x magnification

A callus culture *S. baicalensis* was obtained from leaves, stems, and roots at Tomsk State University on modified MS medium with various concentrations of growth stimulants. The progress of flavonoids accumulating in callus culture suggested that during cultivation the content of flavonoids gradually increased by the 30th day up to 1.21% of the air-dry mass [14]. In the studies presented in the paper, the progeny plants of *S. baicalensis* included 5.8±0.2% of flavonoids, and 12.5±1.03 extractives with the tannins to make up 8.25±0.62.

During chromatographic separation of the extracts, two xanthone-like substances were synthesized in the *Scutellaria baicalensis* specimens in question. The xanthone compounds in alcoholic extracts (70% ethyl alcohol) were identified by upward paper chromatography in 40% acetic acid in comparison with tracking mangiferin (Sigma), which has characteristic absorption maxima in the regions 369, 318, 259 and 241 nm. When chromatograms were developed with ammonia vapors in UV light, there were 2 zones at the level of the mangiferin standard: 1 – a luminous yellow spot at the standard level, and 2 – with bright orange fluorescence (Rf 0.55).

Once treated with an alcoholic solution of AlCl₃, the zones acquired a green color, which confirms the xanthone nature of the substances. Based on the developed methodology for quantitative analysis of the amount of xanthones in terms of mangiferin [12], the authors were first to determine the content of mangiferin and the amount of xanthones in *S. baicalensis* in the amount of 1.48±0.01% per ADM.

4. Conclusion
A technique was developed for clonal micropropagation of *S. baicalensis*. A microscopic analysis of *Scutellaria baicalensis* grown in a tissue culture made it possible to establish the following diagnostic features:
for the leaf – diacytic stomatal complex, simple warty hairs and capitate hairs; essential oil glands with 4.6 and 8 excretory cells;
- for the stem – simple warty and capitate hairs, prosenchyme cells in the epidermis.

Biologically active substances of 70% ethanol extracts from progeny plants were screened. Secondary metabolites of \textit{S. baicalensis} were identified through qualitative and quantitative methods. Tanins, xanthisones, flavonoids and other phenolic compounds were found to be present. A quantitative analysis of some biologically active substances made it possible to obtain the following data: the content of extractives amounted to 12.50±1.03%, with the tanins to make up 8.25±0.62% and a total of flavonoids to be in the range of 5.8±0.2%. For the first time, the content of xanthisones was determined in progeny plants of \textit{S. baicalensis} in terms of mangiferin to be 48±0.01% per ADM.

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