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Lignans in *Schisandra chinensis in vitro* cultures

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Contents of schisandrol A and schisandrol B were determined in methanolic extracts of biomass from *in vitro* cultures of *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae) using an HPLC method. The biomass was cultured on six variants of Murashige and Skoog (MS) medium containing different concentrations of growth regulators, cytokinin (BAP) and auxin (NAA). The contents of both lignans were different and dependent on the MS medium variant. The maximal contents of schisandrol A (70.54 mg/100 g d.w.) and schisandrol B (86.41 mg/100 g d.w.) were considerable in comparison with plant material: leaves (29.69 and 34.50 mg/100 g d.w.) and fruits (132.39 and 109.40 mg/100 g d.w., respectively). This is the first report on the quantitative analysis of schisandrol A and schisandrol B in *Schisandra chinensis in vitro* cultures.

Schisandra chinensis is a vine native to eastern Asia (mostly China, Korea, Sakhalin), used in traditional Chinese medicine (TCM) for ages (Chinese Pharmacopoeia 2005) and long ago recognized in North America (United States Pharmacopoeia 2011), but much later acknowledged in European countries (Hencke et al. 1999) as an adaptogenic species, and professionally reviewed in a WHO monograph in 2007 (WHO Monographs on Selected Medicinal Plants 2007).

Its adaptogenic properties, hepatoprotective, antioxidant and anticarcinogenic actions, and other directions of biological activity have been attributed mostly to lignans possessing the characteristic structure of dibenzocyclooctanes, the representatives of which include schisandrol A and schisandrol B (Hencke et al. 1999).

The hitherto conducted studies on the lignans accumulation in plant *in vitro* cultures focused principally on podophyllotoxin and its derivatives in cultures of several *Podophyllum* and *Linum* species (Koulman et al. 2004). We found only one literature report about the qualitative analysis of three selected lignans (Havel et al. 2008) in an *in vitro* culture of *Schisandra chinensis* but without quantitative analysis.

So far, *Schisandra chinensis in vitro* cultures have been studies by researchers from the Czech Republic to establish their potential for biotransformation of different precursors (e.g. hydroquinone) into phenolic glycosides, arbutin and salicin (Dusková et al. 2005). In addition, different methods of micropropagation of this plant species in *in vitro* cultures, mostly by embryogenesis, have been developed (Kim et al. 2005; Smíšková et al. 2005).

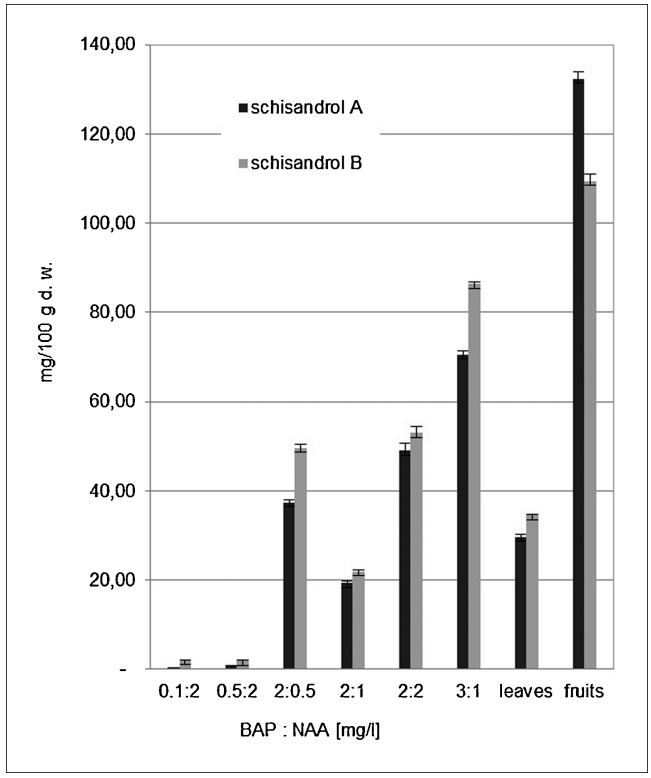

Fig. 1: Contents of studied lignans in biomass from shoot-differentiating callus cultures cultivated on different MS medium variants and in leaves and fruits of *Schisandra chinensis*. The values are the means ± SEM of three experiments.

The aim of the present study was to examine the contents of schisandrol A and schisandrol B in extracts from *Schisandra chinensis* shoot-differentiating callus cultured on six variants of Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with differing contents of cytokinin (BAP) and auxin (NAA), ranging from 0.1–3.0 mg/l.

In addition, we maintained non-differentiating callus culture on one of the MS medium variants supplemented with 2 mg/l BAP and 2 mg/l NAA. The studies aimed also to compare the biosynthetic capacities of cells from *in vitro* cultures with cells of overground parts (leaves, fruits) of plants grown *in vivo*.

In our experiments, we attempted to initiate *Schisandra chinensis in vitro* cultures from seeds and leaf buds. The trials with leaf buds proved successful and we obtained shoot-differentiating callus culture and undifferentiating callus culture.

Dry biomass increments obtained during 4-week growth cycles in shoot-differentiating callus cultured on MS medium variants were diverse and ranged from 2.1- to 6.1-fold. The maximum biomass growth was observed on the MS medium variant containing 2 mg/l BAP and 2 mg/l NAA. In callus cultured on this medium (2 mg/l BAP, 2 mg/l NAA), a 7.6-fold biomass growth was documented.

Schisandrol A contents in extracts from the shoot-differentiating callus cultures widely differed from 0.35 to 70.54 mg/100 g d.w. Schisandrol B contents ranged from 1.86 to 86.41 mg/100 g d.w. The maximum schisandrol A content was obtained on MS medium containing 3 mg/l BAP and 1 mg/l NAA. The medium with the same composition of growth regulators was also conducive to schisandrol B accumulation (Fig.).

Extracts of the callus cultivated on one of MS medium variants contained low amounts of both lignans of the same order of magnitude, namely 2.05 and 2.54 mg/100 g d.w., respectively. Due to low lignans contents, analyses of extracts from the undifferentiating cultures were discontinued.

Table: Maximal contents of studied lignans obtained in the biomass cultured *in vitro* and in leaves and fruits of *Schisandra chinensis*

Analyzed material	Content of lignans (mg/100 g of d. w.)	
	Schisandrol A	Schisandrol B
Callus culture	2.05	2.54
Shoot-differentiating callus cultures	70.54	86.41
Leaves	29.69	34.50
Fruits	132.39	109.40

The influence of biomass differentiation stage in *in vitro* cultures on the accumulation of secondary metabolites is a known phenomenon (Charlwood at al. 1990). Our results confirmed this relationship. The maximum schisandrol A and schisandrol B contents obtained from the shoot-differentiating callus culture were 23.60- and 20.96-fold greater, respectively, than in the callus culture cultivated on the same medium variant.

The effect of concentrations of growth regulators in culture media on the accumulation of secondary metabolites is well documented (Ramawat and Mathur 2007). We have recently evidenced this effect in *Ruta graveolens* cultures during studies of free phenolic acid accumulation (Ekiert et al. 2009). We demonstrated this relation also earlier in the studies of linear furanocoumarins and umbelliferone accumulation in *Ammi majus* (Ekiert and Gomółka 2000b), and *Pastinaca sativa* cultures (Ekiert and Gomółka 2000a).

The contents of both lignans in extracts of leaves from plants growing *in vivo*, analyzed for comparison, were high and amounted to 29.69 mg/100 g d.w. for schisandrol A and 34.50 mg/100 g d.w. for schisandrol B. In fruits the richest source of lignans of this plant species, the contents of the lignans under study were several times (4.46- and 3.17-fold) higher than in leaves and amounted to 132.39 mg/100g d.w. (schisandrol A) and 109.40 mg/100g d.w. (schisandrol B). In this context, lignan contents obtained in the shoot-differentiating callus culture (approximately 71 and 86 mg/100 g d.w., respectively) should be considered to be substantial and interesting from a practical perspective (Table).

Even at this stage of research, the obtained results suggest that the *in vitro* cultures tested in this study, are a good model for further studies and a potential source of biologically active lignans.

These are the first studies on the effect of growth regulators on lignans accumulation in *Schisandra chinensis in vitro* cultures. The obtained results are promising and can be an inspiration for future studies.

Experimental

1. Establishment of in vitro culture

The *in vitro* culture was established from leaf buds of *Schisandra chinensis* (Turcz.) Baill. (*Schisandraceae*) from the Rogów Arboretum - Warsaw University of Life Sciences, Forest Experimental Station in Rogów (Poland). The plant material was sterilized in 0.1% NaOCl for 10 min. and maintained on Murashige-Skoog - MS solid medium (1962) supplemented with growth regulators: 1 mg/ml BAP (cytokinin) and 0.5 mg/l NAA (auxin). After 4 weeks we obtained two different cultures: shoot-differentiating callus culture and undifferentiating callus culture. The cultures were cultivated under constant artificial light (4 W/m^2, LF-40 W lamp, daylight, Piła) at 25 ± 2 °C.

2. Experimental in vitro culture

Experimental shoot-differentiating callus culture was maintained on six MS medium variants differing in concentrations of plant growth regulators, BAP and NAA [mg/l]: 0.1 and 2.0; 0.5 and 2.0; 2.0 and 0.5; 2.0 and 1.0; 2.0 and 2.0; 3.0 and 1.0. The experimental callus culture was cultivated only on one MS medium variant, containing 2 mg/l BAP and 2 mg/l NAA. Both cultures (three series) were grown under constant artificial light (4 W/m^2, LF-40 W lamp, daylight, Piła) at 25 ± 2 °C for four weeks.

3. Plant material

Plant material harvested in Poland in 2010, analyzed for comparison, comprised: leaves and fruits of *Schisandra chinensis* (Rogów Arboretum - Warsaw University of Life Sciences, Forest Experimental Station in Rogów, Poland).

4. HPLC analysis

The dried biomass from *in vitro* cultures collected after 4-week growth cycles and plant material (from 0.2 to 0.5 g.) were extracted by sonication with methanol. In the methanolic extracts, chromatographic quantification of two lignans: schisandrol A and schisandrol B, was performed using the HPLC method developed by Zhang et al. (2009). Separation was performed using an analytical column Kinetex™ 18C (150 x 4.6 mm, 2.6 µm) at 30 °C. The mobile phase consisted of acetonitrile (A) and water (B) at a flow-rate of 0.8 mL min^{-1} (gradient program); injection volume: 5 µl. Detection wavelength was set at 225 nm. Quantification was made by comparison with standards: schisandrol A and schisandrol B (Chromadex™).

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