Influence of medium composition on secondary metabolites synthesis in *Drosera binata*

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Introduction

Plant secondary metabolites are used in medicine and industry. Their larger amounts can be received by the plant *in vitro* cultures. Appropriate plant growth factors promote increased synthesis of biologically active compounds which can be extracted and examined in numerous biological activity tests. The main active compound for *Droseraceae* is plumbagin. Biosynthesis of this compound and other secondary metabolites depends on many factors such as growth medium pH and osmotic pressure. This work aims to test plants' growth conditions and to analyze quantitatively and qualitatively *Drosera binata* ethanolic extracts.

Materials and Methods

Drosera binata from University of Gdansk plant cultures, MS salts (Duchefa Biochemie, Netherlands), plant agar (Duchefa Biochemie, Netherlands), sucrose (Acros, Belgium), C18 column modified with phenyl groups Zorbax SB-Phenyl 3,5µm, 4,6mmx150mm (Agilent, USA), ethanol (Avantor, Poland), acetonitrile HPLC grade (Avantor, Poland), trifluoroacetic acid (Alfa Aesar, USA), deionized water. Plant cultures - *D. binata* plants were cultivated on solid MS medium with a variable amount of macroelements (3/4 MS, 1/2 MS, 1/3 MS) with different pH (5.2; 5.5; 6). Mediums were supplemented by 1%, 2% or 3% of sucrose. Cultures were maintained for six weeks. After the growth period, the material was collected, cleaned from medium and stored at -20°C. Extraction - 1 mg of frozen plant material was extracted three times with 1ml of 95% ethanol for 48 h at room temperature. After this time, extracts were collected and kept in -20°C until HPLC analysis. Extracts were analyzed with use of HPLC system Ultimate 3000 (Dionex, USA) with a C18 column modified with phenyl groups, UV-VIS detector at wavelength 254nm and gradient elution program.

Results

The highest concentrations of secondary metabolites were obtained for 1/3 MS medium at pH 6 with the addition of 3% sucrose. The lowest concentrations of secondary metabolites were obtained for 3/4 MS at pH 5.2 and 3% sucrose and 3/4 MS at pH 5.5 and 1% sucrose. Metabolite with the highest biosynthesis level was plumbagin (concentration ranges 0,17 mg/ml – 0,44

mg/ml). Quercetin has the highest ability to extract from the analyzed plant material. Concentrations of the compound in the first extract are about ten times higher than concentrations in the second extract. Quercetin concentrations in the second and third extract are similar. The lowest level of extraction has droserone, concentration ratio for the first and second extracts is 1.75 and for second and third extracts 1.36.

Discussion

The concentration of plumbagin synthesized in 6 weeks by *D. binata* reached half values of the concentrations obtained for *D. rotundifolia* and *D. intermedia* in growth time from 6 to 24 months (Baranyai, Bäcker, Reich, & Lindequist, 2016). Analyzed in this study secondary metabolites are also synthesized by other *Drosera* spp. (Marczak, Kawiak, Łojkowska, & Stobiecki, 2005). In pharmaceutical production, e.g. *D. capensis* can be a source of 7-methyljuglone and *D. binata* can be used as a good natural source of plumbagin (Samaj et al., 1999). Plant extracts are rich in biologically active compounds, and they have a great antimicrobial potential (Krolicka et al., 2008). They can be used in mixtures with other substances as an antimicrobial agent (Krychowiak et al., 2014) or natural feed additive that supports the development of farm animals beneficial microflora (Elghandour et al., 2018).

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References

- Baranyai, B., Bäcker, C., Reich, C., & Lindequist, U. (2016). The production of 7-methyljuglone, plumbagin and quercetin in wild and cultivated *Drosera rotundifolia* and *Drosera intermedia*. *Mires and Peat*, 18(19), 1–8.
<https://doi.org/10.19189/MaP.2016.OMB.228>

- Elghandour, M. M. M. Y., Kanth Reddy, P. R., Salem, A. Z. M., Ranga Reddy, P. P., Hyder, I., Barbabosa-Pliego, A., & Yasaswini, D. (2018). Plant Bioactives and Extracts as Feed Additives in Horse Nutrition. *Journal of Equine Veterinary Science*, 69, 66–77. <https://doi.org/10.1016/j.jevs.2018.06.004>
- Krolicka, A., Szpitter, A., Maciag, M., Biskup, E., Gilgenast, E., Romanik, G., ... Łojkowska, E. (2008). Antibacterial and antioxidant activity of the secondary metabolites from in vitro cultures of *Drosera aliciae*. *Biotechnology and Applied Biochemistry*, 184, 175–184. <https://doi.org/10.1042/BA20080088>
- Krychowiak, M., Grinholc, M., Banasiuk, R., Krauze-Baranowska, M., Głód, D., Kawiak, A., & Królicka, A. (2014). Combination of silver nanoparticles and *Drosera binata* extract as a possible alternative for antibiotic treatment of burn wound infections caused by resistant *Staphylococcus aureus*. *PLoS ONE*, 9(12), e115727. <https://doi.org/10.1371/journal.pone.0115727>
- Marczak, Kawiak, A., Łojkowska, E., & Stobiecki, M. (2005). Secondary metabolites in in vitro cultured plants of the genus *Drosera*. *Phytochemical Analysis*, 16(3), 143–149. <https://doi.org/10.1002/pca.833>
- Samaj, J., Blehova, A., Repcak, M., Ovecká, M., Bobák, M., Šamaj, J., ... Bobák, M. (1999). *Drosera* Species (Sundew): In Vitro Culture and the Production of Plumbagin and Other Secondary Metabolites. *Medicinal and Aromatic Plants*, 11, 105. https://doi.org/10.1007/978-3-662-08614-8_7