STUDIES ON THE ANTHRAQUINONE PRODUCTION BY *RUBIA TINCTORUM* HAIRY ROOT CULTURES

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European madder (*Rubia tinctorum* L.) is a perennial plant and a member of the family Rubiaceae. It is a source of a natural dye, it produces a variety of anthraquinone pigments in its roots and rhizomes. The main components are di- and trihydroxy-anthraquinones and their glycosides: ruberythric acid (alizarin-primeveroside), alizarin, pseudopurpurin, purpurin, rubiadin, munjistin, lucidin-primeveroside [1]. These substances show some bactericidal and spasmolytic activity and facilitate the loosening of kidney concrements containing calcium and magnesium phosphates. In this connection, madder is used in medicine. Some components show mutagenic activity (lucidin), but purpurin has some inhibitory effect on bacterial mutagenicity [2].

We have investigated the growth and anthraquinone content of the genetically modified hairy root cultures. The hypocotyl of *Rubia tinctorum* was infected by *Agrobacterium rhizogenes* (strain R-1601). After the elimination of bacteria, the hairy roots were cultured on liquid Gamborg B5 [3] and HMS [4] media in Erlenmeyer flasks.

For determination of anthraquinones, the lyophilized tissue samples were extracted with MeOH using an ultrasound device. After evaporation, the athraquinone glycosides were hydrolysed with HCl. The hydrolysate was purified by solid phase extraction. The purified samples were analysed by HPLC method. Alizarin and purpurin were identified with the use of external standards. Investigating the cultures growing on different media we found, that the production of purpurin was more than threefold higher (2.93 mg/g) than that of alizarin (0.87 mg/g) on HMS medium, while on B5 medium the alizarin production was slightly higher (1.12 mg/g).

Crude MeOH extracts were fractionated by flash chromatography method. Fractions were investigated by HPLC. Anthraquinone glycosides and aglycones were separated and ruberythric acid, lucidin-primeveroside, munjistin, pseudopurpurin and lucidin were detected via UV spectrum analysis.

[1] Hagers Handbuch der Pharmazeutische Praxis (1994) Springer-Verlag, Berlin.
[2] Marczylo T. H., Hayatsu T., Arimoto-Kobayashi S., Tada M., Fujita K., Kamataki T., Nakayama K., Hayatsu H. (1999) Protection against the bacterial mutagenicity of heterocyclic amines by purpurin, a natural anthraquinone pigment. Mutat. Res., Genet. Toxicol. Environ. Mutagen. 444 (2): 451-461.
[3] Gamborg, O.K., Miller, R.A., Ojima, K. (1968) Nutrient requirements of suspension cultures of soybean root cells. Exptl. Cell. Res. 50: 151-158.
[4] Kuzovkina I. N., Mantrova O. V., Al'terman I. E., Yakimov S. A. (1996) Culture of Genetically Transformed Hairy Roots Derived from Anthraquinone-producing European Madder Plants. Rus. J. of Pl. Phys. 43 (2): 291-298.