

## DISSERTATION INFORMATION

Title: **Rosmarinic acid production in *Ehretia asperula* Zollinger et Moritzi cell cultures**

Major: Biotechnology

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### ***The main results of the thesis***

Friable callus was successfully formed from leaves *in vitro* and on Gamborg (B5) medium supplemented with 30 g/L glucose, 0.4 mg/L 2,4-D, and 0.1 mg/L BA, as a material for cell suspension cultures.

The best cell suspension proliferation was achieved when 1 g of friable callus was cultured in 20 mL of liquid B5 medium supplemented with 30 g/L glucose, 0.4 mg/L NAA, 0.1 mg/L BA and was rotated at 90 rpm on an orbital shaker under total darkness for 4 weeks

Cell aggregates are not sieved and are cultured in the dark for the highest biomass and rosmarinic acid content. The content of rosmarinic acid in cell suspensions cultured in medium supplemented with 45 g/L glucose was 1.2 times higher than when 30 g/L glucose was used. Chitosan was added to the culture medium as an elicitor for rosmarinic acid biosynthesis. Chitosan 50 mg/L promoted the cell suspension to produce rosmarinic acid after 48 hours of treatment, 1.17 times higher than the treatment without chitosan.

In addition, the activity of *Ehretia asperula* Zollinger et Moritzi extract was also verified through its ability to inhibit oxidation and HEK293 cells. The antioxidant capacity of the extracts was ranked as: extract from leaf *in vitro* > extract from callus derived from leaf *in vitro* > extract from leaf in the garden > extract from suspension cell biomass. At concentrations below 400µg/mL, the extracts were not toxic to HEK293 cell.

### ***The new scientific findings***

A callus cell line (friable, white to pale yellow) obtained from the leaves of *Ehretia asperula* Zollinger et Moritzi *in vitro* is suitable for cell suspension cultures.

Some suitable factors (lighting condition, medium volume, rotation speed, plant growth regulators) were identified for the proliferation of *Ehretia asperula* Zollinger et Moritzi cell suspension.

Some suitable factors (light intensity, cell aggregate size, sugar, chitosan) were identified for the rosmarinic acid synthesis of *Ehretia asperula* Zollinger et Moritzi cell suspension.

The antioxidant capacity of extract from suspension cell biomass was equivalent to that of extract from leaf in the garden and was not toxic to HEK293 cell when using extract concentrations below 400 µg/mL.

**Advisors**

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