Naphthoquinone production in in vitro cultures of *Drosera communis* St. Hil.

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**Introduction**

Sundew herb has been widely used to treat bronchitis and cough and until today it is part of several prescriptions. Caused to short natural abundance of *Drosera rotundifolia* the Pharmacopoeia allows the use of other *Drosera* species, especially *Drosera ramentacea*, an African plant that contains 0.5% of naphthoquinones with antineoplastic, antimicrobial and antiviral activities [1, 2]. We established the in vitro culture of *Drosera communis* (Fig. 1) to study the formation of naphthoquinones and succeeded in the scale up to a 251 airlift-type fermentor, which produced up to 0.17% dry wt of naphthoquinones, an amount corresponding to that prescribed by the German Pharmacopoeia (AB-DE-7) for *D. rotundifolia* herba.

**Results and Discussion**

Cultures of *Drosera communis* were first subcultured on Murashige-Skoog (MS) [3] medium solidified with 0.2% Gelrite, because fast growth of *D. rotundifolia* and *D. intermedia* cultures in this medium was described by Bonnet et al. in 1984 [4]. In addition we tested several other media for growth, including Gamborg B5 [5] and Woody-plant [6] medium. The fastest proliferation of the cultures was observed in Woody-Plant liquid medium containing 2% sucrose under continuous light (Fig. 2). Concomitant with the growth the naphthoquinone production in the cultures increased with the change of the media up to 0.17% of the dry material (Fig. 3). It is interesting, that the fast growing cultures in this case were those with the highest content of natural products, i.e., with the most active secondary metabolism. Maintaining the culture conditions optimized for naphthoquinone production, we transferred the cultures to a 251 round bottom flask equipped with a pipe for air supply (Fig. 4). After 3 month of culture in this fermentor approx. 1.2 kg of *D. communis* plants were obtained and extracted for naphthoquinones resulting in 200 mg of the products with plumbagin as the main compound (identified by capillary GLC-MS).

**Material and Methods**

Plant material: Cultures of *Drosera communis* were obtained from Dr. T. E. Macek, Prague. The plants were subcultured monthly on MS liquid medium, or other media (B5, WP). For growth kinetics cultures were harvested at 7 day intervals, starting 10 days after the first inoculation. After 30 days plants were subcultured in the same medium and the experiment was repeated. Cultures were maintained under continuous light on a rotary shaker (200 ml Erlenmeyer flask, 50 ml medium). For mass culture a 251 round bottom flask, containing 201 WP medium and 3% sucrose was equipped with an air pipe (flow 20 ml/min) and approx. 5 g plants were inoculated. Cultures were harvested after 3 month.

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HPLC analyses: Harvested cultures (approx. 1 g fresh plants) were extracted exhaustively with petroleum ether (app. 10 ml) at 45°C. After evaporation the residue was dissolved in methanol and injected into HPLC; column: RP18-100 (12.5×4 mm ID); eluent: 50% acetonitrile/H₂O; detection by UV at 254 nm; the column was calibrated with authentic samples.

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References