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Comparison of extraction methods for isolation of 20-hydroxyecdysone from *Leuzea carthamoides*

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Leuzea carthamoides is an adaptogenic plant and a valuable source of phytoecdysteroids, where 20-hydroxyecdysone (20-HE) is the most abundant ecdysteroid in the majority of plant parts [1]. This ecdysteroid is known for the anabolic and tonic effects on mammals and is used as dietary supplement. Moreover, 20-HE is a steroid hormone responsible for insect moulting and metamorphosis. Therefore, it could be applied to control the harmful insect population in agriculture [2].

Phytoecdysteroids are polar compounds mostly extracted by Soxhlet extraction (SE) or maceration. These widely used methods have some disadvantages such as high temperature causing decomposition of biological compounds (SE), use of large amount of organic solvents (harmful to environment) and the long extraction time. Therefore, supercritical fluid extraction (SFE) and pressurised liquid extraction (PLE) become more often used for extraction from plants. Generally, SFE using supercritical CO₂ as a solvent is carried out at low temperatures and is used for the extraction of nonpolar compounds, but it is also suitable for the extraction of polar compounds by adding a small amount of organic solvent into the system (as modifier). This method is more selective and provides purer extract than conventional method. In contrary to SFE, PLE takes only few minutes (5-20 min.) and uses a small amount of organic solvents. It is conducted at high temperatures, above the normal boiling point of solvents, and under increased pressures keeping the solvents liquid. In spite of the high temperature, thermolabile substances are mostly preserved, because of the short extraction time. PLE is also suitable for extraction of 20-HE due to its thermo stability. Further, the combination of these methods can increase the concentration of polar compounds in the final extract. Firstly, SFE removes the nonpolar compounds, and then PLE provides a more concentrated extract using the same material.

This work is focused on the extraction of 20-HE from roots and seeds of *Leuzea carthamoides* using SFE, PLE and Soxhlet extraction. The SFE and PLE conditions (pressure, temperature, type and concentration of solvent, extraction time) were optimized to improve extraction yields and concentrations of 20-HE in extracts. These methods were compared with a conventional Soxhlet-type extraction. The SFE experiments were carried out at temperatures from 40°C to 60°C, pressures from 30 to 65 MPa and with addition of 5-20% of co-solvent (ethanol, methanol and acetone). PLE with ethanol, methanol and acetone was made at temperatures from 70°C to 100°C, pressures from 7 to 20 MPa and extraction time from 5-15 min. Soxhlet extraction with ethanol was used to evaluate the total content of 20-HE in roots and seeds. The concentration of 20-HE in extracts was determined by HPLC.

Acknowledgments:

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References:

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- [2] L. Dinan, *Phytochemistry* 2001, 57, 325-339