PhD thesis summary:

Contributions to the biological activity of natural extracts obtained from lignocellulosic plant matrices

PhD student: Sanda-Liliana Coșarcă

Supervisor: Daniela-Lucia Muntean, Full Professor, PhD.

Identification and exploitation of sustainable methods for the obtaining of bioactive products from plant matrices, as well less known plant by-products, represents an actual trend in the field of applied sciences, including the medical research.

The first part of the thesis encompasses a comprehensive up-to-date review regarding the phenolic compounds in general, with a focus on the potential use of *Fagus sylvatica* (L) (beech) and *Picea abies* (L) Karst (spruce) rhytidome as sources of polyphenols. There were summarized the recent advances regarding conventional and non-conventional extraction methods suitable for the recovery of phenolic constituents from this matrices, analytical methods used for the determination of total phenolic and tannins content of *Fagus sylvatica* (L) (beech) and *Picea abies* (L) Karst (spruce) rhytidome, as well the medicinal use of the extracts obtained from the above-mentioned matrices based on their already proven biological properties.

Personal contribution to the actual knowledge in the field consists in:

The acquirement of plant material and optimization of extracts *F. sylvatica* (L) and *P. abies* (L) Karst rhytidome extracts which was realised using lignocellulosic biomass originating from Gurghiu Mountains, Topliţa area, Harghita county, achieved as waste by-product from commercial unit Copet Dinex, Subcetate, Harghita, România, in February 2017

- Collection, drying and grinding of plant material;
- Classical aqueous and organic solvents (ethanol, methanol) assisted extraction;
- Extraction and optimization of the ultrasonic-assisted and microwave-assisted extraction methods with different solvents (ethanol, methanol).

For the statistical analysis of the obtained numerical responses, a D-optimal experimental model (Design of Experiments /DoE) was employed, using *Modde* software (version 11.0, Sartorius Stedim Dta Analytics AB, Umea, Sweden). The optimal conditions that led to the highest value of the total phenolic content (TPC) (76.57mg GAE/g) were achieved by using a 300W microwave power, an extraction time of 4 minutes, and an extraction solvent made up of a 50:50 ratio (*v/v*) of ethanol and distilled water. In the ultrasonic-assisted extraction, the optimal conditions for achieving the highest total phenolic content (TPC) were achieved by using a mixture of ethanol and distilled water in a 70:30 (*v/v*) ratio as the extraction solvent at a temperature of 60°C with an extraction time of 15 minutes.

Characterization of the optimized extracts in terms of total phenolic (TPC) and total tannin (TTC) content, qualitative and quantitative identification of some individual phenolic constituents

The highest TPC value obtained by UAE using a 70:30 *v/v* hydroethanolic mixture as solvent was observed in the *Picea abies* rhytidome extract, with a value of 171.21 mg gallic acid/g of plant

material. A similar value (165.95 mg gallic acid/g of plant material) was obtained using the same method, with distilled water was used as solvent. Compared to UAE, MAE has led to higher results, regardless of the solvent used. Among the individual phenolic constituents analyzed by the LC-MS/MS method, *p*-coumaric acid, ferulic acid, quercetin and isoquercetin were identified in the extracts, ferulic acid being the most common phenolic compound.

Determination of the biological activity of optimized extracts of beech and spruce:

- *In vitro* and *in vivo* determination of antioxidant activity;
- Cell toxicity evaluation;
- Study of microbiological activity.

The antioxidant activity of beech rhytidome extracts obtained through the microwave-assisted extraction method was evaluated by using DPPH, FRAP and TEAC assays. The strongest antioxidant activity was recorded for the extract with the highest phenolic content.

The spruce extract showed similarities to the antioxidant effect of vitamin E and may showcase a vitamin E-like effect (although not as intense) in maintaining NO concentrations, although the mechanism of action may be different. In contrast, the beech extract did not shown a beneficial effect as an antioxidant during Cr(VI) administration.

Beech and spruce rhytidome extracts decreased the viability of A375 melanoma cells, the most significant effect being observed at the highest dose used (2.5 mg/mL). In lung carcinoma A549 cells, the extracts showed no cytotoxic effect. The effects observed on both tumor cell lines were dose dependent.

The antibacterial activity of *Fagus sylvatica* rhytidome extracts (BBE) was tested on four types of bacteria. *Staphylococcus aureus* was the most sensitive strain to all the tested samples, with similar values of MIC (minimum inhibitory concentration) (1.56 mg/mL) and MBC (3.12mg/mL). Regarding the antifungal activity of beech extracts obtained through microwave-assisted extraction, *Candida* species exhibited the highest sensitivity to the beech rhytidome aqueous extract (BBE) with a MIC value of 25 mg/mL and a value of MBC of 50 mg/mL.

The original aspects of this thesis consist of:

- **optimization of the extraction processes** of phenolic constituents from the *Fagus sylvatica* rhytidome using modern technologies, such as microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE). This optimization was achieved with through a carefully designed experimental plan which involved the evaluation and precise adjustment of critical parameters such as microwave power, temperature, extraction time and solvent type, which led to maximum yields of polyphenols, contributing significantly to the efficiency of the extraction process;
- ***in vivo* study of the antioxidant capacity** of the extracts, a relatively unexplored field for the studied species;
- *toxicity assessment of the extracts on lung carcinoma (A549) and human melanoma (A375) cell lines*, aspects that have not been previously addressed in the available literature.