Doctoral thesis abstract:

The aim of the doctoral thesis was to isolate the triterpene saponin phytocompounds from the leaves of *Hedera helix* L. (ivy), known to have therapeutic properties, their physico-chemical characterization and biological evaluation of biocompatibility and antiproliferative effect activity. In a first step, extracts variants from *Hedera helix* leaves were qualitatively analyzed by physico-chemical methods and the specific classes of phytocompounds were established. Ivy extracts were obtained by four extraction processes: refluxation, ultrasonication, maceration and Soxlet continuous extraction for which the total saponin content was comparatively estimated. The isolation of the component saponins was performed from ethanolic extract obtained by maceration of *Hedera helix* L. Crude hederagenin was isolated from ethanolic extract resulted from repeated maceration of ivy leaves, followed by acid hydrolysis and purification with acetonitrile, and also from ethanolic extract obtained by refluxation, subjected to acid hydrolysis and purification with lead acetate. Based on the LC-MS chromatograms, has been determined the amount of hederagenin in the tested samples. (Method II) was 20.50 μg/mL of approx. 5 times more than in the original extract. The crude Hederagenin tested *in vitro* showed biocompatibility with normal mouse fibroblast NCTC cells L929, at concentrations of 2-200 μg/mL and had antitumor activity on Hep-2 human cervix epithelial tumor cells, at concentrations of 100-400 μg/mL, with an IC50 value of 320 μg/mL, close to standard hederagenin value (IC_{50} of 250 μg/mL). The fractions of refluxed hydroethanolic extract from ivy leaves were separated by flash chromatography with UV-Vis detection, being biologically and biochemically characterized. The antiproliferative activity of these fractions was dependent on the saponin content, and their antioxidant activity was correlated with the content in polyphenols and flavonoids. The fractions rich in saponins tested *in vitro* showed a strong antiproliferative effect on human cervical cells (IC50 181 μg / mL and 115.7 μg/mL, respectively) and were non-cytotoxic on normal mouse fibroblast NCTC cells over the concentration range of 2-200 μg/mL. The optimization of a saponins mixture with maximum antiproliferative effect, consisting of α-hederin, hederagenin and hederacoside C, was achieved by mathematical modeling using the response surface method (RSM) generated by the Design Expert 11 software. 14 mixture variants of the three components, was obtained which were subsequently tested on cell cultures. The resulting data indicated as the optimal variant was the mixture of α-hederin, hederagenin and hederacoside C with the mass ratio of 3,863: 100,000: 596,137 (μg). This mixture recorded the maximum antiproliferative effect on Hep-2 tumor cells (34.83% viability) and was biocompatible with normal NCTC L929 cells (79.59% viability). The *in vitro* studies have shown that the mixture in optimal variant of the three saponins has the expected antitumor effect on human cervical tumor cells, wich indicates its potential to be used in antitumor therapy. Also, the obtained data provide basic information on the possible use of hederagenin isolated from *H. helix* leaves, respectively of the extract fractions separated by flash chromatography, with optimal saponin content, as possible antitumor agents in cancer treatment. The obtained results were published in the form of 3 scientific articles in ISI listed journals.

Keywords: *Hedera helix* L.; phytochemical constituents; α-hederin; hederagenin; hederacoside C; flash chromatography; saponins mixture optimization; *in vitro* cytotoxicity; antiproliferative activity.