

ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department: Pharmaceutical Botany and Ecology

Candidate: Mgr. Eliška ŠTEJNAROVÁ

Supervisor: Doc. RNDr. Lubomír OPLETAL, CSc.

Title of doctoral thesis: **The Influence of Compounds from Selected Higher Plant Taxons on Platelet**

Aggregation

As a part of phytochemical study of secondary metabolites of plants from family Asteraceae, 32 species from this taxonomical unit were tested for platelet aggregation and antioxidant activity. Platelet aggregation was evaluated *in vitro* on human thrombocytes in platelet rich plasma. The primary used concentration of extracts was $500 \mu\text{g}\cdot\text{mL}^{-1}$, arachidonic acid (AA), adenosine diphosphate (ADP), collagen (COL), and thrombin (TR) were used as agonists. Out of the tested species, *Leuzea carthamoides* (leaves) was the only species which exhibited antiplatelet activity, the extract inhibited platelet aggregation induced by ADP and COL by $33 \pm 6 \%$ and $95 \pm 5 \%$, respectively. None of the tested taxons showed proaggregatory effect. From 10.5 kg of dried leaves of *L. carthamoide* the extracts were prepared sequentially; five compounds: LC-1 (cynaropicrine), LC-2 (chlorojanerin), LC-3 (repiolid), LC-4 (jaceosidin), LC-5 (hispidulin) were isolated from the most active extract (chloroform) by column and thin layer chromatography. The antiplatelet activity of compounds (IC_{50}) was for LC-1: $336 \pm 17 \mu\text{M}$ – ADP, $300 \pm 33 \mu\text{M}$ – COL, $1050 \pm 34 \mu\text{M}$ – AA, aggregation induced by TR was not affected; for LC-2: $552 \pm 19 \mu\text{M}$ – COL, aggregation induced by TR, ADP, AA was not affected; for LC-3: $630 \pm 12 \mu\text{M}$ – ADP, $1050 \pm 25 \mu\text{M}$ – COL, $490 \pm 30 \mu\text{M}$ – AA, aggregation induced by TR was not affected by repiolid; for LC-4: $1190 \pm 31 \mu\text{M}$ – ADP, $382 \pm 13 \mu\text{M}$ – COL, $136 \pm 16 \mu\text{M}$ – AA, aggregation induced by TR was not affected; for LC-5: $1060 \pm 54 \mu\text{M}$ – ADP, $100 \pm 24 \mu\text{M}$ – COL, $522 \pm 13 \mu\text{M}$ – AA, aggregation induced by TR was not affected. Compounds LC-1 and LC-2 were practically inactive in DPPH radical scavenging assay ($\text{EC}_{50} > 1000 \mu\text{M}$), while LC-3 was

significantly more active ($EC_{50} 259 \pm 14.2 \mu\text{M}$). Both flavonoids were also less active in comparison with standard antioxidants: LC-4 ($EC_{50} 411 \pm 17.8 \mu\text{M}$) and LC-5 ($EC_{50} 796.6 \pm 25.7 \mu\text{M}$), trolox ($EC_{50} 27.8 \pm 1.7 \mu\text{M}$), quercetin ($EC_{50} 25.3 \pm 1.6 \mu\text{M}$). In case of FRAP test the highest value showed LC-4 ($39.2 \mu\text{M}$ after 60 mins exposition) in comparison with standards: trolox ($22.2 \mu\text{M}$) and quercetin ($98.3 \mu\text{M}$).

Key words: Antiplatelet activity, DPPH assay, FRAP test, Asteraceae, *Leuzea carthamoides*, cynaropicrine, chlorojanerin, repdiolid, jaceosidin, hispidulin.