ABSTRACT

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Title of Doctoral Thesis: **Study of chemical constituents of taxons from order Laurales and Ranunculales with potential neuroprotective activity.**

Key words: *Berberis vulgaris, Peumus boldus, Hydrastis canadensis*, isoquinoline alkaloids, acetylcholinesterase, butyrylcholinesterase, prolyl oligopeptidase.

Commercial goldenseal extrakt, boldo leaves and barberry root bark were selected as sources of isoquinoline alkaloids for study of their biological activity. Mixtures of summary tertiary alkaloids were prepared by standard extraction and subsequently fractionated in aluminium oxide chromatography column using the step gradient elution with petrol, chloroform and ethanol. Repeated column chromatographies, preparative TLC and crystallizations led to the isolation of 28 isoquinoline alkaloids, 6 of them were identificated as new structures (some of them were propably artifacts). The chemical structures of isolated compounds were determined on the basis of spectrometric techniques (NMR, MS) and by comparison with literature. Isolated alkaloids in sufficient amount were tested on ability to inhibit human erythrocyte AChE and serum BuChE and POP (IC_{50} was ascertained).

The cholinesterase inhibitory activity was determined *in vitro* by modified spectrophotometric Ellman's method. (+)-Canadaline was the most potent inhibitor of AChE and also weak inhibitor of BuChE in a dose-dependent manner with IC_{50} values of 32,9 ± 4,9 μM and 105,4 ± 15,6 μM respectively. The compound BV02 (IC_{50} 55,3 ± 6,6 μM), berlambine (IC_{50} 62,4 ± 11,5 μM), (+)-bersavine (IC_{50} 68,2 ± 10,5 μM), (+)-obamegine (IC_{50} 97,4 ± 3,4 μM) and (+)-berbostrejdine (IC_{50} 65,9 ± 7,5 μM) inhibited AChE, the last one inhibited BuChE (6,9 ± 1,0 μM) as well. The most potent inhibitor of BuChE was (+)-aromoline with IC_{50} value of 0,82 ± 0,1 μM, it also inhibited horse serum BuChE with IC_{50} value of 8,7 ± 0,1 μM in a mixed manner; other effective inhibitors were

(+)-*N*-methylcoclaurine, (+)-retikuline, (+)-chenabinol and (-)-muraricine (IC_{50} values 15,0 ± 1,4 μM, 33,6 ± 3,0 μM, 44,8 ± 5,4 μM and 67,5 ± 5,9 μM respectively). Other isolated alkaloids were considered to be inactive (IC_{50} > 100 μM).

The POP inhibition activity was determined spectrophotometric method using *Z*-Gly-Pro-*p*-nitroanilid as substrate. The strongest inhibition activity was shown by (+)-bersavin (IC_{50} value of 67,3 ± 6,2 μM), inhibition activities of (+)-aromoline, berlambine, (+)-*N*-methyllaurotetanine and (-)-sinoacutine was comparable to the standard of berberine (IC_{50} 142,3 ± 21,1 μM), nevertheless any of the alkaloids gained activity of the standard *Z*-pro-prolinal. Other isolated alkaloids were considered to be inactive (IC_{50} > 200 μM).