ABSTRACT

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Title of Doctoral Thesis: **Study of biological activity of isolated alkaloids from *Corydalis cava* (Fumariaceae).**

Key words: *Corydalis cava*, isoquinoline alkaloids, DPPH, AChE, BuChE, BACE1, IMER.

Tubers of *Corydalis cava* were selected on the basis of bio-guided spectrophotometric Ellman´s method as a source of isoquinoline alkaloids for study of their selected biological activities. The tubers of *Corydalis cava* were extracted with ethanol and the mixture of summary tertiary alkaloids (fraction A) was fractionated in silica gel chromatography column using step gradient elution with hexane, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of fifteen isoquinoline alkaloids. The chemical structures of isolated compounds were determined on the basis of spectroscopic techniques and by comparison with literature data. Isolated alkaloids were tested on ability to inhibit AChE, serum BuChE (IC_{50}), BACE1 and for its free-radical scavenging activity (EC_{50}).

The cholinesterase inhibitory activity was determined *in vitro* by modified spectrophotometric Ellman's method. (+)-Canadaline inhibited AChE as well as BuChE in a dose-dependent manner with IC_{50} values 20.1 ± 1.1 μM and 85.2 ± 3.2 μM, respectively. (+)-Canadine with an IC_{50} value 12.4 ± 0.9 μM was the most potent inhibitor of acetylcholinesterase, whilst (±)-corycavidine and (+)-bulbocapnine were effective inhibitors of butyrylcholinesterase with IC_{50} values 46.2 ± 2.4 μM and 67.0 ± 2.1 μM, respectively. Other isolated alkaloids were considered inactive (IC_{50} > 100 μM).

The free-radical scavenging activity of isolated alkaloids was tested *in vitro* by means of the DPPH test. The highest activity was exhibited by (–)-scoulerine, (–)-sinoacutine and (+)-bulbocapnine with EC_{50} values 102 ± 6.2 μM, 209 ± 8.1 μM and 279 ± 16.7 μM, respectively. Other isolated alkaloids were considered inactive (EC_{50} >1000 μM).

BACE1 inhibitory activity of isolated alkaloids was measured by two different assays: multi-well plate format (FRET assay) and hrBACE1-IMER assay. FRET assay in solution studies was carried out on a multi-well spectrofluorimeter using M-2420 and Panvera substrates. The assay was performed by reading the fluorescence signal at $\lambda_{ex}/\lambda_{em}$ = 320/405 nm and 544/590 nm, respectively. Panvera substrate was used for four compounds which were found to interfere with substrate M-2420 at λ = 405 nm. The hrBACE-IMER was inserted into a HPLC system and chromatographic analyses were performed using substrate M-2420. Fluorescence detector was set at 320 and 405 nm for excitation and emission wavelengths, respectively. (–)-Corycavamine and (+)-corynoline established their BACE1 inhibitory activity in both FRET assay (IC_{50} = 41,16 μM and IC_{50} = 33,59 μM, respectively) and in the hrBACE-IMER assay (IC_{50} = 1690 μM and IC_{50} = 59,07 μM, respectively), other compounds were inactive or did not show a correlation between inhibitor concentration and enzyme inhibition. None of tested compounds can be considered as a good lead compound for BACE1 inhibition in comparison with BACE1 standard inhibitor IV (IC_{50} = 0.02 μM). Use of the above mentioned methods represents a suitable approach for the screening and determination of BACE1 inhibitory activity of tested compounds.