# 5. Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Botany and Ecology

Candidate: **Mgr. Daniela Hulcová**

Supervisor: **Doc. Ing. Lucie Cahlíková, Ph.D.**

Title of Doctoral Thesis: **Biological activity of alkaloids *Narcissus pseudonarcissus* L. cv. Dutch Master (Amaryllidaceae)**

Keywords: *Narcissus pseudonarcissus* L. cv. Dutch Master, Amaryllidaceae, alkaloids, AChE, BuChE, POP, GSK-3β, biological activity.

Bulbs of *Narcissus pseudonarcissus* L. cv. the Dutch Master of the Amaryllidaceae family, along with other species of the genus *Narcissus*, was subjected to a bio-guided study. This study evaluated summary alkaloid extracts using spectrophotometric Ellmans method and GC-MS analysis as a possible source of biologically active Amaryllidaceae alkaloids. *Narcissus pseudonarcissus* L. cv. Dutch Master was selected as a suitable source for isolation of alkaloids. The fresh bulbs of this daffodil were extracted with ethanol and the crude extract was separated into individual fractions by column chromatography using alumina and silica gel as a stationary phase. Subsequently a stepwise elution was performed, where the mobile phase was a mixture of different ratios of petrol - chloroform and chloroform - ethanol. Some fractions had to be repeatedly partitioned by column chromatography. This was followed by preparative TLC and crystallization by which pure compounds were isolated. The chemical structures of the obtained substances were determined by spectrometric techniques (MS, 1D- and 2D-NMR analysis, optical rotation) and by comparison of the obtained data with the literature. From the fresh bulbs, 21 already known substances and one new alkaloid (narcimatulin) were isolated. Alkaloids isolated in sufficient amount were tested for various biological activities associated with possible therapy of Alzheimer's disease and cancer (inhibition against AChE, BuChE, POP, GSK-3β, AKR3C1, cytotoxicity).

Cholinesterase inhibitory activity was determined *in vitro* by the spectrophotometric modified Ellmans method. POP inhibition was determined using Z-Gly-Pro-*p*-nitroanilide as a substrate. To determine inhibitory activity against GSK-3β, an *in vitro* luminescence method was used according Baki et al. (2007).

Some of the isolated compounds showed an interesting biological activity. Galanthamine is known for its anticholinesterase activity. It is worth to mention also homolycorine, where for AChE $IC_{50}$ = 64 ± 4 µM and for BuChE $IC_{50}$ = 151 ± 20 µM. Very promising results have also been demonstrated

by the newly isolated compound narcimatuline, which has a strong inhibitory potential against BuChE $IC_{50}$ = 5.90 ± 0.23 μM. This compound also very well inhibits POP $IC_{50}$ = 29.2 ± 1.0 μM and GSK-3β $IC_{50}$ = 20.7 ± 2.4 μM. Interesting inhibitory activity against GSK-3β was also shown by masonine $IC_{50}$ = 27.9 ± 0.8 μM and caranine $IC_{50}$ = 30.8 ± 0.3 μM. Plant *Narcissus pseudonarcissus* cv. Dutch Master, based on the results, is an interesting source of alkaloids.