ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmacognosy and Pharmaceutical Botany

Candidate: **Tereza Buchalová**

Supervisor: **PharmDr. Daniela Hulcová, Ph.D.**

Title of Thesis: **Isolation of alkaloids from *Hippeastrum* cv. Ferrari and their biological activity**

Many different species, varieties, and cultivars of the genus *Hippeastrum* are popular indoor ornamental plants, but they also represent a rich source of important secondary metabolites called Amaryllidaceae alkaloids. These structurally unique compounds are known for their wide spectrum of biological activities such as anticancer, antiviral, and inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which represent important part in therapy of Alzheimer´s disease (AD).

AD is a neurodegenerative disease that is identified as one of the most frequent causes of dementia in the world. Deficit of the neurotransmitter acetylcholine (ACh) in the cortex participates on the development of the AD, which results in the damage of cholinergic functions, and this is responsible for the memory loss and behavioural changes. AChE and BChE are enzymes involved in the termination of impulse transmission by rapid hydrolysis of ACh. Therefore, AChE and BChE inhibitors are nowadays the most important goal in the treatment of AD.

Ethanolic extract was obtained from 25 kg fresh bulbs of *Hippeastrum* cv. Ferrari. This extract was purified by liquid-liquid extraction and fractionated by column chromatography to 15 individual fractions, which were used to isolate pure alkaloids. The fraction HF-14 was processed by preparative thin layer chromatography and 6 pure compounds were isolated from fraction HF-14: tazettine, 11-hydroxyvittatine, hamayne, 9-*O*-demethyl-7-*O*-ethyllycorenine, 9-*O*-demethylhomolycorine and eugenine.

Isolated alkaloids were tested for their biological activities associated with AD, cytotoxic and antimalarial activity. The inhibitory activity against human cholinesterases was measured *in vitro* by the Ellman's spectrophotometric method, but all tested substances were determined to be inactive against the enzymes (IC_{50} > 100 μM). The most active alkaloid against prolyl oligopeptidase appeared to be eugenine (IC_{50} = 130 ± 8 μM). The majority of isolated alkaloids were screened for cytotoxic activity. The screening study was performed on a total of 10 cell lines (Jurkat, MOLT-4, A549, HT-29, PANC 1, A2780, HeLa, MCF 7 and SAOS 2,

MRC-5). Among tested alkaloids, hamayne showed promising cytotoxic activity against HT-29, A549, PANC 1 and HeLa cells, tazettine also showed moderate cytotoxic activity against HT-29 cell line. *In vitro* activity against the hepatic stage of *Plasmodium berghei* was also determined, but none of the tested alkaloids showed significant inhibitory activity.