CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Pharmacognosy and Pharmaceutical Botany

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2024

Student:	**Simona Hašanová**
Thesis Tutor:	PharmDr. Daniela Suchánková, Ph.D.
Consultant:	PharmDr. Jana Křoustková, Ph.D.
Opponent:	Maafi Negar, Ph.D.
Thesis title:	**Amaryllidaceae alkaloids of Narcissus poeticus var. recurvus and their implication to Alzheimer's disease and anticancer activity**

Scope of work, number of 90 pages, 46 figures, 18 tables, 101 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Excellent
b)	The complexity of the methods used:	Excellent
c)	Preparation of the methodological part (clarity, comprehensibility):	Excellent
d)	The quality of the experimental data obtained:	Very good
e)	Processing of results (clarity):	Excellent
f)	Evaluation of results, including statistical analysis:	Excellent
g)	Discussion of results:	Excellent
h)	Clarity, conciseness, and adequacy of conclusions:	Very good
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Very good
l)	Formal level of the work (text structure, graphic design):	Very good

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

Simona Hašanová's diploma thesis focuses on the extraction of alkaloids from the bulbs of Narcissus poeticus var. recurvus, followed by fractionation of the entire extract using advanced chromatographic techniques. Three alkaloids were isolated and purified from selected fractions. These compounds were structurally identified using methods such as 1D and 2D NMR, GC/MS-EI, and optical rotation measurements. These alkaloids, known from Amaryllidaceae plants, were subjected to biological assays to evaluate their potential activity, mailny against cancer and Alzheimer's disease. Additionally, in silico studies were conducted to explore their possible interactions with the acetylcholinesterase enzyme, along with six semi-synthetic derivatives of the same structural class.

The work conducted for this thesis is comprehensive, thorough, and, in many aspects, physically demanding. The flow of the study is consistent and logical, beginning with the extraction of grams of alkaloidal extract, followed by fractionation, isolation, and purification of compounds, and concluding with the determination of biological activity, supported by in

silico studies. The predicted outcome of the work is sufficiently met by achieving the isolation of three pure compounds and reporting one of them as a potential scaffold for inhibiting human butyrylcholinesterase, for the first time. Furthermore, the written thesis is well-organized, with sufficient introductory information and a detailed methodology. Typos are quite rare in the text, and despite a few linguistic mistakes, the writing is comprehensible.

My main comments concern the list of abbreviations, a few writing style issues, and the need for more spectroscopic data.

It is understandable that since the isolated alkaloids were already known, the NMR spectra and such were not repeatedly published. However, I would personally prefer to see the spectra and a discussion of their structural correspondence to the literature in a phytochemical study, particularly for lycorine after recrystallization using DMSO. Since the potential instability of lycorine after storage is mentioned in the text, it would be valuable to discuss its stability following the conditions required to remove DMSO. Publishing the mass spectra of this impure fraction and discussing it would be complementary.

The list of abbreviations appears unnecessarily long, for example many bacterial and fungal strains are mentioned only once throughout the whole text. Additionally, some abbreviations, like PS-1, are used but not defined in the list. The consistency of the writing style is sometimes lost, particularly in the in silico study section. For instance, minus signs are inconsistently represented (en-dash, short dash, or an unknown symbol) in Table 16 and the descriptions of Figures 34 and 37.

Despite the mentioned issues, the overall quality of the thesis is excellent, and congratulations to the author are well-deserved.

Questions and comments to student:

Commemnts: As with any genuine human effort, some mistakes may occur. I would like to draw the author's attention to the following comments:

Page 10: Table 1, It is necessary to choose a consistent form and style for certain terms, such as "Leukemia" or "Leukaemia." Similarly, the formatting of "in vitro" should be consistent—either italic (as used primarily) or non-italic (as seen on p25 and p75). The use of the decimal point should also be standardized. In English-speaking countries, a point is used instead of a comma (e.g., p33, last paragraph: 1.710 and 0.065 vs. 42,301 and 0,065).

Page 14, Table 2, It is advisable to use up-to-date, cumulative references. When discussing alkaloids from the Narcissus genus and their potential in addressing Alzheimer's disease, it is a pity not to mention the newer group of promising alkaloids, such as Carltonine B from Narcissus pseudonarcissus cv. Carlton.

Page 14: Since the short form "AA/AAs" for Amaryllidaceae alkaloids is defined in the list of abbreviations, avoid repeating it in the text unless necessary. The same applies to p17, p38, and p75.

Page 24: Figure 5. Better to define the stereochemistry for position 10b in the depicted structure of lycorine.

Page 33: Section 4.2.3.1, What do ATChI and BuTChI stand for? The chosen short forms for enzymes are different in the abbreviation list.

Page 35: I would recommend not including an NMR spectrum in the methodology section related to the determination of cytotoxic activity against the MCF-7 cell line. Instead, the NMR and the intresting possibility of lycorine degradation could be discussed separately in the results and discussion.

Page 60: In the final paragraph, it would be better to provide a complete profile of the IC50 value. For example: "...for the hBuChE inhibitory potential of alkaloid extracts of Narcissus poeticus var. recurvus (IC50 = 23.0 ± 1.0 μM) initially..."

Page 62: Table 12, If compound 2 hasn't been studied for Jurkat, mark it with *ND, and in the table description, add: *ND = not determined. Or it is just a typo?

Page 67: bold formatting for compound numbers is inconsistently applied, as seen in Figures 34, 35, and 37, compared to other sections.

Page 68: Figure 36, colors of two superimposed molecules need to be defined in the description of the figure. Same applies for Figure 38, p69.

Page 69: Descrption of Figure 38 is not sufficient.

Questions:

1- I would like to ask about following details concerning using DMSO for purification process of lycorine mentioned in page 36:

a- "The DMSO was evaporated by a rotavapor with an oil pump." Could you explain the conditions you used for evaporating DMSO, given that higher temperatures or reduced pressure are necessary for its evaporation? Was there any residue remained in the sample after the filteration and washing with MOH in the NMR spectrum?

b- As given in the text, "Using DMSO as a solvent for the preparation of NMR samples, instability was observed. This instability occurred after a few hours and manifested as a colour change from colourless to yellowish." Therefore I would like to ask, why did you choose DMSO as a solvent for crystalization and purification?

2- Could you please elaborate on the factor LE (ligand efficiency)? Can we draw any conclusions from the LE factors reported in Table 15?

3- Since you have introduced the affinity of cherylline to inhibit hBuChE, and all the three compounds and the galanthine derivatives were completely inactive in AChE, is there a specific reason for conducting or presenting in silico studies only on the AChE protein?

Evaluation of the thesis: Excellent		**For the defense:**	**Recommend**
In Hradec Králové	12. září 2024	signature of the opponent	