OPPONENT’S REVIEW of Ph.D thesis
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“Study of the inhibitory (toxic) effect of the alkaloids from chosen plants of Amaryllidaceae family on some human enzymatic systems (in vitro study) II.”

CHOICE OF RESEARCH TOPIC
The choice of research topic is relevant from multiple perspective:
- ability of Amaryllidaceae alkaloids to inhibit human choliesterases (AChE, BuChE) and their possible usage for treatment of neurodegenerative diseases especially Alzheimer’s disease (AD)
- inhibition of prolyl oligopeptidase as a new approach for supportive treatment of AD
- evaluation of antitumor activity of alkaloids

The presented work is thus a contribution to a wide range of research results in this field. For all these reasons, I consider the aim of dissertation to be very up-to-date and extremely important.

LITERATURE REVIEW
The thesis refers to a large number of references (211) and summarizes recent knowledge in the field. The comprehensive literature review relies on classifications and the results and methodological reviews of a specific literature relevant to the research topic from the international field.

METHODOLOGY
During her Ph.D Nina Vaněčková focused her effort on isolation and identification of alkaloids from the fresh bulbs of Nerine bowdenii Watson and to evaluate their different biological activities. The doctoral thesis contents obvious methods used within the doctoral research in the field of Pharmacognosy and natural compounds isolation. Analytical methods for alkaloid analysis, separation and isolation (GC, column chromatography, preparative chromatography on different carriers and different mobile
phases) MS, NMR spectra and statistical analysis during evaluation of biological activities have been chosen adequately and the interpretation of the results is also appropriate.

FORMAL REQUIREMENTS
Overall dissertation extent is 119 pages. Dissertation is divided into several parts obvious for this kind of work. The style is appropriate, the tables and figures are presented with care and rigor. Formal adjustment, clarity and language level of the text parts fit well with the general requirements.

FULFILLING THE OBJECTIVES
The main goal of the dissertation was the isolation and identification of the alkaloids from the above mentioned plant and their screening for biological activities connected to AD and oncological diseases, with respecting several partial aims defined in Chapter 2: Aim of the dissertation. As a result, 21 alkaloids were isolated and identified – two of them for the first time in this plant species. Attention was paid to the study of the inhibitory activity against human cholestereases and prolyl oligopeptidase. Twelve of the isolated alkaloids were tested for cytotoxic activity against p53-mutated Caco-2 and HT-29 colorectal adenocarcinoma cells and antiproliferative activity (by WST-1 assay) in the screening panel of 17 human cell lines.

To conclude, the objectives of the dissertation were successfully fulfilled in all respects.

PUBLICATIONS OF THE AUTHOR
Publications of the author presented in the thesis Chapter 10: Compendium of publications is strongly related and relevant for the research topic and have been published in international journals (especially Natural Product Communications 6x, RSC Advances, Phytochemistry Letters) and monography: Studies in Natural Products Chemistry
It could be appreciated that the dissertation has contributed to the several scientific research projects of Charles University, of the Faculty of Pharmacy, and European project all of them mentioned in Acknowledgements of Dissertation.
REMARKS TO THE INDIVIDUAL PARTS OF DISSERTATION AND QUESTIONS TO THE CANDIDATE

- page 47: table 6 is coming before table 5
- It is a little bit strange to mention that structural elucidation was realized on the basis of performed experiments (optical rotation) with note, that optical rotation was not measured due to lacking of compound chiral centre (page 59, 69, 70)
- I would like to clarify Figure 10 (chromatogram of the summary alkaloid extract). Is it GC of crude extract or one of the 8 combined fractions obtained after CC? What represent red marked abbreviations A 13, A 14 and A 22?
- page 49: 51 g of extract was fractionated by CC and the result of this fractionation is summarized in table 6. Eight combined fractions were obtained in total amount of approx. 28 g. What about the rest of the extract? Is that column still in progress or the rest of extract is irreversibly attached to the solid phase?
- Several fractions were stored for further analysis due to content of minor compounds (page 50, 51). Do you intend to analyze them? Is there possibility to identify some new structures?
- Cytotoxic activity against two cancer cell lines was evaluated (page 85). Is it possible to reach out effective µM concentrations in organism with respect of solubility of tested compounds?
- According to your knowledge are some of Amaryllidaceae alkaloids in some stage of clinical trials?

SUMMARY

The Ph.D student with this dissertation thesis demonstrated the ability to work independently, and to analyze obtained results. Candidate prepared an original work which fulfill criteria and conditions of a scientific work and published results in scientific international journals. The comments stated above do not significantly reduce its positive contribution. I consider that the dissertation addressed all objectives and I recommend the PhD thesis for acceptance and defense.

Bratislava, 1. 10. 2018

Prof. PharmDr. Pavel Mučaj, PhD.