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: 2022/2023

Student:	**Elizabeta Hristiyanova Dulendarova**
Thesis Tutor:	RNDr. Jaroslav Jenčo, Ph.D.
Consultant:	-
Opponent:	PharmDr. Jana Křoustková, Ph.D.
Thesis title:	**Phytochemical studies of potential phytopharmaceuticals using separation methods**

Scope of work, number of 63 pages, 27 figures, 16 tables, 88 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):	Very good
d)	The quality of the experimental data obtained:	Excellent
e)	Processing of results (clarity):	Excellent
f)	Evaluation of results, including statistical analysis:	Excellent
g)	Discussion of results:	Very good
h)	Clarity, conciseness, and adequacy of conclusions:	Excellent
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Very good
k)	Language level (stylistic and grammatical level):	Very good
l)	Formal level of the work (text structure, graphic design):	Excellent

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

The diploma thesis of Elizabeta Hristiyanova Dulendarova is focused on the bio-guided isolation of alkaloids from Papaver rhoeas. The thesis is well written and conceived in the usual way, where the Theoretical part consists of a brief summary dedicated to the Papaveraceae family and Papaver rhoeas itself, introducing skeletons of isolated alkaloids and their respective molecules, including several unnecessary references and errors in the structure description. As this work is focused on isolation guided by biological activities, a short introduction is devoted to Alzheimer's disease and cancer. In my opinion, this part could easily be shorter, because, as Ms. Dulendarova stated, the biological assays were analyzed in collaboration and are not even discussed in detail in the Experimental part. The theoretical part is then closed by a chapter describing separation methods in phytochemistry, which is my favorite part of the introduction not only because of the writing style but also in connection with the overall content of the work.

If I have to choose the best part of this work, it is the Experimental part, especially the Results and Discussion. The description of HPLC separation is thorough, including scaling-

up and optimization of the analytical mode to the preparative mode of HPLC instrumentation. Several chromatographs are shown and described. Analyses of HPTLC, GC-MS, and EI-MS data are also included; thus, the design of the isolation was chosen appropriately. In the end, from the total amount of 107 mg, eight alkaloidal fractions were isolated revealing that caaverine was the main component (43.7 mg) that was also responsible for the fraction's activity against butyrylcholinesterase. In addition to this alkaloid, two other substances were identified.

I see the actual structure identification as the weakest point of this part. To describe known compounds only by EI-MS and optical rotation without any reference to the literature is insufficient. The conclusion is brief and to the point. The list of references, whose level is not very good, is more of a cosmetic flaw.

Although the overall similarity is high (35% in Turnitin and 37% in Thesis), none of the similarities has a conclusive value because individually they are matches of up to 1%, and therefore proves that this thesis is an original work.

Questions and comments to student:

COMMENTS

In general, I found several mistakes regarding the classic nomenclature of organic compounds, typos, incorrectly depicted chiral centers, etc. But as this work is focused on separation methods, I do not consider these errors to be fundamental to this thesis.

p.19 - Table 12 has very poor resolution, and the nomenclature is not consistent with the style used in the thesis. I find mentioning tacrine redundant since the reference is dated to 2010, therefore probably, not as recent as possible in Alzheimer's disease topic.

p.23-24 - The family name Papaveraceae should not be in italics.

p.36 - An abbreviation of ethyl acetate is usually EtOAc, not EToAc.

p.37 and 42 - I am missing information on what part of the alkaloidal extract was processed, providing these fractions PPR-11 and PPR-12. It would be great to have it stated right in the thesis, not only in a reference, to make it more comprehensive.

p.51-52 - The temperature should be reported for optical rotation value. In addition, a comparison with the reported value would complete the picture. Anyway, I would recommend using a consistent style for EI-MS and optical rotation for both described compounds, e.g. decimal places, dots/commas, units, etc.

The format of references would need an intervention, especially the names (sometimes only initials, sometimes a family name, etc.), but since it is possible to find the article by title, I accept it.

A drawn molecule with a reference connected to only a database is not valuable (ref. 16-19, 29, 46, 52-55). Databases can and do contain errors, so the best is to connect the molecule with one experimental article or a review, where everything is clearly stated.

QUESTIONS

Page 45 - What do you think by "aforementioned work [88]" when you first cite the article from Breiterová et al.? How does this article relate to your work?

Table 2 - Can you explain how galanthamine is a phenanthrene alkaloid or isoquinoline alkaloid (scaffold mentioned in Table 3)?

Figure 3 - Is this really the structure of tetrahydroisoquinoline? If not, what is it and what is the correct structure of tetrahydroisoquinoline?

What techniques would be best to use for structure analysis of isolated alkaloids?

Please briefly describe what the two chromatograms in Figure 21 show - comparison of peaks, retention time, and so on.

Despite all these facts, the diploma thesis fulfills all requirements, and I recommend it for the defense.

Evaluation of the thesis: Excellent   **For the defense:**   **Recommend**

In Hradec Králové   24. května   signature of the opponent
                           2023