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STATEMENT

by Prof. Petko Penkov Marinov, MD, PhD
Department "Pharmacology, toxicology and pharmacotherapy",
Medical university - Varna
of a dissertation work for the acquisition of the educational and scientific degree "Doctor"
in a professional field *7.3. Pharmacy*
Doctoral programme "Pharmacology (including Pharmacokinetics and chemotherapy)"

Author: *Kristina Yulianova Stavrakeva, MPharm*
Form of PhD study: full-time
Department: Pharmacology, toxicology and pharmacotherapy
Title: "Study of biological effects of methanolic exctract of *Micromeria frivaldszkyana* (*Degen*) Velen. (*Lamiaceae*)"
Scientific supervisors: Assoc. Prof. Elisaveta Apostolova, PhD and Prof. Anelia Bivolarska, MD, PhD

1. **General presentation of the procedure and the PhD student**

In relation to the defence of the doctoral dissertation, the PhD student has submitted the following set of documents:

- Application to the Rector of MU-Plovdiv for the opening of the procedure for the defence of a dissertation
- Curriculum Vitae in European format with the PhD student`s signature
- Notarized copy of a higher education diploma
- Orders for enrolment in doctoral studies, interruption of studies (due to maternity), and continuation of studies; for withdrawal with the right of defence
- Order for conducting an exam from the individual plan and a corresponding protocol for a passed exam or doctoral minimum in the specialty
- Protocol of the department council for preliminary discussion of the doctoral thesis and the decisions taken to open a procedure and for the composition of a scientific jury
- Dissertation work
- Abstract of the dissertation
- List of scientific publications on the topic of the dissertation
- Copies of the scientific publications
- List of participations in scientific forums
- Declaration of originality and authenticity of the attached documents
- Other documents related to the course of the procedure – certificate of earned credits

The PhD student has applied 3 publications.

The presented set of materials on electronic media is in compliance with Article 70 (1) of Section I. Acquisition of the educational and scientific degree 'DOCTOR' at Medical University of Plovdiv; Regulations of the Medical University of Plovdiv from 28.01.2021.

Biographical data and professional qualificaton

MPharm. Kristina Yulianova Stavrakeva was born on October 21, 1996. In 2015, she graduated from high school with a humanitarian profile and intensive learning of the English language. From 2017 to 2022, she was a student at the Faculty of Pharmacy, Medical University of Plovdiv. Upon completing her Master's degree in Pharmacy, she worked in a community pharmacy in Plovdiv. Since January 2025, she has been an assistant professor at the Medical University of Plovdiv, Department of Pharmacology, Toxicology, and Pharmacotherapy. Since 2023, she has been a full-time PhD student in the doctoral program "Pharmacology (including Pharmacokinetics and Chemotherapy)" at the Department of Pharmacology, Toxicology, and Pharmacotherapy, Faculty of Pharmacy, Medical University of Plovdiv.

2. Relevance of the topic

Currently, there has been an increased interest in plant-based medicinal products and nutritional supplements. *Micromeria frivaldszkyana* is a species endemic to Bulgaria, and its phytochemical composition and therapeutic activity are insufficiently explored. Enriching the information regarding the phytochemical content of *M. frivaldszkyana* will increase knowledge about its composition and contribute to the characterization of its biological potential. Revealing unknown properties may serve as a source for the synthesis of new pharmaceutical drugs or dietary supplements containing extracts from the plant. Studying its toxicity will determine its relevance for use and application. The present experimental study consists of examining the qualitative and quantitative composition of the methanolic extract from *M. frivaldszkyana*, acute toxicity, analgesic and anti-inflammatory effects, impact on cognitive functions, and hepatoprotective action.

3. Awareness of the subject matter

The dissertation by the PhD student demonstrates a sound assessment and a well-structured summary of the data from the literature review. Based on this, the formulated aim and objectives are fully appropriate. Numerous experimental methods have been proposed and implemented to achieve the aim and objectives. All of the above reflects a thorough understanding of the existing data to date and contributes to enriching knowledge for the potential future practical application of *M. frivaldszkyana*.

4. Research methodology

In the dissertation, the PhD student has proposed and conducted a variety of experimental methods, which I believe contribute significantly to the successful achievement of the set aim and objectives.

5. Characterisation and evaluation of the dissertation and its contributions

The dissertation paper contains 132 pages and is illustrated with 45 figures and 12 tables. The bibliography includes 257 references, of which 2 pages are in Cyrillic script and 255 pages are in Latin script. The dissertation is structured according to the classical model and includes: Title page, table of contents, and abbreviations – 9 pages; Introduction – 1 page; Literature review – 40 pages; Aim and objectives – 1 page; Materials and methods – 13 pages; Results and discussion – 43 pages; Conclusion – 1 page; Summary – 1 page; Contributions – 1 page; References – 22 pages.

Introduction

The author briefly presents the currently known data on *Micromeria frivaldszkyana* and the potential to determine its suitability for use and application through further toxicity studies. The proposed experimental investigation aims to study the qualitative and quantitative composition of the methanolic extract of *M. frivaldszkyana*, determine acute toxicity, as well as its analgesic, anti-inflammatory effects, influence on cognitive functions, and hepatoprotective action.

Literature review

In this chapter, the PhD student examines the botanical characteristics and distribution range of *M. frivaldszkyana*, its phytochemical composition, and its applications. The candidate presents data on the antioxidant activity of the plant, methods for investigating this activity, methods for studying the analgesic effect, experimental models of inflammation, the impact on cognitive functions, and the models for influencing them, as well as the effect on liver function and experimental models of hepatotoxicity. The literature review is written in over 40 pages and illustrated with 22 figures. It has been meticulously prepared and presented, providing a solid foundation for the dissertation topic.

Aim and objectives

The aim of the study is to obtain a methanolic extract from the aerial parts of *Micromeria frivaldszkyana* (*Lamiaceae*), investigate the chemical composition of the extract, and determine some of the biological effects of the obtained extract. To accomplish this objective, seven tasks of predominantly experimental nature have been outlined.

The aim and the tasks are clearly defined and appropriately articulated, reflecting a well-structured approach to the research.

Materials and methods

The materials used in the dissertation include collection of aerial parts of *Micromeria frivaldszkyana*, methanol extraction, and identification of compounds, as well as experiments conducted on male Wistar rats. The experimental methods include determination of acute toxicity and LD50, analgesic and anti-inflammatory effects, the impact on cognitive functions, and the investigation of hepatoprotective action in various models of hepatotoxicity.

The experimental, laboratory, and statistical methods support and facilitate the achievement of the set aim and objectives.

Results and discussion

In this chapter of the dissertation, the PhD student conducts a thorough investigation of the biological effects of the methanolic extract from *M. frivaldszkyana*. A significant number of compounds present in the plant have been identified by using modern methods. GC-MS analysis identified 83 compounds, while UPLC-MS-MS analysis of the methanolic extract samples from *M. frivaldszkyana* revealed 192 compounds. The results indicate the absence of toxicity following the oral administration of the methanolic extract to male Wistar rats. In the assessment of the analgesic effect, no analgesic activity was observed in the hot plate and analgesimeter tests. Experimental data showed an anti-inflammatory effect. Recorded anti-inflammatory activity of the extract is likely attributable to its high flavonoid content. Regarding cognitive functions, it was found that the application of the methanolic extract from *M. frivaldszkyana* did not enhance spatial working or episodic memory in naïve rats. Furthermore, it was established that the methanolic extract of *M. frivaldszkyana* influences oxidative stress in experimental models of hepatotoxicity, primarily through the suppression of free radical production.

Conclusions

The 8 conclusions formulated by the author represent a logical and coherent culmination of the findings presented in the dissertation.

Contributions

Based on the dissertation, MPharm. Stavrakeva delineates and articulates two distinct groups of contributions, which I fully support. The first group pertains to the scientific-theoretical domain, marking a pioneering study on the acute toxicity of the methanolic extract from *M. frivaldszkyana* following oral administration in rats. Furthermore, it represents the inaugural comprehensive metabolomic analysis of the methanolic extract, which uncovers a notable presence of phenolic acids and flavonoids, compounds likely responsible for the observed biological activities. The second group of contributions is within the scientific-applied field, as it establishes, for the first time, the potential hepatoprotective effects of the methanolic extract of *M. frivaldszkyana* in liver toxicity models in rats, and additionally, it identifies the extract's novel anti-inflammatory effect in a rodent model of hind limb inflammation.

These contributions significantly enhance the field of pharmacological knowledge in our country and bear both substantial theoretical and practical implications for future research and application.

6. Evaluation of publications and personal participation of the PhD student

The PhD student has presented 3 articles related to the dissertation. One of the articles was published in a peer-reviewed international journal with a high impact factor (4.9) for 2023, and it is in the Q1 quartile. The second article was published in a peer-reviewed national journal. The third article appeared in a national journal. In two of the publications, the PhD student is the first author, while in the third, she is the sole author, demonstrating her personal contribution to the development of the current work. The doctoral candidate has also presented 3 contributions at scientific forums: a poster presentation at an international conference in English, where the candidate is the second author, and two participations in national scientific forums, where they are the first and sole author.

7. Abstract of the PhD thesis

The abstract has been prepared in accordance with the requirements and fully aligns with the aims, objectives, and results presented in the dissertation work.

Conclusion

The dissertation titled "Study of the biological effects of the methanolic extract of *Micromeria frivaldszkyana* (*Degen*) Velen. (*Lamiaceae*)" is relevant and addresses current issues in the field of pharmacology. The presented work fully meets the requirements in the Law on the Development of the Academic Staff in the Republic of Bulgaria, the regulations for its implementation, and the regulations for the development of academic staff at the Medical University of Plovdiv, as well as the criteria for the award of the educational and scientific degree "Doctor."

Due to the reasons outlined above, I confidently give my positive assessment. I propose to the honourable members of the Scientific Jury to vote positively for the defence of the dissertation and award MPharm. Kristina Yulianova Stavrakeva the educational and scientific degree "Doctor" in the doctoral program "Pharmacology (including Pharmacokinetics and Chemotherapy)."

14.03. 2025

Reviewer: ...

Prof. Petko Marinov, MD, PhD