MEDICAL UNIVERSITY – SOFIA
FACULTY OF PHARMACY
DEPARTMENT OF PHARMACOLOGY, PHARMACOTHERAPY AND TOXICOLOGY

REVIEW

on a dissertation for obtaining the educational and scientific degree "Doctor" in a doctoral program in the field of higher education: 7. "Health and Sports", Professional field 7.3. "Pharmacy", Doctoral Program "Pharmacology (incl. Pharmacokinetics and chemotherapy)"

Author of the PhD Thesis: Kristina Yulianova Stavrakeva, PhD student at the Department of Pharmacology, Toxicology and Pharmacotherapy at the Faculty of Pharmacy, Medical University of Plovdiv, with supervisors Assoc. Prof. Elisaveta Apostolova, PhD and Prof. Dr. Anelia Bivolarska, PhD

PhD Thesis Title: "Study of biological effects of methanol extract of *Micromeria frivaldszkyana* (*Degen*) Velen. (*Lamiaceae*)"

Reviewer: Prof. Virginia Yordanova Tzankova, PhD, ERT, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy at MU-Sofia; appointed as a member of the scientific jury by Order R-946/25.02.2025 of the Rector of MU-Plovdiv.
The review has been prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation at Medical University, Plovdiv.

Short CV

Kristina Yulianova Stavrakeva conducts her studies in the Master's course in Pharmacy at the Faculty of Pharmacy of the Medical University of Plovdiv. In 2022 she was graduated as a Master of Pharmacy. From 2019 to 2024 she worked as an intern and later as a pharmacist in open-type pharmacy, which allowed her to gain a practical experience in the processes of dispensing of medicinal products (Rx and OTC products), medical devices, nutritional supplements and cosmetics.

Since December 2023, she became a full-time PhD student at the Department of Pharmacology, Toxicology and Pharmacotherapy at the Faculty of Pharmacy at the Medical University of Plovdiv, and since January 2025 she has been promoted as an assistant professor at the same department. During this period, assistant professor K. Stavrakeva actively participated in the teaching and research activities of the department, in which she conducted practical classes with students, conducting colloquia, participating in exam sessions, etc.

Mag. Pharm. Kristina Stavrakeva was granted with the right of defense her PhD thesis, based on the positive decision of the Departmental Council (Minutes No. 10/04.10.2024) and of Order No. R-899/31.10.2024 of the Vice-Rector for Research and Development of MU-Plovdiv.

Relevance of the scientific problem

The development of new herbal medicinal products and dietary supplements is in the focus of increased scientific and practical interest, especially in the recent years. The presented dissertation is aimed at studying the chemical composition of methanol extract from the aerial parts of *Micromeria frivaldszkyana (Lamiaceae)* and provides new data on the biological effects of the extract. *Micromeria frivaldszkyana* is of particular scientific interest due to the fact that it is endemic plant in Bulgaria. For this reason, information on its phytochemical composition and biological activity is limited. Scarce phytochemical data on the metabolites of the plant and on potential antioxidant and antimicrobial properties are available in the literature. This make the research topic a promising from scientific point of view.

The dissertation is written on 132 typewritten pages. It corresponds to the traditionally adopted structure in Bulgaria and contains the following sections: introduction, literature review, goals and objectives of the dissertation, results and discussion, experimental part, conclusions and contributions. The work is illustrated with 45 figures and diagrams and 12 tables. The list of cited literature includes 257 titles, most of which have been published in the recent years. The structure and layout of the dissertation corresponds to the procedural requirements, and the technical implementation is good.

Degree of knowledge of the state of the problem and interpretation of the literature review

The literature review is thematically divided into 7 sections: general characteristics and phytochemical composition of *Micromeria frivaldszkyana*, methods for assessing antioxidant activity, incl. antioxidant enzymes and biomarkers for oxidative stress, pain and methods for studying the analgesic effect, inflammation and experimental models of inflammation, the influence of oxidative stress and inflammation on cognitive functions, models for experimental

induction of hepatotoxicity and assessment of hepatoprotective properties. The main issues related to the purpose and objects of research are considered. Special attention is paid to experimental methods and models for pharmacological study of new biologically active substances.

The literature review summarizes the review and evaluation of a sufficient volume of literature sources, most of which are from the last few years. This supports the relevance of the scientific topic and shows a good knowledge of the specialized literature. The presentation and systematization of the information shows the ability of K. Stavrakova to use the literature sources.

Purpose, tasks, hypotheses and methods of research

The scientific hypothesis is formulated logically, based of the data in the literature overview. **The goal and tasks** are clearly formulated, presenting the stages of the study and indicating specific approaches and methods.

The materials and methods are presented in detail, citing the relevant literature sources. Their diversity shows a very good methodical preparation of the PhD student and is a guarantee for the successful completion of the tasks set in the dissertation. The wide range of experimental methods and models is impressive. Methods for extraction and identification of compounds, as well as for analysis of the data are used in a proper way. *In vivo* models of induced pathological conditions include the determination of analgesic activity, such as "hot plate" tests (thermal stimuli) and an "Analgesimeter" (mechanical stimuli). The anti-inflammatory effect of the substances has been studied in a model of inflammation of the hind leg of rodents, and a battery of tests, including reflex and active learning method, spatial working memory study, anxiety behavior study, recognition memory test, etc. The hepatoprotective action of the active substances was monitored *in vivo* in models of paracetamol-induced and t-BHP-induced hepatotoxicity, by determining markers for liver function, oxidative stress and antioxidant protection, and also the levels of pro-inflammatory cytokines in liver homogenate. The statistical analysis was carried out using statistical methods suitable for the experiments, which allows for an adequate interpretation of the results.

The experimental methods used are well selected, which allows scientific research to be carried out in accordance with the goals and objectives. The described experimental protocols show that the PhD student has used multi-stage methodological procedures, which is a good prerequisite for obtaining reliable scientific results.

Visualization and presentation of the results

The experimental data are presented clearly and concretely, which shows that the K. Stavrakeva has acquired skills to present and interpret the scientific results. The results are divided into two main sections – phytochemical characterization and study of the pharmacological and toxicological properties of biologically active substances.

By UPLC-MS-MS analysis of samples of methanol extract from *M. frivaldszkyana* , 192 compounds were detected, 123 of which were identified. Among the secondary metabolites with the highest concentrations, flavonoids, mainly flavonoid glycosides, were identified, and rosmarinic acid was among the most significant substances detected. Using GC-MS analysis, 83 compounds were classified as amino acids, organic acids, sugars and sugar alcohols. A total of 163 lipid compounds have been identified by the methods of lipidomimic examination of the nonpolar fraction, divided into 10 classes.

Pharmacological and toxicological testing is an integral element of the dissertation, and poses as the main scientific problem the study of the effects of methanol extract from *M. frivaldszkyana*. Based on the determined LD50 values, it was found that the extract has a good safety profile and does not cause toxic effects in rats at doses up to 5000 mg/kg body weight (p.o). The analysis of the results from the tests with mechanical and thermal pain stimulus showed that the plant extract did not exert significant analgesic effects. In contrast, in a model of acute exudative inflammation induced by carrageenin the methanol extract of *M. frivaldszkyana* showed a good anti-inflammatory effect. Based on the established phytochemical composition, an effect can be expected in the models of impaired memory.

It has been proven that methanol extract of *M. frivaldszkyana* affects oxidative stress, mainly by mechanisms of inhibition of free radical production, and not by increasing the activity of antioxidant enzymes. A good protective effect of *M. frivaldszkyana* extract can be expected in oxidative free radical damage to the liver.

The scientific results are presented systematically and in a logical order. They are comprehensively illustrated with 12 tables and 22 figures, which are sufficiently clear and informative. The original results are derived personaly from the experimental work of Kristina Stavrakeva. After each section, she summarizes and discusses the results, and correctly compares them with the data from scientific literature.

The conclusions of the dissertation are clearly reflected to the thesis goals and objectives.

Contributions of the dissertation

Recently, there has been an upward trend towards the development and use of herbal medicinal products and food supplements, but according to the literature data, only a small part of the higher plants are well studied and used as a source of biologically active substances. The doctoral thesis of Kristina Stavrakeva deals with an interesting scientific field, such as a study of the therapeutic potential of new plant species. In order to solve the main scientific issues, a comprehensive analysis of methanol extract from *M. frivaldszkyana* was carried out and the main classes of biologically active substances were identified. Among them, sucrose, glucose, mannose, fructose, polyphenols and sugar alcohols, as well as triacylglycerols from the lipid group were found to be primary metabolites in the largest quantity. The extract is rich in linarin, chlorogenic and rosmarinic acid, rutin, eupatorin, kaempferol-3-O-rutinoside and apigenin. The pharmacotoxicological analysis performed *in vivo* shows a good safety profile and no toxic effects. The high content of phenolic compounds (chlorogenic and rosmarinic acid, rutin, eupatorin, kaempferol-3-O-rutinoside and apigenin) is probably the basis for the observed anti-inflammatory activity of the extract, and the antioxidant activity of the flavonoids in the extract determines the good hepatoprotective properties proven in the models of oxidative liver damage.

The scientific contributions of the dissertation are presented as a theoretical and applied contributions. I accept the main contributions of the dissertation. They present a new knowledge in the field of plant phytochemistry and pharmacology.

Critical remarks and questions

During the reading, I posed some questions about the methodology of the research. All questions were answered, and the notes are correctly reflected in the dissertation.

Publications related to the dissertation

The assistant professor K. Stavrakeva presented 3 publications related to the dissertation: one was published in an journal with impact factor (International Journal of Molecular Sciences, IF 2022 - 5.6, Q1).

In three of the publications, K. Stavrakeva is the lead author. The publications are sufficient in volume and fully reflect to the content of the dissertation. An information about participation in 1 international and 2 national conferences and congresses is presented.

The presented data fully meet the criteria for acquiring the educational and scientific degree "Doctor".

The short version (summary) of the thesis is prepared in accordance with the regulatory requirements. It is sufficiently comprehensive and presents in the main results, conclusions and contributions obtained in the course of the scientific development. On this basis, I believe that the short version of the thesis reflects well the structure of the dissertation and corresponds to its content.

CONCLUSION

The dissertation on the topic "Study of biological effects of methanol extract from *Micromeria frivaldszkyana* (*Degen*) Velen. (*Lamiaceae*)" by assistant professor Kristina Yulianova Stavrakeva is a scientific study on the analysis of the phytochemical composition of methanol extract from the aerial parts of *Micromeria frivaldszkyana (Lamiaceae)* and provides new data on the pharmacotoxicological properties of the extract in different pathological models. The study was conducted at a good experimental level, the results of which were critically and thoroughly discussed. I accept the scientific contributions and consider that they correspond to the professional field of the topic being developed.

Based on the scientific analysis of the dissertation, I believe that it meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation at Medical University, Plovdiv, which permits my POSITIVE assessment.

I strongly recommend to the scientific jury to vote positively and to award Mag. Pharm. Kristina Yulianova Stavrakeva the educational and scientific degree "DOCTOR" in the doctoral program in the field of higher education 7. "Health and Sports"; Professional field 7.3. "Pharmacy"; Doctoral program "Pharmacology (incl. Pharmacokinetics and chemotherapy)".

17. 03. 2025

G. Sofia

VIRDJINIA IORDANOVA TZANKOVA

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REVIEWER:

/Prof. Virginia Yordanova Tsankova, PhD, ERT/