МЕДИЦИНСКИ УНИВЕРСИТЕТ
ПЛОВДИВ
Вх. № Р-6375 / 14. 07. 2021 г.

OPINION

by Assoc. Prof. Dr. Eng. NADEZHDA TRAYCHEVA PETKOVA
Department of "Organic Chemistry and Inorganic Chemistry", Faculty of Technology, University of Food Technology,
member of the Scientific Jury according to Order № P-786 / 25.05.2021

in a competition for dissertation for the educational and scientific degree **"PhD"**
in **Field of higher education: 4. "Natural sciences, mathematics and informatics, "Professional field: 4.3. "Biological Sciences"**
PhD program: "Bioorganic chemistry, chemistry of natural and physiologically active substances"

Author: Yoana Pencheva Georgieva
Form of PhD studies: independent preparation
Department: "Bioorganic Chemistry" at the Faculty of Pharmacy, MU-Plovdiv
Title: "*Scutellaria altissima* L. (Lamiaceae) - a source of biologically important flavonoids and diterpenes"
Scientific advisers: Assoc. Prof. Stella Dimitrova, Ph.D., MU-Plovdiv,
Assoc. Prof. Petko Bozov, PhD,
Paisii Hilandarski University of Plovdiv

1. General presentation of the procedure and the PhD student

The dissertation contains clearly separated sections, presented on 127 standard pages, structured as follows: Content - 3 pages, List of abbreviations - 1 page, Introduction - 1 page, Introduction - 28 pages, Aims and tasks of the dissertation - 1 page, Materials and methods - 11 pages, Results and discussion - 41 pages, Conclusions - 2 pages, Contributions - 1 page, List of publications in connection with the dissertation and noted citations, as well as participations in scientific forums - 3 pages; References - 15 pages and 2 Appendices - 19 pages. The used references contains 196 sources, 7 of them are in Cyrillic, and the remaining 189 are written in Latin, 58 of them are contemporary sources from the last 10 years. The dissertation includes 22 figures, 3 infrared spectra (FTIR), 5 NMR spectra (^{1}H and ^{13}C), 3 HPLC chromatograms and 11 tables. The dissertation is well structured and contains all the sections inherent in the dissertation.

The PhD dissertation was approved and directed for public defense by an extended department council at the Department of Bioorganic Chemistry at the Faculty of Medicine of the Medical University of Plovdiv, held on 29th March, 2021. The PhD student was elected with the right to defense by order № P-556/20.04.2021 of the Rector of MU-Plovdiv Prof. Dr. Mariana Murdzheva, MD, MSc. The presented set of materials on paper is in accordance with Article 115 (1) of the Procedure for Acquisition of educational and scientific degree "PhD" in MU - Plovdiv; Regulations of MU-Plovdiv from 06.11.2014 and includes all necessary documents. The doctoral student has submitted 5 scientific publications.

2. Actuality of the topic.

The presented PhD thesis by Yoana Georgieva considers an extremely modern topic related to the phytopharmacy and pharmacological action of plants of the genus *Scutellaria* L. and the biologically active compounds (flavonoids and diterpenes) that they contain. Until now in Bulgaria, the interest in this genus is mainly focused on the study of the composition of deterpents and the results of their effects, however, there is no data about their content flavonoids, which are important for the determination antioxidant and antimicrobial activity. The topic is relevant, enriching the knowledge about the phytochemical composition and biological activity in Bulgarian species of the genus *Scutellaria* L. and especially for the Bulgarian species *Scutellaria altissima* appears from three floristic regions in Bulgaria. Of great interest is a representative of flavonoids in this species, determining the broad spectrum of biological activity.

The introduction part is presented in-depth, it is comprehensive, accessible and the scientific information is interpreted with understanding. Based on this literature review, conclusions have been drawn and the lack of scientific information on some of the members of the genus *Scutellaria* L. is indicated. This inevitably leads to a clear formulation of the aim of the dissertation, namely - to study the content of flavonoids and diterpenes in extracts of *Scutellaria altissima*, as well as to study their biological activity. In order to achieve the formulated aim, 7 main tasks are clearly defined. The research conducted in this scientific work reveals a number of biologically active substances with different chemical structure (mostly flavonoids, carbohydrates, organic acids) of the species *Scutellaria altissima*. The main contribution of the PhD thesis is the evaluated antimicrobial effect of *Scutellaria altissima* extracts against the growth of *Streptococcus mitis*.

3. Degree of knowledge of the problem.

A detailed botanical and phytochemical characteristic of the representatives of the genus *Scutellaria* L. is performed in the introduction part. The phenolic compounds and terpenes are described and classified in detail, the biological activity of some of these compounds and their pharmacological effect are considered. An up-to-date bibliography was used, as it consists of 196 literature sources. It is noteworthy that the literature used is in Latin - 189 sources and only 7 in Cyrillic. The PhD student has used contemporary literature on the research topic, as the main part of the sources (over 77%) is after 2000. Based on the literature reference, the aim and tasks of the PhD thesis are accurately formulated.

4. Research methodology.

The materials and methods used in the PhD thesis are appropriately selected and correspond to the implementation of the set aims and tasks. The methods for botanical characterization of the collected plant material of the species *Scutellaria altissima* are described in detail and well illustrated. Modern methods have been used, both for the analysis of the phytocomponents and for their activities, as well. Spectral methods for the structural characterization of the isolated compounds are applied.

5. Characteristics and evaluation of the PhD thesis and contributions.

The data obtained in the PhD thesis are neatly formed in tables and figures, thoroughly discussed, which shows that the PhD student has acquired skills to handle a huge set of data and skillfully summarize the results. As a result of this study, 8 conclusions were summarized and formed. I accept the conclusions and contributions formulated in the author's abstract and the dissertation as reliable and personal work of the doctoral student. Five original scientific contributions were made, mainly of scientific and applied nature, as follows:

Scientific contributions:

1. From the areal parts of *Scutellaria altissima* are isolated and spectroscopically characterized four neo-clerodans diterpenes identified as: skutekiprin, skupolin H, clerodin and skutekiprol A, one sterol identified as β-sitosterol and one glycosidically bounded iridoid identified as globularin. All substances have been proven and isolated for the first time from this plant species.

Scientific and applied contributions:

2. The developed and validated HPLC method is possibly applicable for quality control of plant substances, extracts and phytopreparations obtained from species of the genus *Scutellaria* L.

3. For the first time the flavonoid composition of species of the genus *Scutellaria* L., distributed in Bulgaria, was studied. The presence of biologically active flavonoids characteristic of the plant genus has been proven. The highest content of them is found in the species *Scutellaria altissima*, which makes it a promising species for future research.

4. For the first time the content of carbohydrates and organic acids in dry plant material of species of the genus *Scutellaria* L. growing in Bulgaria was studied.

5. The antimicrobial effect of *Scutellaria altissima* extracts against *Streptococcus mitis* has been demonstrated for the first time.

6. Assessment of the PhD student's publications and personal contribution

The PhD student has presented ***5 scientific publications*** on the PhD in 4, of which she is the first author. Particularly impressive is the fact that 4 of the publications have been published in referenced and indexed publications in the global databases ***Web of Science*** **and** ***Scopus***. It is worth emphasizing that the PhD student has one publication in *Plants*, **MDPI** publishing house with **IF 2.762** and ***quartile - Q1*** according to ***SCImago Journal & Country Rank, one in Q2 journal, one in Q3 journal and one with Q4, respectively***. So far, a total of seven citations from three of the presented publications have been noticed, which shows the actuality of the considered issues and the international interest that the results of the dissertation have been received. The results of the PhD thesis are presented at four scientific conferences, three took place in Bulgaria and one internationally organized in Italy. In three of the presented participations she is in first place and is leading auhtor.

The PhD candidate also participated in ***a project*** of MU-Plovdiv, Project № NO - 01/2018 on the topic: "Study on chemical composition, antioxidant and antimicrobial activity of extracts of *Scutellaria altissima*", which is directly related to the dissertation.

The active publishing activity of the PhD student fully meets and even exceeds the requirements for acquiring educational and scientific degree "PhD".

7. Recommendations and notes.

The PhD thesis is very well structured and written according to the national criteria for this type of work. The results are illustrated in tables and figures. The main conclusions are clearly and precisely formulated. I have no critical remarks on the work. But I have a few recommendations related to some technical and microbiological inaccuracies. Antimicrobial activity was tested against Gram-positive bacteria, Gram-negative bacteria and *Candida albicans*, as the last mentioned are yeasts. Therefore, the terms bacterial growth and determination of the minimum bacteriostatic concentration (MBSC) and the minimum bactericidal concentration (MBCC) are correct only to the studied Gram-positive bacteria and Gram-negative bacteria, but not for yeasts. I recommend replacing these names with the themes of minimal inhibitory activity concentration. The statement "The other strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans* tested did not show a bactericidal or bacteriostatic effect" is terminologically incorrect, as *Candida albicans* is a yeast and not a bacterium. I recommend corrections, keeping the same meaning: "The other strains of *Staphylococcus aureus, Escherichia coli and Candida albicans* tested did not show an antimicrobial effect."

The term carbohydrates could be replaced by sugars or sugar composition. The reason for this is the fact that only monosaccharides with five and six carbon atoms and disaccharides without oligosaccharides and polysaccharides were studied.

8. Questions.

I have the following questions for the doctoral student:

1. The difference in the melting points of scotequiprine isolated by you and that reported by Bruno (Bruno et al., 1992) differ dramatically. How do you explain this difference? Is this related to the purity of the compound or is it due to something else?

2. How did you determine the sensitivity and precision of the HPLC method you developed?

Despite of these recommendations, this PhD theis is a serious scientific and complete study. I recommend to the PhD student Yoana Georgieva to continue working in the field of phytochemistry, upgrading her research with other plant species in her future scientific career.

9. Abstract work

The abstract work is made according to the requirements and reflects the main results achieved in the dissertation.

CONCLUSION

The presented PhD thesis is an original and completed study. The PhD student Yoana Georgieva possesses in-depth theoretical knowledge and acheives professional skills in the

PhD program "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances", demonstrating qualities and skills for independent research.

The PhD thesis contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet all the requirements of the Law for development of the academic staff in the Republic of Bulgaria (LDASRB), the Rules for application of LDASRB and the Rules of MU – Plovdiv. The presented materials and PhD thesis results completely to the specific requirements, adopted in connection with the Regulations of MU - Plovdiv for application of LDASRB. Having in mind the relevance and importance of the PhD thesis, the implementation of the aim, the used modern chromatographic and spectral methods of analysis, the contained scientific and scientific-applied results, which represent an original contribution to science and the significant number of published scientific publications, I give a **positive** assessment and recommend to the esteemed Scientific Jury to award the educational and scientific degree ***"PhD"*** to **Yoana Pencheva Georgieva** in **4. "Natural Sciences, Mathematics and Informatics" Professional field: 4.3. "Biological Sciences", Doctoral Program: "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances".**

28.06.2021

Jury Member: .....

(Assoc. Prof. Dr. Eng. Nadezhda Petkova)