

REVIEW

By Prof. Mariana Dimitrova Argirova, DSc

Member of the academic jury set to render a decision on a procedure for the acquisition of academic degree “Doctor of Philosophy” (PhD) according to the Classifier of the areas of higher education 4. “Natural sciences, mathematics and informatics”; Professional field 4.3. “Biological sciences”; Doctoral program “Bioorganic chemistry, chemistry of natural products and physiologically active substances”

Author: Yoana Pencheva Georgieva

Form of doctoral studies: independent study form

Department: Bioorganic chemistry

Topic: “*Scutellaria altissima* L. (Lamiaceae) – a source of biologically important flavonoids and diterpenes”

Supervisors: Assoc. Prof. Stella Dimitrova, Ph.D. and Assoc. Prof. Petko Bozov, DSc.

1. General presentation of the procedure

This review is prepared in response to order № P-786/25.05.2021 issued by the Rector of the Medical University (MU) of Plovdiv. The set of materials presented to me in electronic format complies with the Development of Academic Staff in the Republic of Bulgaria Act (DASRB), the Rules for the application of the DASRB Act, as well as the Regulations for academic development in MU – Plovdiv (2021) and includes the following documents:

- Doctoral student’s CV.
- Copy of the diploma for completed master's degree.
- Orders for enrollment in doctoral study and for accomplishment of the doctoral study.
- Order for conducting an examination of the individual plan and the respective protocol for completed doctoral minimum in the specialty.
- Certificate for completed training in the Doctoral School of MU - Plovdiv.
- Records of the departmental council for preliminary discussion of the dissertation and the decisions taken for opening the procedure for public defense of doctoral thesis.
- Doctoral thesis.
- Extended abstract of the doctoral thesis.
- List of scientific works.
- Copies of five scientific publications.
- List of participations in scientific forums.
- List of citations.
- Declaration of originality and authenticity of the attached documents.

2. Biographical information about the candidate

The author of dissertation submitted for review – Yoana Pencheva Georgieva, is a graduate of Paisii Hilendarski University of Plovdiv, where in July 2008 she received a bachelor's degree in Biology and Chemistry, and in May 2013 she graduated with a master's degree in Medical Biology. Since November 2011, she has worked as a biologist at the Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy of MU – Plovdiv and is involved in preparing practical classes with students in Pharmaceutical Botany, Pharmacognosy and Phytochemistry, as well as in research activities of the Department. She was enrolled as a PhD student at the Department of Bioorganic Chemistry on July 30, 2019, and a procedure for public defense of the doctoral thesis was opened on April 24, 2021. The presented dissertation is a logical extension of her thesis “Phytochemical study of *Scutellaria altissima* L for the presence of diterpenoids” for obtaining a master's degree.

3. Assessment of topicality of the dissertation and relevance of the aims and objectives to the field of research

The subject of research in the dissertation of Yoana Georgieva is the plant *Scutellaria altissima* L. (Lamiaceae) as a possible source of biologically important flavonoids and diterpenes. After the end of the Golden age (1930-1960) of synthetic organic chemistry, which led to the production of completely new classes of medications, nowadays we see a real renaissance of the research on natural sources for both, isolation and identification of new bioactive compounds, and templates for design of new drugs. Phytotherapy is traditional for Bulgarian folk medicine, but most often, the claims for a certain healing effect are poorly supported by scientific evidence. That is why phytochemistry has enjoyed a well-deserved research interest in the last two decades and logically the doctoral student is interested in the scantily studied species of Bulgarian origin *Scutellaria altissima*, as well as the possibilities for practical application of some of its secondary metabolites, mainly *neo*-clerodane diterpenes.

4. Characteristics and evaluation of the dissertation

The dissertation is 186 pages long and is classically structured in the following main chapters: introduction, literature review, aims and objectives, material and methods, results and discussion, conclusions, contributions, and references. The dissertation is supplemented by 18 pages of appendices containing spectral data of compounds isolated from *Scutellaria altissima*.

The chapter Introduction and the well-structured Literature Review, covering 196 sources, seven of which in Bulgarian language, demonstrate a good acquaintance of the known data on the type and structure of the flavonoids and *neo*-diterpenes constituents of the plant, as well as their known pharmacological effects. The aim is clearly defined, and the objectives for its achievement are specifically set. Chapter Material and Methods describes in detail: the studied plant material, methods for isolation and identification of the compounds of interest, methodology of antioxidant, antimicrobial and antifeedant assays. The Results and Discussion chapter summarizes the PhD student's own research with an emphasis on the isolation and identification of secondary metabolites. Six known clerodane diterpenoids, which are first demonstrated in this species of Bulgarian origin, are isolated from *Scutellaria altissima* by preparative chromatography and identified by comparing infrared and ¹H-NMR and ¹³C-NMR spectra with available data. An accurate, precise, and sensitive chromatographic method is

developed for the simultaneous determination of eight flavonoids (glycosylated or as aglycones) in plant extracts; a comparison is made with the flavonoid composition of other members of the genus *Scutellaria*.

Considerable attention is paid to the potential biological properties of the extracts obtained and some individual compounds. Since very often these properties of secondary metabolites are associated with their antioxidant activity, it is measured using three different methods complementary to each other. Studies on biological effects are targeted and guided by previously known bioactivities of the genus – antimicrobial and antifeedant effects. The antimicrobial properties of aqueous and 70% aqueous-ethanol extracts from roots and aerial parts of *Scutellaria altissima* from two populations are tested against some common clinical isolates of Gram-positive (*Staphylococcus aureus*, *Streptococcus mitis*), Gram-negative bacteria (*Escherichia coli*) and the fungus *Candida albicans*. The evaluation of the antimicrobial activity is carried out by determining the minimum bacteriostatic concentration and the minimum bactericidal concentration. The author discusses the shown moderate antimicrobial effect of the extracts to *Streptococcus mitis* and especially 70% ethanol extract from the aerial part, in the light of the flavonoids content.

Antifeedant activity against larvae of *Leptinotarsa decemlineata* Say (Colorado potato beetle) of 14 *neo*-clerodane diterpenoids isolated from genus *Scutellaria* is also studied. The tested natural diterpenoids have shown very good inhibition of the *Leptinotarsa decemlineata* Say larvae feeding at a dose of 1000 ppm. The relationship between the structural characteristics of the compounds and their antifeedant activity, which corresponds to that proposed by other authors, has been confirmed.

5. Assessment of the scientific and research accomplishments of the candidate

The dissertation presented for review contains several novelties, reported for the first time in the scientific literature:

- The composition and amount of secondary plant metabolites is highly dependent on soil, climatic, environmental, and other factors. That is why the first data on the phytochemical composition of the Bulgarian population of *Scutellaria altissima*, is of significant scientific merit. The illustrated morphological and microscopic analysis of the plant would serve for the more accurate identification and future standardization of the plant material as a phytoproduct.
- The developed chromatographic method for simultaneous determination of eight typical flavonoids in extracts of *Scutellaria altissima*, in addition to scientific value, is also of practical importance, as it can be used in future standardization and quality control of the extract.
- The flavonoid constituents of several species of the genus *Scutellaria* L., widespread in Bulgaria, is studied for the first time, which opens new perspectives for biomedical applications of their aqueous or organic extracts.
- The comparative studies made with several members of the genus *Scutellaria* allow making a chemotaxonomic link between the individual species.
- The antimicrobial activity of aqueous and 70% ethanol extracts of *Scutellaria altissima* against *Streptococcus mitis* is a good ground for their further analysis and purification from

inactive compounds (e.g., carbohydrates and organic acids) in a perspective to use the extracts in oral care products.

- Finally, the very good antifeedant effect of 14 *neo*-clerodane diterpenoids isolated from *Scutellaria altissima* has been confirmed.

6. Assessment of meeting the minimal criteria in accordance with DASRB, the Rules for the application of the DASRB Act, as well as the Regulations for academic development in MU – Plovdiv (2021)

The results obtained by the doctoral student have been published in four international journals and one full-text report from a scientific forum. The PhD student fully meets the criteria of DASRB Act and the Regulations for obtaining PhD degree in MU – Plovdiv. Based on the impact factor and quartile of the journals in which the results of the dissertation are disseminated, she collects a total of 70 points for the required 30. The Regulations for academic development at MU – Plovdiv (2021) requires published 3 articles on the content of the dissertation, two of which in journals referenced in international databases of scientific literature (WoS and/or Scopus); criteria that the doctoral student also meets. In four of the publications, the doctoral student is the first author. However, I would recommend, she to take on the responsibility of a corresponding author in future publications. Totally seven foreign researchers' quotations of three papers confirm the scientific value of the results obtained. Yoana Georgieva has presented these results at four scientific forums, one of which in Italy.

7. Assessment of the extended abstract of the doctoral thesis

The abstract is written according to the requirements and accurately reflects the main scientific findings and contributions of the dissertation.

8. Opinions, notes and recommendations

The thesis is written concisely, logically and its graphic design is impeccable. I have some minor editorial remarks - for example, the use of both, decimal point and decimal comma as a decimal separator; a discrepancy between the spectrum shown in Figure 22 and its description in the text; some inaccurate expressions (e.g., on page 52 of the dissertation and page 18 of the extended abstract – an isolated and well-characterized individual compound cannot be called "a mixture"); some unnecessary duplications in the text and appendices, etc. There are no data in the thesis about tests on antifeedant effect of total extract of *Scutellaria altissima*, but I would recommend carrying out such experiments and comparing the results with those obtained for the individual *neo*-clerodane diterpenoids.

CONCLUSION

According to DASRB, PhD is an educational and scientific degree. The content of the reviewed thesis convincingly shows that the doctoral student Yoana Georgieva has not only upgraded the knowledge obtained in the master's degree and has covered the educational component of the degree "doctor", but also has in-depth theoretical knowledge and professional skills in the field of bioorganic chemistry, demonstrating qualities and skills for independent scientific work.

Based on the analysis of the dissertation and the published articles, I give my positive assessment of the developed thesis and consider reasonable to propose to the honored scientific jury to award the educational and scientific degree "Doctor" to Yoana Pencheva Georgieva in Area of higher education 4. "Natural sciences, mathematics and informatics", Professional field 4.3. "Biological sciences", Doctoral Program "Bioorganic chemistry, chemistry of natural products and physiologically active substances".

July 1, 2021,

Reviewer: