

STATEMENT

by prof. Ginka Atanasova Antova, PhD,

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On dissertation for awarding the educational and scientific degree “Doctor of Philosophy”
(PhD)

Professional field: 4.3. „Biological sciences”

Doctoral program: „Bioorganic chemistry, chemistry of natural and physiologically active substances”

Author: Yoana Pencheva Georgieva

Form the PhD: independent preparation

Department: Bioorganic chemistry

Thesis: *Scutellaria Altissima* L. (Lamiaceae) - source of biologically significant flavonoids and diterpens

Supervisors:

Assoc. Prof. Stela Zdravkova Dimitrova, PhD, Medical University – Plovdiv

Assoc. Prof. Petko Ivanov Bozov, DSc, The “Paisii Hilendarski”, Plovdiv University

1. General presentation of the dissertation

This opinion is prepared on the basis of Order (P-786/25.05.2021) of the Rector of MU – Plovdiv and is in accordance with the requirements of the Law for development of the academic staff in the Republic of Bulgaria, the Requirements for its implementation and the Regulation for development of the academic staff of the MU – Plovdiv. The presented set of materials on electronic media is in accordance with Article 115 (1) of the Regulation for acquisition of Educational and Scientific Degree "Doctor" at MU Plovdiv, in the Regulations of MU Plovdiv of 06.11.2014, and includes all the necessary documents and materials.

The doctoral student has submitted 5 publications.

Yoana Georgieva graduated with a Bachelor's degree in Biology and Chemistry and a Master's degree in Medical Biology. In 2019 she was enrolled as a doctoral student in independent preparation, and on 14th April 2021 completed her studies with the right to defend the dissertation. She currently works as a biologist in the Department of Pharmacognosy and Pharmaceutical Chemistry at the Medical University Plovdiv.

The dissertation consists of nine chapters, presented on 127 standard pages, the structure is as follows: content - 3 pages, list of used notations and abbreviations - 1 page, introduction - 1 page, review and conclusion - 28 pages (~ 22%), aim and tasks of the dissertation - 1 page, materials and methods used in the experimental research - 11 pages (~ 9%), results and discussion - 41 pages (~ 32%), conclusions - 2 pages, contribution - 1 page, scientific publications, citations and participation in scientific forums and projects - 3 pages, reference

list - 15 pages, 2 appendices (Appendix 1 - Isolated *neo*-clerodane diterpenes of the genus *Scutellaria L.* with established biological activity, Isolated *neo*-clerodane diterpenes of species of the genus *Scutellaria L.* in Bulgaria and Annex 2 - Spectral data of the isolated substances) - 19 pages. The reference list includes 195 literature sources. The dissertation includes 33 figures and 11 tables.

2. Relevance of the topic

The dissertation presented by Yoana Georgieva examines an extremely important and relevant in recent years for pharmacy problem regarding research on the composition and pharmacological potential of a number of medicinal plants in our country in order to outline possible pharmacological applications of various biologically active substances isolated from them. The studies in this work lead to the discovery of biologically significant substances of the Bulgarian species *Scutellaria altissima* from the family Lamiaceae. This paper provides for the first time scientific data on the flavonoid composition and the content of carbohydrates and organic acids in the studied species of the genus *Scutellaria L.*, found in Bulgaria. The antimicrobial effect of *Scutellaria altissima* extracts against *Streptococcus mitis* has also been proven.

3. Knowledge of the problem

In the literature review a detailed description of the botanical characteristics of the genus *Scutellaria L.* and in particular of *Scutellaria altissima* is made, the phytochemical composition of the representatives of this species, and in particular the content of phenolic and terpene compounds, as well as the biological activity and pharmacological potential of genus *Scutellaria L.* are discussed. The literature used contains 195 sources, of which 7 are in Cyrillic, the remaining 188 - in Latin. The bibliographic reference shows that a large part (77%) of the literature are contemporary sources (after 2000) and about 37% are after 2010. Based on the modern and orderly literature review and the conclusions made by it, the purpose and objectives of current research are properly formulated.

4. Research methodology

The section Materials and methods describes the plant material used, the methods for the botanical characterization of the collected plant material from *Scutellaria altissima*, as well as for the isolation of biologically active substances from the studied representatives are given in great detail. The spectral methods for studying the structure of the obtained compounds, the analytical methods for determining flavonoids, polyphenols, carbohydrates and organic acids, as well as the methods for determining the antioxidant, antimicrobial and antifeedant activity are also described. The selected research methods allow achieving the goal and fulfilling the set tasks.

5. Characteristics and evaluation of the dissertation and contributions

The main contributions of the presented dissertation are the following:

- for the first time, four *neo*-clerodane diterpenes (scutecyprine, scupoline H, clerodin and scutecyprol A), β -sitosterol and globularin were isolated and proven from the aboveground parts of *Scutellaria altissima*;

- for the first time the flavonoid composition of species of the genus *Scutellaria* L., found 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.
- an HPLC method for quality control of plant substances, extracts and phytoproducts obtained from species of the genus *Scutellaria* L. has been developed and validated.
- 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.
- the antimicrobial effect of *Scutellaria altissima* extracts against *Streptococcus mitis* has been demonstrated for the first time.

The research was conducted with modern scientific equipment, which guarantees the receipt of objective results, but also the data obtained was discussed correctly, which shows that the doctoral student has acquired skills for presenting results.

Eight conclusions were drawn from the study and 5 original contributions are listed. The conclusions and contributions fully correspond to the obtained results.

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

The doctoral student has presented 5 publications, 4 of them are in peer-reviewed journals, as 3 are in journals with impact factor, and 1 is in a journal with SJR factor. Yoana Georgieva's personal participation in these works is illustrated by the fact that in 4 of the publications the doctoral student is the first author, and in the 5th she is the second author. She has participated in 4 scientific forums, 1 of which has a poster at a conference abroad. To assess the quality of the research, 7 citations from three of the articles were noticed, and it should be noted that all of them are by foreign authors. The publishing activity meets the requirements for acquiring the Educational and Scientific Degree "Doctor".

The dissertation is written according to the requirements for this type of work, including all necessary sections. The dissertation is very well formed and structured, the obtained experimental results are illustrated in well-formed figures and presented in tables. The main conclusions from the dissertation work are clearly and precisely presented, as well as the scientific contributions are clearly and precisely formulated.

I have no remarks and recommendations to the conducted research and the presented materials. I strongly recommend Yoana Georgieva to work hard and deeply in the field of phytochemistry.

7. Abstract on dissertation

The abstract, which is 58 pages, corresponds to the content of the dissertation and is an abbreviated version of its essence, fully and accurately reflects the results achieved in the dissertation, and their analysis.

CONCLUSION

The dissertation, in respect of its relevance, use of modern research methods, scientific and scientific-applied results and their interpretation, volume of work and the number of

publications presented, *fully meets* the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDARB), the Regulations for application of ASDARB, and the Regulations of Medical University - Plovdiv. The presented materials and dissertation results fully comply with the specific requirements adopted in connection with the Regulations of MU Plovdiv for application of ASDARB.

The dissertation shows that the doctoral student Yoana Georgieva *has* in-depth theoretical knowledge and professional skills in the doctoral program "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances", *demonstrating* qualities and skills for independent research.

Due to the above, I confidently give my *positive assessment* of the research presented in the dissertation and abstract, as well as the results achieved and contributions, and *I propose to the esteemed scientific jury to award the educational and scientific degree 'Doctor' to Yoana Pencheva Georgieva* in professional field 4.3. "Biological Sciences", in the Doctoral programme "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances".

21.06.2021 г.

Opinion prepared by:
Prof. Ginka Antova, PhD