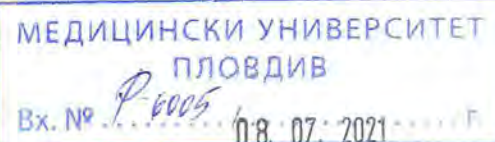


REVIEW



from Prof. Panteley Petrov D.Sc
dep. "Organic Chemistry and Inorganic Chemistry" - UFT Plovdiv

of a dissertation for the award of **PhD**

doctoral program: **Bioorganic chemistry, chemistry of natural and physiologically active substances**

Author: *Joanna Pencheva Georgieva*

Department: Bioorganic Chemistry

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

Supervisors: *Assoc. Prof. Stella Dimitrova, Ph.D., MU-Plovdiv,*
Assoc. Prof. Petko Bozov, D.Sc, P. Hilendarski University of Plovdiv"

Relevance of the topic and expediency of the set goals and objectives

The dissertation presented by Joana Georgieva examines the phytochemical and pharmacological studies of the genus *Scutellaria* L. and the contained biologically active compounds - mainly diterpenes and polyphenols, which are the main and effective chemical compounds contributing to the pharmacological efficacy of medicinal plants of this genus. The topicality of the topic can be emphasized by the fact that in our country the interest in this genus has been focused mainly on studying the composition of diterpenes and their effects, but there is no data on the flavonoids contained in them, which have radical scavenging abilities and are an important factor. determination of antioxidant and antimicrobial activity.

The doctoral student summarizes that the establishment of the qualitative and quantitative composition of extracts of the Bulgarian *Scutellaria altissima*, as well as the establishment of the biological activity, is an initial step in the study of its potential as a source of biologically significant metabolites.

Knowledge of the problem

PhD student Joana Georgieva has given a critical review of 196 research articles and communications, most of which have been published in recent years. The

dissertation review presents briefly and clearly the current state of the scientific literature on the biological activity and pharmacological potential of the genus *Scutellaria* L., as well as the associated anti-oxidant, antitumor, antimicrobial, antifidant and antiviral activity, and the anxiolytic effect.

A significant contribution to the educational part of the doctoral program is the correct assessment, discussion and summarization of the correctly cited scientific facts and the identification of problems that justify the clearly formulated main goal. The literature review enables the doctoral student to orientate correctly in the subject and to highlight the significant problems, both in scientific and in scientific-applied aspect. The set research tasks are specific, feasible and correctly arranged and their solution builds the experimental part of the dissertation.

Research methodology

After the analysis of the literature data and the generalized conclusions, the main goal of the dissertation is formulated: to study the content of flavonoids and diterpenes in extracts of *Scutellaria altissima*, as well as to study their biological activity.

To achieve this goal, specific tasks are set: collection and determination of plant material from *Scutellaria altissima* from different floristic regions of Bulgaria; isolation and identification of diterpenes from aboveground parts of *Scutellaria altissima*; development and validation of HPLC method for quantitative determination of biologically active flavonoids; application of the developed method for establishing and comparing the flavonoid constituent extracts of species of the genus *Scutellaria* L. distributed in Bulgaria; study of anti-oxidant activity of extracts of species of the genus *Scutellaria* L.; study of antimicrobial activity extracts of *Scutellaria altissima* against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mitis* and *Candida albicans*; study of antifidant activity of isolated neo-clerodan diterpenes from *Scutellaria altissima* against *Leptinotarsa decemlineata* Say.

To perform the set tasks, a large set of modern physicochemical, spectrophotometric and chromatographic methods was used to determine the specific indicators.

The experimental studies were conducted in the Departments of Bioorganic Chemistry, Pharmacognosy and Pharmaceutical Chemistry, Microbiology and Immunology of the Ministry of Finance at the Medical University of Plovdiv and LBAV at the Plovdiv Academy of Sciences. The presented results and the scientific analysis of these results are original, performed with the necessary repeatability and are at a good scientific level.

Characteristics and evaluation of the dissertation

The dissertation is presented on 127 standard typewritten pages and contains 33 figures, 11 tables. 196 sources are cited. The construction of the dissertation meets the requirements, containing the necessary main parts: title page, content, introduction, literature review (25 pages), purpose and tasks, materials and methods (12 pages), results and discussion (36 pages), conclusions, contributions and literature, which follow a logical sequence, allowing the doctoral student to study and analyze the problems chosen with the chosen scientific methods.

The dissertation is written in very good professional-scientific language, with good style, conciseness and clarity. The material is easy to read and understand. The individual parts are well balanced in volume.

The section "Materials and methods" describes precisely the main methods used in performing the tasks in the dissertation: extraction in laboratory conditions and preparation of samples for HPLC analysis of organic acids, carbohydrates and polyphenols, infrared spectroscopy (IR), magnetic resonance imaging (¹H NMR and ¹³C-NMR) of terpenes, ORAC and HORAC methods for determining antioxidant activity; experiments to study acute toxicity and anxiolytic action of model extracts. The methods, parameters, columns with which the analyzes are performed are indicated. The doctoral student has skillfully selected and mastered a large number of classical and modern physico-chemical and technological methods, applicable and in full accordance with the goal and objectives of the dissertation. Joana Georgieva has gained the necessary methodological experience in conducting the scientific experiment, summarizing the reliability of the results and deriving the patterns.

The section "Results and discussion" clearly and accurately describes the experiments conducted to analyze the taxonomic traits, based on which the botanical identity of the plant species *Scutellaria altissima* from different floristic regions of Bulgaria was confirmed.

Methodologically correct and correctly developed methods have been applied to characterize the isolated diterpenes from aboveground parts of *Scutellaria altissima*, which have a neo-clerodan structure, containing tetra- or hexa-hydrofurofuran, HPLC method for the determination of biologically active flavonoids, characteristic of the genus *Scutella* L: scotelarin, baicalin, baicalein, vo-gonin, vogonoside, luteolin, chrysin and verbascoside. Based on the obtained results, reliable analyzes and conclusions are made.

The biological activity of the substances in the extracts of *S. altissima*, *S. albida* and *S. galericulata* was studied by established and standardized methods. Antioxidant activity was determined by three independent methods ranging from 652 to 1280 μmol TE / g by ORAC method; from 201 to 583 μmol GAE / g by HORAC method and from

7.9 to 10.1 AOA by "Electrochemical method". The antimicrobial activity of *Scutellaria altissima* extracts against *Streptococcus mitis* was studied and found to be most pronounced in 70% of ethanol extracts.

Contributions and significance of the development for science and practice

The results of the dissertation have primarily scientific and applied value and are directly aimed at solving specific problems in the processing and supply of food supplements. Conclusions are made with theoretical and practical methods for determining the composition and quality of *Scutellaria* extracts and their application as biologically active additives.

Contributions of scientific and applied value

- From the aboveground parts of *Scutellaria altissima* were isolated and spectrally characterized four neo-clerodan diterpenes, identified as: skutekiprin, skupolin H, clerodin and skutekiprol A, one sterol identified as β -sitosterols, a glycosidically linked iranidoid, iridoid. All substances have been proven and isolated for the first time from this plant species.
- The developed and validated HPLC method has a possible application for quality control of plant substances, extracts and phytopreparations obtained from species of the genus *Scutellaria* L.
- 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.
- 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 proven for the first time.

Evaluation of dissertation publications

There are 4 scientific papers on the dissertation, which are published in journals indexed in Thomson Reuters-WEB OF SCIENCE and SCOPUS, for which documents are attached and one in a foreign journal. The materials from the publications are included in the dissertation. The doctoral student presented a list of four participations in international and our scientific conferences with international participation. In most scientific reports and communications, Joana Georgieva is the lead author, which shows that she is the main executor of the scientific experiment. The doctoral student is also included in a project funded by the Science Fund of the Medical University of Plovdiv, whose topics are related to the dissertation.

These data give me reason to claim that the presented scientific developments cover the scientometric requirements for obtaining the educational and scientific degree "Doctor" of the Medical University of Plovdiv.

Abstract

The abstract is 58 pages long and contains 11 tables and 20 figures, which accurately reflect the main results and contributions of the dissertation and meets the specific requirements of MU - Plovdiv.

CONCLUSION

During the development of the dissertation, Joanna Pencheva Georgieva has acquired knowledge and ability to interpret and analyze what is known in the literature, to independently set scientific tasks and to solve them correctly. Demonstrates in-depth knowledge in the field of biologically active substances, masters, applies and interprets modern methods for analysis of these substances. The results obtained in the course of the development are correctly interpreted and represent a certain interest and contribution in a scientifically applied and applied aspect. My overall assessment of the dissertation, based on its relevance, structure, content and theoretical summaries, gives me reason to believe that the dissertation contains scientific, scientific-applied and applied results, which represent an original contribution to science and speak to all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for implementation of ZRASRB. The presented materials and dissertation results correspond to the specific requirements described in the "Procedure for acquiring ONS" Doctor "in MU - Plovdiv" to the "Regulations of MU - Plovdiv".

Based on the analysis, I give a positive assessment of the dissertation and propose to Joana Pencheva Georgieva to be awarded the educational and scientific degree "Doctor" in the scientific field "Natural Sciences, Mathematics and Informatics", professional field "Biological Sciences" in the doctoral program "Bioorganic chemistry, chemistry of natural and physiologically active substances.

05.07.2021

Reviewer:

Prof. Panteley Denev, D.Sc