OPINION

from Prof. Dr. Iliyan Ivanov
Faculty of Chemistry, University of Plovdiv "Paisiy Hilendarski"

on Doctor of Sciences Thesis for acquiring the scientific degree "Doctor of Sciences"
in the field of higher education: 4. Natural sciences, mathematics and informatics
professional field: 4.2. Chemical Sciences (Organic Chemistry)

Author: Assoc. Prof. Dr. Petko Ivanov Bozov
Topic: Clerodane diterpenoids from species of the *Lamiaceae family*

1. General presentation of the procedure and the dissertant

The set of materials on electronic media presented by Assoc. Prof. Dr. P. Bozov is correct and in full compliance with the RAS in the Republic of Bulgaria, the regulations for its application, and Article 45 (4) of the Regulations for the development of the academic staff of the University of Plovdiv. All conditions and requirements for obtaining the scientific degree "Doctor of Science" are met.

The results of the research are described in a 290-page dissertation, including 33 tables, 137 figures, 3 diagrams, and 23 appendices. The bibliography covers 276 literary sources mainly after 2000. In structural and compositional terms, the dissertation includes an introduction; three chapters - literature review, materials and methods, results and discussion; conclusions and contributions of the dissertation; bibliography and applications.

2. Actuality of the thematic research direction

The actuality of the presented topic is predetermined by the continuing increased interest in the application of biologically active compounds of natural origin in various spheres of human activity. The dissertation of Assoc. Prof. Bozov for obtaining the scientific degree Doctor of Science is dedicated to the study of clerodan diterpenoids - compounds with proven biological activity, contained in species of the genus Scutellaria, Teucrium polium subsp. vincentinum L. (Rouy) D. Wood, Teucrium scordium subsp. scordioides (Schreb.) Maire et Petitmengin, Salvia splendens Ker.-Gawl. and Salvia nemorosa L., distributed on the territory of Bulgaria. It is a logical extension of the topic of obtaining the educational and scientific degree of doctor.

3. Knowledge on the problem

Assoc. Prof. Bozov's many years of experience in the field of phytochemistry, in particular in research on the composition of plants containing clerodan diterpenoids, as well as continuous monitoring of literature sources are a prerequisite for excellent knowledge of the topic and related scientific challenges. Good knowledge of the problem and the accumulated

personal experience in the study of clerodan diterpenoids allows the correct formulation of the research goal and the tasks for its achievement.

4. Methodology of the investigation

The chapter Materials and methods accurately describe the materials used, methods and approaches applied for solving the set tasks, and the realization of the set goal. The use of the most modern methods and techniques for revealing the structure of the isolated compounds, including their stereochemistry, makes an excellent impression. Practically all available modern spectral methods are applied, the main role is given to NMR in its various variants. Current methods and practices have also been used in the extraction of compounds from plant material, their isolation, and purification, as well as the testing of their biological activity. The methods are described in sufficient detail in the dissertation.

5. Characteristics and evaluation of the doctoral thesis and its contributions

The results obtained in the course of the research and described in the dissertation are presented in the chapter *Results and discussion*. The contributions of research can be determined in several directions.

a. **Isolation of clerodan diterpenoids.** After phytochemical analysis for the presence of clerodan diterpenoids in 15 Bulgarian plant species of 5 genera of the *Lamiaceae family* (8 from Scutellaria, 3 from Salvia, 2 from Teucrium, 1 from Ajuga, and 1 from Stahys) 48 diterpenoids were isolated and spectrally characterized. Twenty-two of the compounds (diterpenes) are new to science and are being described for the first time.

b. **Proving of the structure of isolated clerodans and signal assignment in ^{1}H and ^{13}C NMR spectra.** All isolated diterpenes underwent complete signal assignment in ^{13}C-NMR spectra. The spectral data for some of the isolated diterpenoids have been confirmed, corrected, or supplemented. The configuration of all asymmetric centers in the isolated compounds was proved by interpretation of the binding J constants and the NOESY studies.

c. **Biological activity.** The antifidant activity of extracts from 8 species of the genus *Scutellaria* and 43 clerodane diterpenoids isolated from *S. alpina*, *S. galericulata*, *S. altissima*, *S. splendens*, *T. polium* and *T. scordium* against larvae of *Leptinotarsa decemlineata* was studied. The cytotoxic activity of 12 neo-clerodan diterpenoids isolated from members of the genus *Scutellaria* was studied against two cell lines: carcinogenic cells from human lung tumors and normal umbilical cord cells, as well as the antimicrobial activity of 22 clerodan diterpenoids against nineteen strains belonging to eleven different types of pathogenic and hygienic indicator microorganisms in food products and against two strains of yeast.

6. Evaluation of the publications and personal contributions of the candidate

The publications submitted in connection with the thesis defense in renowned specialized scientific journals directly correspond to the topic of the dissertation.

According to Art. 1 (2) of the rules of application of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the implementation of the minimum national requirements are as follows:

Group of indicators	Content	Doctor of science	Performance
A	Indicator 1	50	50
B	Indicator 2	100	100
C	Sum of indicators from 5 to 10	100	183
D	Sum of points in indicator 11	100	152

The total number of publications included in the dissertation is 28 and they are distributed as follows:

a. Publications in scientific journals, referenced and indexed in world-famous databases with scientific information (ISI Web of Knowledge and/or SCOPUS) - 18 articles, of which Q1 - 1 article; Q2 - 8 articles; Q3 - 4 articles; Q4 - 5 articles. The total impact factor is 14,621.

b. Publications in refereed editions - 8 articles;

c. Publications from conferences – 1 article;

d. Cited publications - 1 article.

The results obtained in the development of this dissertation are popularized among the scientific community in Bulgaria and abroad through twelve participations in scientific forums.

For the publications included in the dissertation at the time of its writing 141 independent citations were noticed, as the total number of citations of the author is 217, and the h-index is 7.

I know Assoc. Prof. P. Bozov as a colleague and his personal contribution to the research and their originality for me is not in doubt.

7. Scholarly essay

The scholarly essay of the dissertation accurately and completely reflects the dissertation, highlighting the essential aspects and deriving the main results of the research.

8. Recommendations for future use of the contributions and results of the thesis

My recommendations for future use of the dissertation contributions and results are in the direction of Assoc. Prof. Bozov to continue expanding the research while continuing to share and pass on the experience gained with his younger colleagues.

CONCLUSION

The dissertation thesis contains original scientific and applied-research scientific results that correspond to modern achievements and represent a significant and original contribution to the chemistry of natural compounds. The presented materials and dissertation results fully comply with the requirements of the Law for the development of the academic staff in the Republic of Bulgaria (LDASRB), the State Regulations for the application of LDASRB, and the regulations for the application of LDASRB in the University of Plovdiv "Paisiy Hilendarski" for observing LDASRB in professional direction 4.2 Chemical Sciences and research field "Organic chemistry".

Due to the above, I confidently give my ***positive assessment*** of the research presented by the above-reviewed dissertation, abstract, results, and contributions, and ***I offer the esteemed scientific jury to award the degree of "Doctor of Science"*** to Petko Ivanov Bozov in the field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.2. Chemical Sciences (Organic Chemistry).

19 April 2021

Opinion submitted by:.....

(Prof. Dr. Iliyan Ivanov)