REVIEW

by Dr. Nikolay Georgiev Vassilev,

Professor at the Institute of Organic Chemistry with the Center for Phytochemistry, BAS

of a dissertation for the award of the scientific degree **"Doctor of Science"**

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 - Department of Biochemistry and Microbiology at the Faculty of Biology of Plovdiv University "Paisii Hilendarski"

Topic: *Clerodane diterpenoids from species of the Lamiaceae family*

1. Subject of review

By order № P33-902 of 11.03.2021 of the Rector of Plovdiv University "Paisii Hilendarski" (PU) I was appointed a member of the scientific jury to provide a procedure for the defense of a dissertation on "Clerodan diterpenoids of species of the family *Lamiaceae*" for obtaining the scientific degree 'Doctor of Science' at the University of Plovdiv in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field: 4.2 Chemical Sciences (Organic Chemistry). The author of the dissertation is Assoc. Prof. Dr. Petko Ivanov Bozov from the Department of Biochemistry and Microbiology at the Faculty of Biology of the University of Plovdiv (PU).

The set of paper materials presented by Assoc. Prof. Dr. Petko Ivanov Bozov is in accordance with Art. 45 (4) of the Regulations for development of the academic staff of PU and includes the following documents:

- request to the Rector of the University of Plovdiv for disclosure of the procedure for defense of the dissertation;
- CV in European format;
- Minutes of the preliminary discussion of the dissertation in the department;
- abstract;
- abstract in English;
- list of scientific publications on the topic of the dissertation;
- dissertation work;

- reference for fulfillment of the minimum national requirements;
- declaration of originality and authenticity of the attached documents;
- copies of scientific publications on the topic of the dissertation;
- a copy of the diploma for the educational and scientific degree "Doctor";

The dissertation has enclosed 27 publications, and one publication from a scientific conference. Of these publications, 18 are in publications, referenced and indexed in world-famous databases of scientific information (ISI Web of knowledge and / or Scopus).

2. Brief biographical data

Associate Professor Dr. Petko Ivanov Bozov is a graduate of the University of Plovdiv and in 1986 he graduated with a master's degree in chemistry. In 1994 he obtained the scientific-educational degree "Doctor" by defending a dissertation on "Di and triterpenoids in members of the family *Lamiaceae* and their biological activity." After that for some time he was a teacher, engaged in drug trade and was a part-time lecturer in general and inorganic chemistry at the Medical University - Plovdiv. Since 2010 he has been working at the Faculty of Biology of the University of Plovdiv, first as an assistant, then as a senior assistant and since 2015 he has been an associate professor at the Faculty of Biology of the University of Plovdiv.

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

The isolation, purification and characterization of new substances from natural plant products is an extremely important task for Bulgaria, as we are a country rich in herbs and are among the top five European countries in the export of herbs and spices to the EU. Although the first in exports, the financial revenues from these exports are incomparably less than the value added, which is realized if a finished product is exported on the basis of some active ingredient in natural plant products. This determines the relevance of the problem developed in the dissertation in scientific and scientific-applied terms. The aim of the dissertation is to isolate clerodan diterpenoids from species of the *Lamiaceae* family, to study the structure, stereochemistry and antifidance activity of the compounds, to test the cytotoxic and antimicrobial action of selected diterpenoids. Thus, this dissertation can be considered as a stage in the transition from the export of herbs and spices to the export of high-value and science-intensive end products.

4. Knowledge of the problem

Associate Professor Dr. Petko Ivanov Bozov has made an overview of the composition, structure, classification and biosynthesis of terpenes on about 30 pages. He then turned to clerodan diterpenes and their classification. The following is a structural classification of the neo-clerodan

di-terpenes isolated from species of the genus *Scutellaria* and their biological action, as well as isolated clerodan diterpenes from species of the genus *Teucrium* in Bulgaria. The chemical composition of the essential oil of *Ajuga laxmanii Benth, Salvia amplexicaulis Lam* is presented and subspecies of *Stachys cretica*, as well as isolated polyphenols of species of the genus *Scutellaria*. The literature review concludes with the results of the phytochemical study of *Scutellaria alpina, Ajuga salicifolia* and *Lavandula spica*, included in the author's procedure for acquiring "Doctor" degree. The thorough and critical review of the literature, the numerous literary sources convince me of the in-depth knowledge of the literature on the topic of the dissertation and it is not by chance that the author has clearly set the goals and realistically the tasks of the dissertation. The use of modern physical methods to prove the structure and stereochemistry of substances is impressive.

5. Research methodology

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation. Methods for isolation and separation of biologically active substances in the studied plant material are standard for such cases. A combination of different spectral methods (elemental analysis, IR, MS, 1D and 2D NMR techniques) was used to determine the structure and stereochemistry of the compounds.

6. Characteristics and evaluation of the dissertation

After a detailed literature review in Chapter 1 of the dissertation and presentation of the materials and methods in Chapter 2, in Chapter 3 the results of the research are presented and the obtained results are discussed. The structure and stereochemistry of the isolated biologically active substances of species of the genus *Scutellaria*, *Teucrium* and *Salvia* were determined. The systematic description of the analysis of the obtained results from the different methods until the achievement of the complete structural and stereochemical characterization of the obtained known and new structures is impressive. In addition, the description is illustrated by the original IR, HPLC, MS, ^{1}H and ^{13}C NMR spectra. Techniques such as DEPT-135, COSY, HSQC, HMBC and NOESY were used to fully describe the NMR spectra and were skilfully analyzed. Following are the tests for the antifidant activity of extracts, for the antimicrobial activity of clerodan diterpenoids, for the cytotoxic activity of isolated neo-clerodan diterpenoids, as well as for the isolation and characterization of other organic compounds (Determination of the chemical composition of essential oil from *Ajuga laxmanii Benth, Salvia amplexicaulis Lam.* and *Stachys cretica subsp. bulgarica Rech. Fil*, Quantification of polyphenols in *Sculellaria altissima*, Isolation and characterization of sterols, cleroindicins and glucoside-related iridoids). In this part of the dissertation the original IR and NMR spectra are given as supporting info.

The presentation of the original spectra and their skilful analysis leave no doubt in the reader about the authenticity of the material on which the contributions of the dissertation are based.

7. Contributions and significance of development for science and practice

The research presented in the dissertation has original scientific contributions both in a purely scientific aspect and in applied science. Based on these contributions, several conclusions can be drawn. Therefore, the author has correctly separated the conclusions from the contributions of the dissertation. The conclusions are formulated in four directions: I. In isolation and characterization of the structure and stereochemistry of clerodan diterpenoids (9 conclusions), II. Conclusions on the relationship structure / antifidant activity of clerodan di-terpenoids (5 conclusions), III. Conclusions on the antimicrobial and cytotoxic activity of clerodan diterpenoids (3 conclusions), IV. When isolating and identifying other natural organic compounds (6 pins). The original scientific contributions are in two directions: I. In the isolation of clerodan diterpenoids (4 contributions), II. In proving the structure of isolated clerodans and the transfer of signals in ^{1}H and ^{13}C NMR spectra (6 contributions). 6 practical contributions, 3 in isolation of other organic compounds and 3 confirmatory contributions were also defined.

8. Evaluation of dissertation publications

The publications that reflect the results of the dissertation and are not used in the procedures for "Doctor" and "Associate Professor" are 11. Of these, 2 were published in the specialized journal *Phytochemistry Letters* (Q2, SJR = 0.452 and Q2, IF = 1.418), 3 in the specialized journal *Natural Product Communications* (Q2, SJR = 0.199 and Q3, IF = 0.884 for the two earlier publications and Q3, SJR = 0.199 and Q4, IF = 0.468 for the 2020 publication), 1 in the specialized journal *Journal of Chemistry of Natural Compounds* (Q3, SJR = 0.299 and Q4, IF = 0.653) and 5 are in the international specialized journal *Bulgarian Chemical Communications* (Q4, SJR = 0.142). The four publications in journals with Q2 provide 80 (4 * 20) points, the two publications in journals with Q3 provide 30 (2 * 15) points and the five publications in journals without IF, but with SJR provide 50 (5 * 10) points. Thus, the total number of points on indicator D is 160 instead of 170 points, but it is significantly above the required minimum of 100 points. The total number of citations on the topic of the dissertation is 141, and the ones not used in other procedures are 77. Thus, indicator D is 154 (77 * 2) points, again significantly above the required minimum of 100 points.

9. Personal participation of the author

I do not know the candidate personally, but I am impressed by the volume of research, which is an indication of diligence and dedication to scientific research. My assessment of the personal participation of the author in the dissertation research is based on the content of the dissertation. The way

the introduction and the literature review of the dissertation are written shows that he is very familiar with the available literature, which helped him to plan clearly and accurately the scientific research and to choose methods and approaches to achieve the goals of the dissertation. Reading the dissertation convinces me of the in-depth knowledge of the developed problems.

The dissertation includes the work of five graduates and two doctoral students, but this in no way diminishes the value of the research, but only shows the ability of the candidate to be a successful supervisor and mentor.

10. Abstract

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

11. Critical remarks and recommendations

The author has applied many experimental spectra of IR, MS and NMR studies. In my opinion, most of them are better to go in the supporting info, and where they are discussed in the text to indicate the discussed signals. It does not make much sense to present two-dimensional NMR spectra without projections on both axes. In some places the presented NOESY spectra are not phased. When describing proton NMR spectra, the number of protons is represented by an integer, not as on page 62: 0.5H, 1.5H, etc.

Despite these minor critical remarks, the dissertation is at a very high scientific level, the results of which have been published in renowned journals in the field.

12. Personal impressions

I have no personal impressions.

13. Recommendations for future use of dissertation contributions and results

My recommendations for the future use of the results of this work is in the search for practical application of both extracts and isolated substances, including cooperation with multiple groups that test a variety of biological activity.

CONCLUSION

The dissertation 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 (ZRASRB), the Regulations for application of ZRASRB and the respective Regulations of Plovdiv University "Paisii Hilendarski".

The dissertation shows that the dissertation Assoc. Prof. Dr. Petko Ivanov Bozov has in-depth theoretical knowledge and professional skills in the scientific specialty Organic Chemistry by demonstrating qualities and skills for conducting research with original and significant scientific contributions.

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

25.04.2021 Writing the review: .....

Prof. Dr. Nikolay Vassilev