

## OPINION

by **Prof. Dr. Velizar Kostadinov Gochev,**

Head of the Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski",

of a dissertation for the award of the scientific degree "Doctor of Sciences" (DSc) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.2 Chemical sciences (Organic chemistry)

**Candidate:** Assoc. Prof. Dr. Petko Ivanov Bozov

**Title:** "Clerodane diterpenoids from species of family *Lamiaceae*"

### ABBREVIATIONS USED:

**ASDARB – Academic Staff Development Act in the Republic of Bulgaria**

**RIASDARB – Regulation on the Implementation of the Academic Staff Development Act in the Republic of Bulgaria**

**RPUIASD - Regulations of the University of Plovdiv "Paisii Hilendarski" on the Implementation of the Academic Staff Development**

### 1. General presentation of the procedure and the candidate

By Order № P33-902/11.03.2021 of the Rector of the University of Plovdiv "Paisii Hilendarski" (PU) I was appointed as a member of the scientific jury of a dissertation entitled "**Clerodane diterpenoids from species of family *Lamiaceae***" for the award of the scientific degree "Doctor of Sciences" (DSc) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.2 Chemical sciences (Organic chemistry) by Dr. Petko Ivanov Bozov, who is Associate Professor at Department "Biochemistry and microbiology", Faculty of Biology.

The set of digital materials presented by Assoc. Prof. Dr. Bozov is in accordance with Article 45(4) of RPUIASD, including: request to the Rector of PU for disclosure of a defense procedure (№ K1-6/04.03.2021); CV (European format); copy of diploma for PhD degree (candidate of Chemical Sciences); protocols of departmental councils related to the opening procedure; doctoral thesis; thesis abstract; list of scientific publications related to the dissertation; copies of scientific publications; declaration of originality and authenticity of the attached documents; reference for compliance with the minimum national requirements for reaching scientific degree "Doctor of Science". Based on the documents and the declarations enclosed it is clear that formal requirements for reaching scientific degree "Doctor of Science"

have been fulfilled, which allows me to determine the procedure as lawful and to proceed to a detailed evaluation of the dissertation and the contributions presented by Assoc. Prof. Dr. Bozov.

## **2. Actuality of the subject**

The widespread level of antibiotic resistance among pathogen microorganism; the increased desire of development of bioagriculture and replacement of synthetic chemical preparations for plant defense by natural and biodegradable compounds, combined with increased needs of effective substances with cytotoxic activity influence intensive screening of new substances with wide spectrum of biological activities, such as antimicrobial, antifungal, antitumor, antifeedant and etc. Bulgarian flora is extremely rich of medicinal and aromatic plants as a source for potential isolation of new bioactive substances. One of the most intensively studied Family is *Lamiaceae*, but the scientific studies which are focused on the clerodane diterpenoids are scarce, both on the national and international level. Based on the above-mentioned reasons the dissertation of Assoc. Prof. Dr. Bozov is important and actual not only from practical point of view, but from fundamental point of view, too.

## **3. Familiarity of the problem**

The literature review is based on 276 publications, pointing the major achievements in the studied problem for more than 30 years period, which allows me to determine the literature review as detailed and comprehensive. The review is focused on the clerodane diterpenoids, isolated from different species of genus *Scutellaria*, species *Teucrium polium* and *T. scordium* and also the polyphenols, isolated from genus *Scutellaria*. The classification, nomenclature, biosynthesis and bioactivities of clerodane terpenoids are presented. The major skeleton structures, functional groups and ester substitutes, detected in natural compounds isolated from plants of genus *Scutellaria* wildgrowing in Bulgaria are also discussed. The literature data about chemical composition and bioactivity of essential oils from *Ajuga laxmannii*, *Salvia amplexicaulis* and *Stachys cretica*, wild growing on the Balkan Peninsula are compared. The major scientific achievements of the research groups of Assoc. Prof. Dr. Malakov and Prof. DSc Papanov, dedicated to the phytochemistry of species belonging to genera *Scutellaria* и *Ajuga*, are discussed briefly. These results are base for the long-time studies carried out by Assoc. Prof. Dr. Bozov and applied in the present dissertation.

The scope and the way of organization of the literature review allows me to conclude that Assoc. Prof. Dr. Bozov knows the worldwide achievements on the research problem in details.

#### 4. Research methodology

To reach the major scientific goal and tasks of the study a wide range of analytical methods, which allow reaching correct and reproducible results are used. The methodology section is to some extent unnecessarily detailed.

#### 5. Characteristics and evaluation of the dissertation

Dissertation is structured in accepted order, including: Introduction, Literature review, Goal and tasks, Methodology, Results and discussions, Conclusions, Contributions, Reference list and 23 Applications. Ratios among sections are optimal. Forty-one secondary metabolites are isolated from genus *Scutellaria*, two of them are sterols, two are glucoside-bounded iridoides, two cleroidicines and 35 *neo*-clerodane diterpenoids. Fifteen of the isolated diterpenoids demonstrate new chemical structures. Neoajugapyrin A is a new diterpene for genus *Scutellaria*. Ten of the isolated compounds are new for the botanical species. Eleven furoclerodan lactoditerpenoids are isolated from the aerial parts of two species belonging to genus *Teucrium*. A clerodane teulamifyn B is proved for the first time in *Teucrium polium*. Eight of the compounds isolated characterized by *neo*-clerodane skeleton. Capitatyn and auropolyn are not detected in *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood. Five of the isolated diterpenoids differ from all the 11 clerodanes, proved by Malakov et al. in the diterpene fraction of *Teucrium polium* subsp. „*polium*“. The compounds isolated from genus *Teucrium* characterized by furofuran ring. In the molecules of the diterpenes, isolated from genus *Teucrium*, acetate groups are detected, meanwhile in the *neo*-clerodane diterpenoids isolated from genera *Scutellaria* and *Ajuga* different types of ester groups are detected. It is proved that in genera *Teucrium* and *Salvia* clerodanes with furofuran rings are not biosynthesized. *neo*-Clerodane diterpenoids with lactone ring in decalin nucleus demonstrate strong antifeedant activity. The influence of chemical structure of the isolated *neo*-clerodane diterpenoids and antifeedant activity is proved. Tested *neo*-clerodane compounds are not prospective as antitumor and antimicrobial agents.

Important and original scientific contributions of the dissertation can be highlighted. Forty-eight natural diterpenoids are isolated and spectrally characterized. One of these compounds belongs to labdane skeleton type (sclareol), three compounds are from 19-*nor*-clerodane skeleton type, and the rest 44 belong to the group of *neo*-clerodane diterpenoids. Thirteen other compounds are proved for the first time in the studied botanical species. Chemical structure of *neo*-ajugapyrin A (published as 1- $\beta$ -hydroxyscutecyprin) and 3- $\beta$ -hydroxyscutecyprin is revised and corrected. The real structure of 1- $\beta$ -hydroxyscutecyprin

(trivial name scutegalerin A) is isolated and characterized. In the molecules of 11 different clerodane diterpenoids the presence of epimeric couples is proved. Five of these compounds are spectral characterized. A *neo*-Clerodane (scutaltysin C) with unusual C-11R configuration is isolated. For the first time epimer mixture of scutecolumnin C (11S configuration) and 11-*epi*-scutecolumnin C (with 11 R configuration), which is published earlier as unseparated, is partially separated in the current study. For all of the isolated diterpenes <sup>13</sup>C-NMR spectrums are totally characterized. The missing literature data about <sup>13</sup>C-NMR spectrum of ajugapyrin A and 6-ketoteuscordin are supplemented. The major part of the contributions, achieved in the dissertation, submitted by Assoc. Prof. Dr. Bozov are original and fundamental.

## **6. Evaluation of the publications and the personal contribution of the candidat**

The results of the dissertation are presented in 11 papers, which are published in scientific journals indexed in Scopus and Web of Science. Five of the papers belong to quartile Q2 journals, 1 - to Q3 and 5 - to Q4, with total impact factor 8,789. Five of the papers are published in journals, which are not indexed in Scopus and Web of Science. Only 1 paper is published in Conference Proceedings Book. Assoc. Prof. Dr. Bozov presented the results of his studies at 5 international and 7 national scientific forums. I am deeply convinced that the greatest attestation for the quality of a scientific publication is not the value of the impact factor or the quartile of the journal, but its citation. For this reason, I appreciate the fact that the papers of Assoc. Prof. Dr. Bozov, based on the results published in the current dissertation, are cited 141 times (according to Scopus). Assoc. Prof. Dr. Bozov is a leading author (first, corresponding or last author) of all papers, listed in dissertation. This fact undoubtedly proves his leading role for the realization of the publications.

## **7. Summary of the dissertation**

The summary of the dissertation fulfills all of the formal requirements and adequately reflects its essence and achievements.

## **8. Recommendations for future use of dissertation contributions and results**

I have no significant critical remarks on the dissertation and the quality of the results obtained. I think that the link between the studies related to diterpenoids from *Scutellaria*, *Teucrium* and *Salvia* and the studies related to the composition of essential oils and phenolic compounds of *Ajuga* and *Stachys* is insufficiently highlighted. I recommend, if it is possible, to expand the biological experiments, especially those concerning the insecticidal activity of the

newly isolated compounds, so that the results of the dissertation will find not only theoretical and fundamental, but even practical applications.

## CONCLUSION

The dissertation contains scientific and applied results, which represent an original contribution to science and meet the requirements of the RIASDARB and RPUIASD. The presented materials and dissertation results correspond to the specific requirements of the Faculty of Biology, adopted in connection with the RIASDARB and RPUIASD. The dissertation shows that Assoc. Prof. Dr. Petko Ivanov Bozov has in-depth theoretical knowledge and professional skills in the scientific specialty Organic Chemistry, demonstrating qualities and skills for conducting research with original and significant scientific contributions. Due to the above, I give my positive assessment of the research presented in the dissertation, summary, results and contributions, and I propose to the esteemed scientific jury to award the scientific degree "Doctor of Science" to Assoc. Prof. Dr. Petko Ivanov Bozov in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field 4.2. Chemical Sciences (Organic chemistry)

12.04.2021

Prepared the opinion.

(Prof. Dr. V. Gochev)