

EVALUATION REPORT

From Plamen Angelov Angelov, PhD, Associate professor at the University of Plovdiv "Paisii Hilendarski"

About submitted dissertation for the degree Doctor of Sciences (DSc) in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional classification 4.2. "Chemical Sciences" (Organic Chemistry).

Author: Assoc. prof. Petko Ivanov Bozov, PhD

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

1. General description of the submitted documents

I have been designated as a member of the scientific jury according to order № P33-902/11.03.2021 by the Rector of the Plovdiv University "Paisii Hilendarski", with the task of reviewing and evaluating the thesis described above, submitted for the scientific degree 'Doctor of Sciences'. As such, I confirm that the author of the thesis, Petko Ivanov Bozov, has submitted the complete set of required documents in compliance with the regulations of the Plovdiv University and the Law for the Development of the Academic Staff in the Republic of Bulgaria. The documents include:

- 1.1. Written request to the Rector
- 1.2. CV
- 1.3. Record form the departmental council
- 1.4. Extended abstract of the doctoral thesis (in Bulgarian and English)
- 1.5. List of scientific works
- 1.6. Dissertation
- 1.7. Reference for compliance with the minimal scientific requirements
- 1.8. Copies of the scientific publications

Petko Ivanov Bozov is currently an associate professor at the department of biochemistry and microbiology, Faculty of Biology, University of Plovdiv "Paisii Hilendarski".

2. Brief biographical information about the doctoral candidate

Petko Ivanov Bozov was born in 1962. In 1986 he graduated from the University of Plovdiv "Paisii Hilendarski" with a masters's degree in chemistry. In 1989 he started postgraduate studies in the same university and received his PhD degree in 1994 with a thesis entitled "Di- and triterpenoids from species of the *Lamiaceae* family and their biological activity". From 1993 to 2010 he worked as a chemistry teacher, except for the period 1998 – 2003, when he was employed in the pharmaceutical business. Between 1994 and 2011, along with his main employment, he took part-time teaching positions at the Plovdiv Medical University. In 2010 Petko Bozov joined the Faculty of Biology at the University of Plovdiv "Paisii Hilendarski" as an assistant professor. Later, in 2015, he was promoted to associate professor – a position that he currently holds.

3. Topicality of the dissertation and relevance of the aims and objectives to the field of research

The presented dissertation falls within the fields of Phytochemistry and Organic chemistry. It is aimed at isolation of clerodane diterpenoids from species of the genera *Scutellaria*, *Teucrium* and *Salvia*; study of their structure and stereochemistry; and investigation of their biological activities, such as anti-feedant, antimicrobial and cytotoxic activity.

The use of plants as a sustainable source of bioactive compounds and other feedstock for the fine chemical industry is of great importance for achieving the goals of green economy and sustainable development. Any scientific research aimed at the discovery and study of new bioactive materials from plant sources has relevance not only to the principles of green economy, but could also be important for the development of new drugs and agrochemicals. In view of that, the presented dissertation is topical and contributes to the development of this field of research.

4. Characteristics and evaluation of the thesis

The dissertation is 186 pages long (including Title, Contents and List of abbreviations) and is divided into the following chapters:

- Introductions (3 pages)
- Literature Review (32 pages)
- Aims and Objectives (1 page)
- Materials and Methods (13 pages)
- Results and Discussion (112 pages)
- Conclusions (6 pages)
- References (16 pages)

The dissertation is accompanied by additional 92 pages with IR and NMR spectra of the studied compounds. Declaration of originality and a list of publications and citations occupy 14 more pages at the end of the dissertation.

The concise introductory part is followed by a review of the literature in the field, with a total of 276 references. The literature review is well done. It includes the basics of terpene chemistry, the current knowledge and the latest developments in the field of clerodanes. Such a literature review is indicative of thorough understanding of the chosen research problem and lays the ground for well formulated and substantiated list of aims and objectives.

The next chapter (Materials and Methods) describes in detail the studied plant materials and their origin, as well as the methods for isolation of clerodane diterpenes and the methods for extraction of essential oils. The technical characteristics of the used analytical instruments are listed in sufficient detail and the basics of all spectral methods relevant to the author's own work are presented in brief. State-of-the-art equipment is used for the most important spectral analyses – nuclear magnetic resonance, high-resolution mass spectrometry with electrospray ionization, and infrared spectroscopy. The bioassay methods for antifeedant, antimicrobial and cytotoxic activity are also described here.

The author's own work is presented in the Results and Discussion chapter. Most of this chapter deals with isolation of biologically active substances from species of the genus *Scutellaria*, *Teucrium* and *Salvia* and determination of their structure and configuration. Of utmost importance in this research is the interpretation of the obtained NMR spectral data. The author demonstrates competent application of many contemporary NMR techniques in combination with other methods for structure elucidation, such as IR spectroscopy and mass spectrometry. Analysis of the acetone extract obtained from the aboveground parts of *Scutellaria orientalis* subsp. *pinnatifida* reveals the known *neo*-clerodane diterpenoids, scutorientalin B and scutorientalin D, together with a new *neo*-clerodane, scutorientalin E, the structure of which is elucidated as 7 β -19-diacetoxy-6 α -(E)-cinamoyloxy-4 α ,18-epoxy-8 β -hydroxy-*neo*-cleroda-13-ene-15,16-olide by spectral studies and comparison of data with related compounds. From *Scutellaria altissima* are isolated and spectrally characterized 17 compounds, 14 of which are *neo*-clerodane diterpenoids. Eight known clerodane diterpenoids are found for the first time in this species, and the other six (scutaltisin B, scutaltisin C, scutaltisin D, scutaltisin E, scutaltisin F и scutaltisin G) are new compounds and are described for the first time by the author. From the acetone extracts obtained from the aboveground parts of two *Teucrium* species are isolated 11 furoclerodane lactonediterpenoids – the new polivincins A-C, 6-acetylteucriene F, teucriene E acetate, 3 α -acetoxy-4 scordidesin A and the known teulamifin B, teupolin XII, teucriin A and 6-ketoteuscordin. Clerodane teulamifin B is found for the first time in *Teucrium polium*. The author has successfully recorded and assigned the ¹³C NMR spectrum of 6-ketoteuscordin – data that so far has not been available in the literature. In summary, the author has performed phytochemical analysis of clerodane 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 *Stachys*). 48 diterpenoids have been isolated and spectrally characterized. One of them has labdane skeleton (sclareol), three have a 19-*nor*-clerodane skeleton, and the other 44 are *neo*-clerodane diterpenoids. 22 of the diterpenes are described for the first time by Petko Bozov: two with a 19-*nor*-clerodane skeleton and twenty with a *neo*-clerodane skeleton. Another 13 diterpenoids have been detected for the first time in the studied species.

Considerable attention is paid to the possible biological activity of the studied compounds. The author investigated three types of biological activity, choosing the bioassays by analogy with previously published data for structurally similar compounds. The emphasis is on antifeedant activity against the larvae of *Leptinotarsa decemlineata* Say. This activity is studied for 43 clerodane diterpenoids isolated from *S. alpina*, *S. galericulata*, *S. altissima*, *S. splendens*, *T. polium* and *T. scordium*. Similar antifeedant assays are carried out with extracts from species of the genus *Scutellaria*. The strongest suppression of the larvae is observed with the extract from *S. albida*. Very good activity is shown by extracts from *S. altissima*, *S. galericulata* and *S. alpina*. The antifeedant effect, under dietary conditions of choice, of seven natural *neo*-clerodane diterpenes isolated from *Scutellaria alpina* и *Salvia splendens* has also been measured. These natural products show very good suppression of the larval feeding at concentration 1000 ppm ($\approx 33 \mu\text{g} / \text{cm}^2$).

The antimicrobial activity of twenty-two clerodane diterpenes isolated from acetone extracts from the aerial parts of species of the genus *Scutellaria*, *Salvia* and *Teucrium* (Lamiaceae) has been studied against nineteen strains belonging to eleven different species of pathogenic bacteria (and also against two strains of yeast). Three of the studied compounds (Scutalpin A, Scutalpin E, Scutalpin F) show moderate antimicrobial activity. The diterpenes with furan ring in their structure are found to be weakly active, and all other compounds are not active at all.

The cytotoxicity of some *neo*-clerodane diterpenoids is assayed against human cancer cells (H1299) and normal umbilical cord cells (HUVEC). Three compounds (Scutalpins A, E и F) show weak to moderate cytotoxicity against both cell lines. Among the assayed compounds, the most active is Scutalpin A, with IC_{50} 21.35 and 23.9 μM . All other compounds are found to be inactive in the studied range of concentrations.

The author's own work is complemented with a short subchapter dealing with the isolation and characterization of some other compounds of plant origin. This subchapter describes an analysis of the chemical composition of essential oil from three plant species and quantitative determination of polyphenols in *Scutellaria altissima*. Also described here is the isolation and characterization of sterols, clerodindines and iridoid glucosides.

5. Publications in connection with the dissertation

Results from this dissertation have been published in a total of 28 scientific papers. 18 of these are published in internationally recognized journals, indexed in reputable databases (Scopus and/or Web of Science). One more publication can be added to this group, because it is cited in internationally recognized journal. These scientific articles fully correspond to the results presented in the dissertation.

In most of the publications related to this dissertation Petko Bozov is the first or the corresponding author, which is a good indication for his own contribution.

So far, a total of 142 citations of these publications have been noticed in the specialized literature.

CONCLUSION

This dissertation contains original research results which contribute to the development of the scientific field and satisfy the requirements for quality and novelty imposed by the Law for the Development of the Academic Staff in the Republic of Bulgaria. The dissertation shows that its author, Petko Ivanov Bozov, has sufficient theoretical knowledge and professional skill in the fields of Phytochemistry and Organic Chemistry. The author demonstrates abilities for advanced scientific research. In view of the above, I kindly recommend the scientific jury **to grant the degree** "Doctor of Sciences" to Petko Ivanov Bozov.

23.04. 2021

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Assoc. Prof. Plamen Angelov, PhD