

## STATEMENT OF OPINION

by **Detelina Stoyanova Belkinova PhD**,

**Associate Professor at the Department of Botany and Biological Education, Faculty of Biology, P. Hilendarski University of Plovdiv, Regarding the Dissertation for the Acquisition of PhD Academic Degree in the Field of Higher Education: 4. Natural Sciences, Mathematics, and Informatics; Professional Direction: 4.3. Biological Sciences; PhD Programme: Botany**

**Author: Donika Petrova Gyuzeleva**

**Topic: *Biological and Phytochemical Research on Plants in the Flora of Bulgaria with a Potential for Biotechnological Application***

**Academic Advisors:**

***Prof. Plamen Stefanov Stoyanov – P. Hilendarski University of Plovdiv***

***Prof. Anelia Veselinova Bivolarska – Medical University of Plovdiv***

### **1. General Presentation of the Procedure and the PhD Candidate**

By Order No. ПД-21-2058 from 15 November 2024, of the Rector of Paisii Hilendarski University of Plovdiv, I have been appointed as a member of the scientific jury to provide a procedure for the defence of this dissertation named *Biological and Phytochemical Research on Plants in the Flora of Bulgaria with a Potential for Biotechnological Application* for the acquisition of the scientific and educational **PhD** degree. The author of the dissertation is Donika Petrova Gyuzeleva – full-time PhD Candidate at the Department of Botany and Biological Education.

The set of materials on the procedure, provided by Donika Gyuzeleva, is under Art. 36 (1) of the Regulations of the Academic Staff Development Procedures of the University of Plovdiv, and includes the full set of documents. The materials on the procedure include attachments of 4 (four) printed articles, 3 (three) participations in scientific conferences, and a list of 7 (seven) reported quotations. All the documents and materials on the procedure have been prepared diligently and precisely.

### **2. Relevance of the Topic**

Plants have been used as a source of natural medicine for different diseases since ancient times. In recent decades, interest in new species with pharmacological action has been growing. However, their adequate use in medicinal preparations requires a thorough investigation of their metabolites and biological activities by applying the metabolomics approach. This determines the relevance of the present dissertation, whose object of study are the species *Marrubium friwaldskyanum* (*Bulgarian endemic plant*), *Marrubium peregrinum* (*Lamiaceae*) and *Centaurea thracica* (*Asteraceae*).

### **3. Knowledge of the Subject Matter**

The literature review provides detailed specialist information and is logically structured into four main sections: 1/ Botanical and Morphological Characterization of the Studied Species; 2/ Phytotherapeutic Properties; 3/ Phytochemical Characterization; 4/. Biological Activity of the Phenolic Compounds of the Studied Species.

The reference demonstrates an excellent knowledge of the subject matter and the ability to summarize and critically analyse a large number of literary sources. Both in the literature review and in the Results and Discussion section, foreign sources are discussed skilfully and correctly. The number of references cited in the bibliography is impressive – 257, of which more than two-thirds (175) have been published in the last 15 years. This proves unequivocally the relevance of phytochemical screening in recent decades.

### **4. Methods of Study**

What I do find as strong point of this work is the adopted methodological approach, which includes: 1. A clearly formulated goal from which the specific tasks are derived; 2. Selection of appropriate species; 3. Selection of highly sensitive modern methods that guarantee the quality of the results; 4. Application of appropriate statistical methods, leading to adequate interpretation of results and reliable conclusions. This methodological approach has contributed to the successful solution of the set tasks.

Given the interdisciplinary nature of the dissertation, a wide range of methods have been applied: Classical (comparative-anatomical microscopy), chemical analysis methods (according to established international standards), non-targeted metabolomic analyses (GC-MS, UPLC-MS and UPLC-MS/MS technologies); methods for the determination of biological activities (*in-vitro* cytotoxicity and antitumor activity assays on cell lines, antibacterial activity) and statistical methods (multivariate analysis, principal component analysis). The methods are described in sufficient detail and precision that they can be reproduced.

### **5. Characteristics and Assessment of the Dissertation and the Contributions**

The dissertation is 128 pages long and follows a standard structure: Introduction (1 page), Literature Review (16 pages), Purpose and Objectives (1 page), Materials and Methods (25 pages), Results and Discussion (52 pages), Conclusions (3 pages), Contributions (1 page) and Works Cited (21 pages). 257 titles are cited, of which 7 in Cyrillic and 250 in Latin. The dissertation is excellently illustrated with 39 figures and 12 tables. The following additional sections of the dissertation are attached: Participations in Scientific Forums; a list of the Publications on the Topic of the Dissertation; Citations and Declaration of the author for originality of the results and contributions. I support this statement, as *I did not find plagiarism* during the review.

The dissertation has a clearly formulated aim – through a biological and phytochemical study of the species *Marrubium friwaldskyanum*, *M. peregrinum*, *M. vulgare* and *Centaurea thracica* to establish their significance for application in biotechnological developments and their use as medicinal plants. Specifically, the set tasks are solved by applying modern scientific approaches and methods of analysis, which guarantees a high scientific level of the work. The obtained results are correctly presented and their reliability is beyond doubt. They correspond fully to the set tasks and are synthesized in 10 main conclusions concerning the study of *M. friwaldskyanum* and *M. peregrinum* and 5 conclusions concerning *C. thracica*. The contributions are divided into original scientific contributions and scientific contributions of an applied nature. As the most significant scientific contribution of fundamental nature, I can point out the first for Bulgaria detailed research on the chemical composition of fruits and seeds of *Centaurea thracica*. The established anatomical features of the species *M. friwaldskyanum* and *M. peregrinum* enrich the knowledge about the biology of the genus *Marrubium* and can be used for taxonomic purposes. The proved unique tissue-specific compounds with important bioactivities in *M. friwaldskyanum* and *M. peregrinum* are of the contributions with the characteristics of scientific and applied nature which have significant meaning. In my opinion, another contribution of an applied nature can be added: the high content of biologically active substances (monounsaturated fatty acids, tocopherols, and phospholipids) in the oils of *Centaurea thracica* fruits and seeds makes them a valuable raw material for pharmaceutical products against a number of diseases.

## **6. Assessment of Publications and Personal Contribution of the PhD Candidate**

In connection with the dissertation, four publications in which the PhD candidate is the **lead author** are attached to the materials. The co-authorship declarations prove that she has made a substantial contribution to the overall publication activity and her personal contribution is beyond doubt. The four articles are in journals that are refereed and indexed in Scopus. Three of the articles were published in the reputed journals '*Journal of Molecular Sciences*' (IF: 4.9), '*Heliyon*' (IF: 3.4) and '*Molecules*' (IF: 4.2), with a quartile Q1. Although the results have been published recently, they have already had an international response and 7 citations. With this, the minimum required 30 points of the national criteria for obtaining the educational and qualification PhD degree have been reached and exceeded approximately 3 times – the candidate's performance is 87 points.

## **7. Abstract**

The abstract is properly formatted and correctly reflects the essence and results of the dissertation.

## **8. Recommendations for Future Use of the Dissertation Contributions and Results**

I would like to congratulate Donika Gyuzeleva and her academic advisors for the thorough dissertation. It demonstrates a mastery of a number of modern methods in a topical contemporary

field and developed skills of a scientific researcher. I wish her to continue to apply the mastered methods with the same responsibility and precision to other interesting and promising phytochemical species.

## **CONCLUSION**

The proposed dissertation of Donika Gyuzeleva is a comprehensive and in-depth study that reveals solid theoretical background, methodological knowledge, skills for field and experimental work and the ability to analyse and interpret the results. All the prerequisites necessary for the acquisition of educational qualification PhD degree are present.

The dissertation contains sufficient scientific and applied results that represent an original contribution to science. The presented materials and results meet all the requirements of the Development of Academic Staff in the Republic of Bulgaria Act (DASRBA), the Regulations for the Application of the DASRBA, and the Respective Regulations of Paisii Hilendarski University of Plovdiv.

**The above data and facts give me grounds to give a POSITIVE mark of the developed dissertation and to propose to the honourable scientific jury to award the educational and qualification PhD degree to Donika Petrova Gyuzeleva in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics; Professional Direction: 4.3. Biological Sciences; PhD Programme: Botany.**

January, 3 2025

**This Statement of Opinion was executed by: .....**

/ Assoc. Prof. Detelina Belkinova /