

## REVIEW

By PhD Tsvetanka Georgieva Raycheva -

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Of the dissertation for the award of the educational and scientific degree "PhD"

In: field of higher education 4. Natural Sciences, Mathematics and Informatics

Professional field 4.3. Biological Sciences

Doctoral Programme Botany

**Author:** Donika Petrova Gyuzeleva

**Subject:** Biological and phytochemical study of plants from Bulgarian flora with potential for biotechnological application

**Scientific Supervisors:** Prof. PhD Plamen Stefanov Stoyanov - Faculty of Biology, Paisii Hilendarski University; Assoc. prof. PhD Anelia Veselinova Bivolarska - Department of Medical Biochemistry at Medical University-Plovdiv.

### 1. General description of the submitted materials

By Order No. RD-21-2058 of 15.11.2024 of the Rector of Plovdiv University "Paisii Hilendarski" (PU) I have been appointed as a member of the scientific jury for providing a proprocedure for the defense of a dissertation thesis entitled "Biological and phytochemical study of plants from the Bulgarian flora with potential for biotechnological application" for the acquisition of the educational and scientific degree "PhD" in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.3. Biological sciences

Doctoral Programme Botany

The author of the dissertation is Donika Petrova Gyuzeleva - PhD student in full-time study at the Department of Botany and Biological Education with scientific supervisors Prof. PhD Plamen Stefanov Stoyanov - Faculty of Biology, Paisii Hilendarski University; Assoc. Prof. PhD Anelia Veselinova Bivolarska - Department of Medical Biochemistry -Plovdiv.

The set of paper materials submitted by Donika Petrova Gyuzeleva is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of PU, and includes the following documents:

1. Application to the Rector of PU for the disclosure of the dissertation defense procedure;
2. Curriculum Vitae in European format;
3. Minutes of the departmental council related to the reporting of the readiness for the opening of the procedure and the preliminary discussion of the dissertation;

4. Dissertation;
5. Abstract (in Bulgarian);
6. Abstract (in English);
7. List of scientific publications on the topic of the dissertation;
8. Copies of scientific publications;
9. Declaration of originality and authenticity of the attached documents;
10. Reference of compliance with the minimum national requirements;
11. Opinion of the supervisors on the readiness to open the procedure.

All the documents submitted have been drawn up in a precise manner, which facilitates the examination.

## **2. Brief biographical data about the PhD student**

Donika Gyuzeleva completed her higher education at Plovdiv University (2011-2015), where she obtained her Bachelor's degree with the qualification 'Medical Biologist'. In 2015-2016, she continued her studies in the Master's program in 'Microbiological Control and Food Safety', upgrading her knowledge in the field of biotechnology and bioorganic chemistry and skills in working with research biological equipment. As of 2018, she is the Deputy Director of the Human Resources Management Department at Plovdiv University "Paisii Hilendarski", where she gained administrative and organizational skills. Since 2023, she has been an assistant professor at the Department of Botany and Biological Education.

01.08.2019 Donika Gyuzeleva was enrolled in a fulltime PhD program in Botany (PU, Department of Botany and Biological Education) and after successfully completing the activities and tasks of her individual study plan she was discharged with the right to defend on 01.08.2022.

## **3. Relevance of the subject matter and appropriateness of the set goals and objectives**

In the present dissertation a biological and phytochemical study of the species *Marrubium friwaldskyanum*, *M. peregrinum*, *M. vulgare* and *Centaurea thracica*, to explore their potential as medicinal resources in biotechnological developments.

Anatomical, morphological, phytochemical and biological studies were conducted on the target species. Information on the biology and taxonomy of the genus *Marrubium* gave

enriched. New information was obtained on specific epidermal characteristics and leaf histological structure in species of the genus *Marrubium*.

Detailed phytochemical analyses of *Marrubium friwaldskyanum* and *M. peregrinum* - large amounts of flavonoids, glycosides, 175 lipid compounds were identified. Extracts of both species exhibited inhibitory effects against Gram-positive and Gram-negative bacteria, as well as antitumor activity against carcinoma cells, with the potential to suppress the development of a multicellular aggregate of pathogenic cells. Detailed chemical analyses of *Centaurea thracica* fruits and seeds are being conducted for the first time in this country. The results show high levels of fatty acids, including saturated and monounsaturated, phospholipids and biologically active substances, which define the biological and nutritional value of fruit and seed oils as a resource for the food and pharmaceutical industries. The topic of Donika Gyuzeleva's dissertation is topical and relevant in the context of increased and growing interest in sources for biotechnological production and phytopharmaceutical resources.

#### **4. Knowledge of the problem**

The PhD student's excellent knowledge of the state of the problem is reflected in a comprehensive and detailed literature review summarizing the available information mainly from 250 foreign sources and 7 of our own. The literature review is logically structured and re-provides detailed specialized information, concerning the biological characterization of the target species, phyto-therapeutic properties, phytochemical characterization and biological and cytotoxic activity of phenolic compounds. The review shows that clinical studies on antitumour activity in the genus *Marrubium* are insufficient, especially for the Bulgarian endemic *Marrubium friwaldskyanum*. It is worth noting that the review shows a thorough knowledge of the issues, the ability to summarize and critically analyze a large number of literature sources, with diverse topics, corresponding to the main theoretical propositions. Furthermore, the analytical overview presented has been used further in the research and analyses done in the thesis.

#### **5. Research methodology**

The overall methodological part is detailed and presented in 25 pages of text and richly illustrated with photographs, which gives a good picture of the research implementation and the materials collected by the author from target species from natural habitats in Bulgaria. The chosen research methods are at a modern scientific and applied level, up-to-date and of proven

quality and precision. It is noteworthy that the use of appropriate methods in the studies, allow to obtain reliable results and achieve the set goal in the dissertation.

## **6. Characteristics and evaluation of the thesis**

The thesis submitted for review for the award of the educational and scientific degree "PhD" is in total 128 pages, the main text is arranged on 97 pages, including 12 tables and 39 figures. The dissertation is structured in 8 chapters: Introduction (1 p.), Literature Review (14 p.), Material and Methods (23 p.), Results and Discussion (76 p.), Conclusions (3 p.), Contributions (1 p.) and References Cited (20 p.). The reference list contains 257 references, of which 7 are in Cyrillic. The main weight is devoted to the chapter "Results and Discussion" (51 p. including illustrations), where the results of the conducted research and the discussion of the considered issues are presented. In the chapter "Conclusions" the main achievements of the dissertation are presented in a synthesized form. The main Contributions of the work are presented as a separate section in synthesized form. This distribution is intended to meet the stated aim and the defined tasks in the development of the dissertation. The dissertation is written at the required scientific level, it is language and style-sound. The relatively few technical and grammatical errors, which are inevitable when writing such a work, make a good impression. The work is well laid out, richly illustrated with good-quality photographs, attached "on the spot" in the analysis and discussion, and not relegated to an "Appendix", as is standard in such works, which I consider a good solution, as it allows for quick and convenient reference when reading.

A declaration by the author of the originality of the results and contributions is attached to the thesis.

## **7. Contributions and Significance of the Development for Science and Practice**

Most of the results obtained are of real scientific and especially scientific contributions of applied nature. I have highlighted some of them in section 3 of the review. In the dissertation and the abstract, the PhD student highlights contributions that are in line with the objectives and results. I consider the following contributions to be of the greatest weight:

For the first time in our country detailed studies on the chemical composition of fruits and seeds of *Centaurea thracica* have been carried out. The high content of fatty acids and biologically active substances isolated from the fruits and seeds of *Centaurea thracica* determined the nutritional and biological value of the oils, and can serve as raw material for

the development of dietary supplements and pharmaceutical products for the prevention of the development of a number of chronic diseases.

Unique tissue-specific compounds with high bioactivity have been identified in *Marrubium friwaldskyanum* and *Marrubium peregrinum*, making them suitable for extraction and subsequent application in the pharmaceutical field.

*Marrubium friwaldskyanum* and *Marrubium peregrinum* have a rich metabolic composition with numerous secondary metabolites and trace elements important for human health. This phytochemical composition has proven antioxidant, antibacterial, anticancer and other bioactive effects, making both species valuable sources for biotechnological productions.

The contributions of the present research are original, some of them being post-confirmatory in nature and others representing new data for science. The areas in which Gyuzeleva works are extremely topical and promising. Proof of the importance of the research conducted with her participation are the publications in reputable journals with impact factor, as well as the numerous positive citations.

## **8. Assessment of the publications on the dissertation**

There are 4 publications related to the dissertation. In three of them Gyuzeleva is the lead author. Three of the articles were published in reputable journals with quartile ranked Q1: the first one in "Molecules" with Q1 rank (IF2021 - 4.6; SJR 0.744), the second publication in "International Journal of Molecular Sciences" (IF - 4.9; SJR 1.179); the third one in "Heliyon" Q1 rank (IF - 3.4; SJR 0.617). The fourth publication was in the journal "Ecologia Balkanica", a category with a quartile rank of Q4 (SJR- 0.202; Q4).

Although the results were published recently, they have already had an international response and have been cited in 7 publications, including journals with the prestigious Q1 ("Molecules", "Pharmaceuticals") and Q2 ("Journal of Molecular Liquids").

Thus the minimum national criteria for obtaining the PhD are met and exceeded.

## **9. Personal participation of the doctoral candidate**

I do not doubt the significant personal involvement of the PhD student in the preparation of the dissertation. Donika Gyuzeleva has built up many years of practical experience in field and camera work and competence in working with scientific research equipment. Of course, the high qualification and experience of the scientific supervisors, which

are an undoubted corrective for the quality of the research, should be acknowledged. Under the supervision of Prof. Stoyanov and Assoc. prof. Bivolarska, she successfully builds competences in research work and analytical ability to develop a scientific work.

The results and contributions in this dissertation are original, the conclusions reflect the author's contributions and have practical and scientific value. I have found *no plagiarism* or implausibility in the scientific work.

## **10. Abstract**

The abstract is correctly formatted, in a volume of 47 pages. In terms of content and quality, it fully reflects the essence, the main elements, the achieved results and contributions of the thesis.

## **11. Critical remarks and recommendations**

I have no significant comments on the thesis, apart from a few technical errors: in the spelling of the authors' taxon names when first mentioned in the text (abstract 4 pp. and 6 pp. of the thesis). The dissertation is well laid out, richly illustrated with good-quality photographs and analytical figures, which are very good, but if there were a possibility to present figures of secondary metabolites on a larger scale, they would be more user-friendly. The abbreviation SEM is referred to once as Scanning Electron Microscopy (p. 3) and further as Standard Error of Mean (p. 52) - for the latter, the abbreviation SD (Standard Deviation) is usually used.

I would like to point out that these remarks in no way diminish the scientific value and merits of the thesis.

I have the following questions for the doctoral candidate:

1. In relation to the resource assessment and reproductive potential, has the status of the populations and threatening factors of the Bulgarian endemic *Marrubium friwaldskyanum* been assessed?
2. What are the future research plans of the candidate in the new academic degree?

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

The great scientific interest and the rapid international response demonstrated by the citations of the publications related to the dissertation (7 citations have been identified in a

short period, half of them in high-quartile journals) are proof that the research is relevant and has prospects.

I recommend that in the future the results of the research in the dissertation be published as fully as possible.

## CONCLUSION

The dissertation contains scientific, scientific and applied results, which represent an original contribution to science and meet the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria (LADAPB), the Regulations for the Implementation of the LADAPB and the relevant Regulations of Paisii Hilendarski University.

The dissertation work shows that the PhD student Donika Petrova Gyuzeleva possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Botany, demonstrating qualities and skills for independent scientific research.

Because of the above, I confidently give my *positive assessment* of the conducted research, presented by the above-reviewed dissertation, abstract, results and contributions, and I propose to the honorable scientific jury to award the educational and scientific degree "PhD" to *Donika Petrova Gyuzeleva* in the field of higher education. Natural Sciences, Mathematics and Informatics; professional field 4.3. Biological sciences; doctoral programme Botany.

Reviewer:.....

7.01.2025

(signature)

Assoc. Prof. PhD Tsvetanka Raycheva