REVIEW

by Prof. Dr. Velizar Kostadinov Gochev, Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski"

on a dissertation for the award of the educational and scientific degree of "Doctor"

in: field of higher education 5. Technical Sciences,

professional field 5.11 Biotechnology,

doctoral program Technology of Biologically Active Substances

Author: *Ivanka Kostadinova Koycheva*

Topic: *Antipsoriatic activity of plant in vitro systems from Lavandula angustifolia and Harpagophytum procumbens, and their biologically active metabolites.*

Scientific supervisor: *Prof. Milen Ivanov Georgiev, PhD*

1. **General description of the materials presented**

By Order No. I-171 dated 28 October 2025 of the Director of the Stefan Angelov Institute of Microbiology at the Bulgarian Academy of Sciences (IMicB-BAS), I have been appointed as a member of the scientific jury to ensure the procedure for the defense of a dissertation thesis on the topic "Antipsoriatic Activity of Plant In Vitro Systems of *Lavandula angustifolia* and *Harpagophytum procumbens*, and Their Biologically Active Metabolites" for the acquisition of the educational and scientific degree "Doctor (PhD)" in: field of higher education 5. Technical Sciences, professional field 5.11 Biotechnology, doctoral programme Technology of Biologically Active Substances. The author of the dissertation thesis is Ivanka Kostadinova Koycheva a doctoral candidate in independent training at the "Biotechnology" Department of IMicB-BAS, with academic supervisor Prof. Dr. Milen Ivanov Georgiev from the same institute.

The set of materials submitted by Ivanka Koycheva on an electronic medium complies with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Rules on the Conditions and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at IMicB-BAS (RIMicB-BAS), and includes: a diploma of higher education for the educational degree "Master"; an application for admission to the defense of a dissertation thesis; an enrollment order; protocols and certificates of successfully passed examinations in

accordance with the individual training plan; a report on the credits obtained; a report on the fulfillment of the minimum national scientometric requirements for acquiring the educational and scientific degree "Doctor (PhD)"; protocol from a seminar of the "Biotechnology" Department from the preliminary defense; a preliminary review; the dissertation thesis; copies of scientific publications and a citation report; abstracts in Bulgarian and English; a declaration of originality; a report on plagiarism and the use of artificial intelligence; and a curriculum vitae in European format.

The type and format of the documents submitted allow me to categorically determine that the procedure meets all the requirements of national legislation and subordinate legislation concerning the Institute of Microbiology and Biotechnology at the Bulgarian Academy of Sciences.

2. Brief biographical data about the doctoral candidate

Doctoral candidate Ivanka Kostadinova Koycheva graduated from the Higher Institute of Food and Flavour Industries (now the University of Food Technologies), obtaining the educational and qualification degree "Master" with the professional qualification "engineer-technologist" in the specialty "Technology of Tobacco, Sugar, Vegetable Oils and Cosmetic Preparations." In 2003, she obtained the educational and qualification degree "Master" in the specialty "Macroeconomics," specialization "Organization and Technology of Accounting," from Paisii Hilendarski University of Plovdiv. In 2016, she began work at the "Biotechnology" Department of IMicB-BAS, successively holding the positions of "biotechnological synthesis technologist" (until 2022), and from 2022 to 2025 holding the academic position of "Assistant."

The education and career development of doctoral candidate Ivanka Koycheva unequivocally demonstrate her desire and ability to continuously broaden and further develop the range of her knowledge and professional skills.

3. Relevance of the topic and appropriateness of the stated aims and objectives

According to data from the World Health Organization, psoriasis is a chronic, non-communicable inflammatory disease affecting mainly the skin and joints, which correlates with an increased risk of developing other conditions such as psoriatic arthritis, obesity, hyperlipidemia, diabetes mellitus, sleep apnea, certain autoimmune, cardiovascular, hepatic and psychiatric diseases, squamous cell carcinoma, lymphoma, etc. The pathogenesis of psoriasis is not fully elucidated, but its onset and progression result from the interaction of a wide range of genetic, immunological and environmental factors. The approved medicines used have insufficient efficacy and a number of side effects, which makes the search for new and effective agents particularly relevant. A number of plant-derived biologically active substances with antipsoriatic activity are the subject of intensive research, but the molecular mechanisms of their action remain unclear.

All of this gives me grounds to unequivocally define the topic of the dissertation as relevant and timely.

4. Knowledge of the problem

In assessing the extent to which the problem is known, I base my evaluation on the scope, comprehensiveness, and analytical character of the *Literature Review* section. It is presented on 44 pages and is based on the use of more than 250 up-to-date literature sources. Comprehensive information on the pathophysiology of psoriasis is provided, and a number of biologically active substances such as flavones, glycosides, alkaloids, etc. that have been investigated for antipsoriatic activity are listed. The doctoral candidate has described various experimental models of psoriasis, indicating the advantages and disadvantages of the different models and the type of information that can be obtained from them. The review places emphasis on metabolomics based on NMR spectroscopy, which is natural given its use in the dissertation research. Concise information related to plant in vitro systems and their possibilities for the biosynthesis of biologically active substances is presented analytically, as well as a botanical, phytochemical, and biological characterization of the species *Lavandula angustifolia* and *Harpagophytum procumbens*. The review concludes with an informative summary, which enables the doctoral candidate to justify the need to conduct the present study. My only remark regarding the review is its length, which definitely requires reduction and some restructuring.

The scope of the review, the manner in which the information is presented, and the final summary unequivocally show that doctoral candidate Ivanka Koycheva is familiar with the problem under development in depth and to a degree that allows her to conduct high-quality scientific research.

5. Research methodology

The experimental scheme applied in the dissertation includes all main stages of a biotechnological study: cultivation; isolation of biologically active substances; characterization of the obtained extracts (metabolite profiling); selection of an appropriate model for in vitro investigation of selected extracts and isolated individual biologically active substances; in vivo tests of selected substances and their incorporation into a final bioproduct with antipsoriatic activity. The experimental scheme is logically correct. A wide range of modern molecular biological, immunological, and instrumental methods have been used, which is a prerequisite for conducting high-quality scientific research at a contemporary level.

The broad range of newly acquired and successfully adapted methods shows that the doctoral candidate has successfully fulfilled the educational component of the PhD degree as well.

6. Characterization and evaluation of the dissertation thesis

The dissertation thesis is structured in the conventional manner and includes the following sections: Introduction (1 page); Literature Review (44 pages); Aim and Objectives (1 page); Materials and Methods (12 pages); Results and Discussion (38 pages); Conclusion (1 page); Conclusions/Findings (1 page); Contributions (1 page); and References. Optimal proportions between the volume of the individual sections of the dissertation have been observed. The evaluation of the literature review was presented in item 4. The aim of the dissertation thesis is formulated clearly and precisely, and in order to achieve it, six research objectives have been set, the successful implementation of which allows the aim of the study to be attained. In the Materials and Methods section, the materials used and the methods of analysis are described correctly and in detail, which would allow the reproducibility of the results. The manner of their presentation shows that the doctoral candidate has mastered them and has successfully applied them in the course of the experimental work. The obtained results are presented in 21 figures, an appendix with 8 figures, and data from calculated binding affinities between individual pure components (leukoseptoside and verbascoside) and the proteins AKT, PI3K, pSTAT1 and JAK2. The experimental work begins with a chemical analysis of the composition and an in vitro evaluation of the antipsoriatic activity of an extract of L. angustifolia and pure rosmarinic acid in a psoriasis model in human HaCaT keratinocytes. It was established that the L. angustifolia extract reduces psoriatic inflammation in human keratinocytes by interfering with JAK2/STAT1 signaling, and that its effectiveness is due to its content of rosmarinic acid. Following a similar scheme, studies were also conducted with an extract of H. procumbens, verbascoside, and leukoseptoside. It was found that the H. procumbens extract and the phenylethanoid compounds verbascoside and leukoseptoside affect the JAK2/STAT1 signaling pathway in psoriatic keratinocytes, and that at the protein level verbascoside and leukoseptoside can interfere with PI3K/AKT signaling. The dissertation work continues with validation of an imiquimod-induced psoriasiform dermatitis animal model in mice and in vivo determination of the antipsoriatic potential of rosmarinic acid. The in vivo anti-inflammatory effect of rosmarinic acid was also investigated. A formulation of a cream with rosmarinic acid incorporated into its composition is proposed. The analysis of the obtained data shows that, among the investigated pure monocomponent substances, rosmarinic acid is the compound with the most significant anti-inflammatory and, accordingly, antipsoriatic effects in the conducted in vitro experiments. The proposed mechanism of action of rosmarinic acid is associated with inhibition of inflammatory signaling pathways. The Results and Discussion section concludes with a summary of the obtained results, clearly highlights the limitations of the study, and outlines a future direction for work, namely the conduct of clinical studies to confirm the safety, optimal dosage, and long-term efficacy of rosmarinic acid for the

treatment of psoriasis in humans. Based on the obtained results, seven conclusions have been formulated, which correctly emphasize the main established relationships, although conclusions 1 and 2 could be reformulated so as not to sound declarative.

7. Contributions and significance of the work for science and practice

A total of seven contributions have been formulated, two of which are scientific-fundamental and five are scientific-applied. I accept the wording and categorization of the contributions proposed by the doctoral candidate and unequivocally define them as original.

8. Assessment of the publications related to the dissertation thesis

In connection with the dissertation, five publications have been submitted, and in three of them doctoral candidate Ivanka Koycheva is the lead author, which highlights her substantial contribution to their completion. All materials have been published in Q1 and Q2 journals indexed in the Scopus and Web of Science databases. The total impact factor (IF) of the publications is 39.1, and an impressive 132 citations of the publications in Scopus have been recorded a fact that is rarely encountered in the defense of a dissertation for the acquisition of the educational and scientific degree “Doctor (PhD).”

9. Personal contribution of the doctoral candidate

In three of the five scientific publications presented in relation to the dissertation, the doctoral candidate is the lead author, which unequivocally proves her active participation and a high share of personal contribution in the preparation of the articles, and the conducting of the experiments and the preparation of the dissertation thesis are undoubtedly primarily her personal achievement. In no way do I downplay the support of the academic supervisor, the team of the “Metabolomics” laboratory, and the “Immunology” Department, who have undoubtedly provided valuable support to doctoral candidate Ivanka Koycheva.

10. Dissertation abstract

The dissertation abstract has been prepared in accordance with the accepted requirements and reflects all the more significant results of the dissertation thesis.

11. Critical remarks, recommendations, and questions

I have no critical remarks regarding the dissertation in substance. I consider the presented results and the formulated conclusions and contributions to be correct. The only point I would place in the category of “critical remarks” is my recommendation to strengthen the “biotechnological component” of the dissertation research. The dissertation would definitely sound more biotechno-

logical if it included experiments on cultivation, optimization of process parameters, or conditions for the isolation of biologically active substances.

I have the following question for the doctoral candidate: Can you define more specifically the concept of “antipsoriatic activity” in the context of your study, focusing on the different levels of organization at which you conduct the experiments?

12. Personal impressions

I have known doctoral candidate Ivanka Kostadinova Koycheva both professionally and personally for almost 20 years, which unequivocally allows me to form objective impressions of her qualities. I consider her an ambitious, determined, hardworking, and precise researcher. She combines these qualities with exceptional correctness and integrity, which have become something of a rarity in contemporary society.

CONCLUSION

The dissertation thesis of Ivanka Kostadinova Koycheva contains scientific and applied results that constitute an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Rules on the Conditions and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at IMicB-BAS (RIMicB-BAS). The dissertation thesis demonstrates that the doctoral candidate possesses in-depth theoretical knowledge and professional skills in the scientific specialty 5.11 Biotechnology, and demonstrates qualities and skills for independently conducting scientific research.

In view of the above, I confidently give my positive assessment of the conducted research presented in the dissertation thesis, the dissertation abstract, the achieved results, and the contributions reviewed above, and I propose that the esteemed scientific jury award the educational and scientific degree “Doctor (PhD)” to Ivanka Kostadinova Koycheva in the field of higher education 5. Technical Sciences, professional field 5.11 Biotechnology, doctoral programme Technology of Biologically Active Substances.

17.12. 2025. Reviewer: .....

(Prof. PhD Velizar Gochev)