REVIEW

by Assoc. Prof. Petya Asenova Dimitrova, Laboratory "Experimental Immunotherapy" at the Stephan Angeloff Institute of Microbiology, BAS

appointed as a member of the Scientific Jury with Order I-171/28.10.2025 issued by the Director of the Institute of Microbiology, BAS and as a reviewer by the Scientific Jury with Protocol №№12/28.10.2025

for evaluation of the thesis for academic and scientific degree "Doctor"(PhD)

PhD student: Eng. Ivanka Kostadinova Koycheva
PhD thesis title: ANTIPSORIATIC ACTIVITY OF PLANT IN VITRO SYSTEMS FROM *LAVANDULA ANGUSTIFOLIA* AND *HARPAGOPHYTUM PROCUMBENS*, AND THEIR BIOLOGICALLY ACTIVE METABOLITES
Scientific supervisor: Prof. Dr. Milen I. Georgiev
Research field: 5.11. Biotechnology (Technology of biologically active molecules)

1. General description of the presented materials

The author of the PhD thesis, Ivanka Koycheva, is a PhD student at the Metabolomics Laboratory of the Department of Biotechnology, Institute of Microbiology, BAS, supervised by Prof. Milen Georgiev. She has submitted her dissertation work, in paper and in electronic version, and all relevant documents, according to the requirements of the Regulations for the Development of the Academic Staff of IMikB, BAS, namely: application for admission to thesis defense, copy of completed master's degree, certificate for PhD enrollment, records of exams and courses in the specialty, summary for credits, summary of scores according to the minimum requirements of the Bulgarian law and the additional requirements of IMikB, BAS, protocol and preliminary review of pre-defense, dissertation thesis and dissertation summary in Bulgarian and English, list of publications and citations, declaration of originality and curriculum vitae.

2. Significance of the research topic of the PhD thesis

Psoriasis is a chronic inflammatory autoimmune skin disease that has a strong genetic predisposition. Its incidence is about 2%, but varies in different regions, being lower among Asian and some African populations and reaching 11% among Caucasian and in particular Scandinavian populations. Clinical symptoms are associated with the presence of skin lesions - the so-called psoriatic plaques and with chronic inflammation affecting various tissues and organs, and causing systemic autoimmune conditions. Dermatological manifestations are classified into subgroups, with different phenotype, and are most common among patients with Psoriasis vulgaris. As a result of chronic inflammation, the most common concomitant condition in psoriasis is the psoriatic arthritis, which develops in up to 40% of patients with psoriasis. Psoriatic patients often have hyperlipidaemia, hypertension, concomitant coronary artery disease, type 2 diabetes and an increased body mass index. Several large cohort studies have shown that higher incidence of diabetes and cardiovascular disease (including heart attack) correlate with the severity of psoriasis. Particular attention is currently being focused on childhood psoriasis, due to its increasing incidence and difficulties in diagnosis.

The therapy of the skin manifestations of psoriasis mainly includes vitamin D, retinoids, corticosteroids and biological therapy with antibodies targeting two key cytokines in pathogenesis, IL-17 and IL-23. The goal of psoriasis treatment is to prolong periods of remission, however the long-term therapy has adverse side effects. Therefore, there is still a need for new therapeutic agents to be applied alone and in combination with conventional therapies. In this aspect, Ivanka Koycheva's dissertation work is particularly relevant. The use of biologically active plant metabolites may prove to be a relatively inexpensive but effective therapeutic approach, especially in mild forms of psoriasis and especially in cases requiring long-term corticosteroids administration.

3. Relevance of the PhD thesis goal and tasks

Ivanka Koycheva's dissertation has one goal to investigate the pharmacological potential of extracts derived from in vitro cultivated plants *L. angustifolia* and *H. procumbens*, as well as biologically active substances isolated from the extracts - respectively rosmarinic acid (RA), phenylethanoid glycoside verbascoside (VER) and the structurally similar to VER leukoskeptoside A (LEU). The dissertation uses two model systems - an in vitro model mimicking the inflammatory environment of psoriasis in human keratinocytes and a model of psoriasis induced by topical application of Imiquimod in mice.

Six tasks have been set to achieve the goal and are clearly formulated following the logic when conducting some initial steps in the drug discovery pipeline - namely, the task of obtaining and analysing the content of metabolites in the extract, adapting the in vitro model of inflammation in keratinocytes, treatment with extracts and pure compounds of keratinocytes cultured in inflammatory conditions and of mice with skin inflammation.

4. Level of the author’s competence

Ivanka Koycheva demonstrates excellent theoretical knowledge in the field shown in the competent and concise literature review. The review covers a description of the pathological mechanisms in psoriasis, including changes in keratinocyte function in psoriasis, the role of IL-17 and IL-23, major signalling pathways associated with chronic inflammation, experimental models of psoriasis, modern approaches to psoriasis therapy, ethnopharmacological approach to developing alternative approaches to influence the skin manifestations of psoriasis. Included are a description of biotechnological approaches to obtain valuable biologically active secondary plant metabolites and general information about plants, phytochemical composition and biological activity. Figures (3 figures) have illustrated the mechanisms associated with the pathogenesis of psoriasis, therapeutic approaches and key steps in performing metabolomic analysis. The review shows that the PhD student has an excellent knowledge of the literature and even made a very smooth transition with a summary of current research and a systematization of potential problems that have not been resolved in research up to date.

5. Research methodology

Ivanka Koycheva used a diverse methodologic approaches, such as cultivation and extraction of cell suspensions from *L. angustifolia* and *H. procumbens*, phytochemical analysis of extracts, use of metabolic NMR spectroscopy profiling, chromatographic analysis by high-performance liquid chromatography, in silico docking analysis, cell cultivation of HaCaT cells in the presence of a combination of recombinant cytokines IFN-γ/IL-17A/IL-22, RT-qPCR for a panel of psoriasis-related genes, immunoblot analysis to identify psoriasis-related signaling molecules - AKT, JAK2, PI3K, STAT1 and p-STAT1 and an in

vivo psoriasis model including assessment of psoriasis severity, histological score evaluation of psoriatic skin lesions and analysis of proliferation of splenic T cells isolated from mice with psoriasis.

The methods used are very well described with the inclusion of controls demonstrating precise implementation, as well as good experimental design and careful implementation all having input into the research integrity. The statistical methods are adequate and guarantee the repeatability of the data obtained.

6. Characterization and evaluation of the dissertation work

The dissertation has a structure according to the requirements of the Regulations for the Development of the Academic Staff of the Institute of Microbiology and Biochemistry, Bulgarian Academy of Sciences. It contains the following chapters:

- Title
- Abbreviations used
- Introduction
- Literature review - 50 pages
- Goals and objectives - 1 page, with 6 tasks
- Materials and methods - 11 pages
- Results and discussion - 38 pages, 21 figures and an appendix with 3 figures and data from calculated binding affinities between individual pure compounds with the proteins: AKT; PI3K; pSTAT1 and JAK2.
- Conclusion - 1 page
- Conclusions - 1 page
- Contributions - 1 page - divided into fundamental and scientific-applied nature contributions
- Literature sources - with over 150 sources, over 40% of which in the period 2021-2025.

7. Contributions and research significance

Fundamental contributions to the research field

- The anti-psoriatic activity of an extract of *L. angustifolia*, and of pure substances rosmarinic acid and leucoseptoside A has been demonstrated for the first time.
- Key molecular signalling pathways affected by the action of *L. angustifolia*, rosmarinic acid and leucoseptoside A in HaCaT cells have been identified.

Applied contributions to the research field

- An in vitro model of psoriasis has been optimized, and made applicable for screening the anti-psoriatic potential of plant extracts and natural compounds.
- The in vivo model of psoriasis is suitable for preclinical evaluation of the anti-psoriatic potential of metabolites isolated from *L. angustifolia*, which can serve for application in new medicinal or cosmetic formulations for dermatological application.
- A mechanism of action including L. angustifolia, rosmarinic acid, and leukoseptoside A has been proposed for the first time; this mechanism may be applied to various inflammatory skin conditions.
- Rosmarinic acid has been shown to have potential as an active ingredient for topical psoriasis treatment for the first time.

It is especially appealing to observe that the research's findings have plenty of practical application potential and highlight the value of the dissertation. Additionally, it is noteworthy that modern techniques have been employed to demonstrate the mechanisms of action.

8. Evaluation of publications on the dissertation work and research score of the author

The dissertation ends with an impressive 5 publications, in 3 of which Ivanka Koycheva is the first author. All publications are in Q1 and Q2, and in all of them, the impact factor is above 4. Particularly impressive is the participation of the doctoral student in a review article on rosmarinic acid with an impact factor of 16. In this aspect, the criteria of the Institute of Microbiology for publication activity are **excellently met by Ivanka Koycheva** and show the significance of her study.

9. Critical remarks and recommendations

Question: Can rosmarinic acid be used in combination with other therapies for skin diseases?

CONCLUSION

The dissertation contains fundamental and scientific-applied results that represent **an original contribution to the research field and meet all the requirements** of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of the ZRASRB and the Regulations for the Implementation of the ZRASRB of the Bulgarian Academy of Sciences. The presented dissertation results fully comply with the specific requirements of the Rules of the IMikB for the application of ZRASRB.

Ivanka Koycheva's dissertation work demonstrates skills and characteristics **required for the independent conduct of scientific research**, proving that she possesses in-depth theoretical knowledge and professional skills.

Due to the aforementioned, **I can confidently state my positive assessment** of the research that was done and **I strongly recommend to award** Ivanka Koycheva the educational and scientific degree of "Doctor" in the field of 5.11 Biotechnologies (Technology of biologically active substances).

07/12/2025 Reviewer: .....

Assoc. Prof. Petya Dimitrova