OPINION

by Assoc. Prof. Zhenya Petkova Yordanova, PhD
Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Plant Physiology

member of the Scientific Jury appointed by Order No. I-171/28.10.2025
of the Director of the Institute of Microbiology "Stefan Angelov"

regarding the dissertation thesis submitted for the acquisition of the educational and scientific degree "Doctor" Professional Field 5.11 Biotechnology
(Technology of Biologically Active Substances)

REGARDING:

The dissertation thesis entitled "Antipsoriatic activity of plant *in vitro* systems of *Lavandula angustifolia* and *Harpagophytum procumbens* andtheir biologically active metabolites"

Author of the dissertation thesis: Ivanka Kostadinova Koicheva, independent PhD student, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

Scientific supervisor: Prof. Milen Ivanov Georgiev, PhD, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

1. General overview of the procedure

The materials submitted for the defense of the dissertation thesis by Eng. Ivanka Kostadinova Koicheva - an independent doctoral student in the Metabolomics Laboratory, Department of Biotechnology at the Institute of Microbiology "Stefan Angelov", BAS have been prepared in full accordance with the requirements of the Bulgarian Law on the Development of the Academic Staff (ZRASRB), the Regulations for the Conditions and Procedures for the Acquisition of Academic Degrees, as well as the Regulations of the Bulgarian Academy of Sciences for the application of ZRASRB. All criteria necessary for awarding the educational and scientific degree "Doctor" have been fulfilled.

2. General characteristics of the dissertation and evaluation of the achieved results

The dissertation of Eng. Ivanka Koicheva is dedicated to investigating the antipsoriatic potential of extracts obtained from in vitro plant cultures of *Lavandula angustifolia* and *Harpagophytum procumbens*, as well as the individual biologically active metabolites isolated from them - rosmarinic acid (RA), verbascoside (VER), and leucosceptoside A (LEU) in an in vitro model of psoriasis in human keratinocytes, and the topical application of RA in an IMQ-induced psoriasis model in mice. The work has been conducted within the Metabolomics Laboratory, Department of Biotechnology, Institute of Microbiology "Stefan Angelov", BAS, and is structured in accordance with good international practices for comprehensive biotechnological and pharmacological research.

The dissertation is extensive, clearly structured, and well illustrated, comprising 138 pages, accompanied by 34 figures, 6 tables, and 268 cited references (a substantial portion published between 2021 - 2025).

Psoriasis is a chronic immune-mediated disease of increasing social and medical relevance. Its prevalence (2-3% of the population), the presence of severe cutaneous and systemic manifestations, and the limitations of current therapeutic approaches necessitate the search for new, safer, and more accessible treatment strategies. Conventional medications, although effective, are often associated with adverse effects, whereas biologic therapies are costly and inaccessible to many patients. In this context, the development of natural anti-inflammatory agents and sustainable biotechnological platforms for their production holds significant scientific and practical value. Plant in vitro cultures enable standardized production of metabolites with consistent qualitative profiles - an advantage emphasized by the author both in the theoretical framework and through the obtained experimental findings.

The topic is highly relevant, interdisciplinary, and holds clear applied potential for the development of phytotherapeutic products.

The dissertation represents a substantial advancement of existing scientific knowledge in the field of biotechnological production and pharmacological evaluation of anti-inflammatory plant metabolites, successfully integrating biotechnological, phytochemical, molecular, and in vivo approaches. Unlike most available literature, which is predominantly based on extracts from natural plant materials, the author employs cell suspension cultures of *L. angustifolia* and *H. procumbens* as a sustainable and standardized biotechnological source of secondary metabolites with anti-inflammatory potential. The conducted NMR-based metabolite profiling and validated HPLC analyses reveal the presence and quantitative predominance of key metabolites - rosmarinic acid (RA), verbascoside (VER), and leucosceptoside A (LEU) providing a solid foundation for subsequent biological investigations and contributing valuable data to the metabolomics characterization of both species.

The results clearly demonstrate that the extract from *L. angustifolia* and its major metabolite, RA, are the most potent anti-inflammatory agents in the in vitro psoriasis model. Both significantly reduce the expression of characteristic pro-inflammatory cytokines and chemoattractant molecules (IL-6, CCL2, CCL20) and suppress the activity of key signaling pathways involved in psoriasis pathogenesis, including NF-κB, PI3K/AKT, MAPK, and JAK2/STAT1. Particularly noteworthy is the action of RA, which inhibits JAK2/STAT1 signaling at both the transcriptional and protein levels, and in certain parameters approximates or even surpasses the effect of the reference corticosteroid dexamethasone. In contrast, the individual metabolites from *H. procumbens* exhibit differing levels of activity: LEU shows moderate anti-inflammatory effects, while VER demonstrates limited or inconsistent activity and does not reach the biological significance observed for RA or the *L. angustifolia* extract. This highlights that antipsoriatic potential is specific to certain molecules rather than a universal property of all isolated compounds. These findings provide new insights into the molecular mechanisms of action of plant phenolic compounds and expand current understanding of their role in modulating inflammatory processes in keratinocytes.

The contribution of the dissertation to the in vivo model system is particularly valuable, demonstrating a clear therapeutic effect of a topically applied cream formulation (emulsion) containing RA in an IMQ-induced psoriasiform dermatitis model in mice. The author reports substantial reduction of clinical disease parameters (PASI score), decreased erythema, desquamation, and epidermal thickening, as well as normalization of enlarged lymphoid organs indicative of systemic inflammation. Histological evaluation confirms these findings through visible reduction of acanthosis, parakeratosis, and inflammatory infiltration. The development and stabilization of an O/W emulsion containing various concentrations of RA, tested according to national standards, represents an additional applied scientific contribution with realistic prospects for future implementation in dermatological products.

Overall, the dissertation provides consistent and well-reasoned evidence that biotechnologically produced plant extracts and their active metabolites possess significant antipsoriatic potential, supported by clearly defined molecular mechanisms of action. The combination of in vitro and in vivo data, together with the development of an applicable formulation, underscores both the scientific value and the practical relevance of the research.

3. Contributions of the dissertation

The dissertation contains clearly defined scientific and applied-scientific contributions, grounded in a consistent biotechnological, molecular, and in vivo research approach.

Scientific contributions

- The antipsoriatic potential of biotechnologically produced extracts from *L. angustifolia* and *H. procumbens* has been demonstrated in a model of stimulated human keratinocytes.
- The mechanisms of action have been elucidated, including modulation of NF-κB, PI3K/AKT, MAPK, and particularly JAK2/STAT1, with rosmarinic acid exhibiting the strongest activity.
- Differences in the efficacy of the individual metabolites have been established, with RA outperforming LEU and VER, which is relevant for refining future therapeutic development.
- It has been shown that RA and the *L. angustifolia* extract approach or even surpass the effects of dexamethasone in certain parameters - an important finding for assessing their pharmacological potential.

Applied-scientific contributions

- A stable O/W emulsion containing RA has been developed, demonstrating therapeutic efficacy in an IMQ-induced in vivo model of psoriasiform dermatitis.
- A biotechnological platform has been established for the production of standardized extracts with consistent chemical profiles (NMR, HPLC).

Methodological contributions

- A comprehensive model for evaluating anti-inflammatory agents has been developed, integrating metabolite profiling, gene and protein expression analysis, and in vivo validation.
- An in vitro system for inducing a psoriasiform phenotype in HaCaT cells has been optimized.

4. Publications related to the dissertation

On the topic of the dissertation, the doctoral candidate has published five scientific articles in peer-reviewed and indexed journals, three of which are in Q1 journals and two in Q2 journals, with a total impact factor of 39.1. Eng. Koicheva is the first author of three of these publications, demonstrating her leading role in the planning, execution, and interpretation of the research. The publications reflect the major components of the dissertation and present the results in a logical sequence with strong internal consistency.

The significance and visibility of these studies are further supported by the total of 168 citations in Web of Science (excluding self-citations), which is a strong indicator of international interest in the topic and the author's scientific contribution.

The publication activity associated with the dissertation not only meets but substantially exceeds the minimum requirements for the conferral of the educational and scientific degree "Doctor" and is fully consistent with the scope and quality of the conducted research and the scientific field.

5. Abstract (Author's summary)

The abstract is prepared in accordance with the established requirements and accurately reflects the main results presented in the dissertation.

6. Critical remarks and recommendations

I have no substantial critical remarks regarding the dissertation submitted for review, apart from several minor technical errors observed in certain sections.

7. Question to the doctoral candidate

What, in your opinion, are the reasons for rosmarinic acid exhibiting stronger anti-inflammatory activity compared to leucosceptoside A and verbascoside? Which features of their chemical structure or cellular mechanisms of action would you consider responsible for these differences?

Conclusion

The dissertation of Eng. Ivanka Koicheva addresses a relevant and scientifically significant topic. The work fully complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for its implementation, as well as the corresponding regulations of the Bulgarian Academy of Sciences. The author demonstrates a high level of scientific preparation and skillfully applies contemporary biotechnological, phytochemical, molecular, and in vivo methods. The obtained results are original, convincingly substantiated, and elucidate both the molecular mechanisms of action of the investigated metabolites and their potential for future application in dermatological products. I evaluate the dissertation as substantial, well executed, and characterized by clearly defined scientific and applied-scientific contributions.

In view of the above, I confidently give my positive assessment and recommend that the esteemed scientific jury duly acknowledge the merits of the presented work and vote in favor of awarding the educational and scientific degree "Doctor" in Professional Field 5.11 Biotechnology (Technology of Biologically Active Substances) to Ivanka Koicheva.

18.12.2025 г.
Sofia

Prepared by:
(Assoc. Prof. Zhenya Yordanova, PhD)