O P I N I O N

BY PROF. DR. MILKA MILCHEVA MILEVA

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Regarding the dissertation on the topic: "Antipsoriatic activity of plant in vitro systems from *Lavandula angustifoli*a and *Harpagophytum procumbens*, and their biologically active metabolites"

for the acquisition of the educational and scientific degree "doctor"

Scientific direction: 5.11. Biotechnology (Technology of biologically active substances)

PhD student: Eng. Ivanka Kostadinova Koycheva

Scientific supervisor: Prof. Dr. Milen Ivanov Georgiev

This review was prepared in accordance with Order No. I-171/28.10.2025 of the Director of the Institute of Microbiology "Stefan Angelov" - BAS (IMikB) by decision of the Scientific Council of IMikB, Protocol No. 12 of 28.10.2025, according to which I am included in the scientific jury.

The dissertation work, a set of materials and documents for the defense procedure presented by Eng. Ivanka Kostadinova Koycheva, fully comply with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADRB), the Regulations for its implementation and the relevant Regulations of the Institute of Microbiology, Bulgarian Academy of Sciences.
I do not know the candidate, I have no public publications, and I am not in a conflict of interest, according to the law.

EVALUATION OF THE SUBMITTED MATERIALS IN THE COMPETITION

The dissertation work of Eng. Ivanka Koycheva is written on 138 pages and is structured within the framework of generally accepted standards. The bibliography includes over 200 literary sources. The results are illustrated with 34 figures and 6 tables. The abstract is 53 pages long, includes 23 figures and 5 tables and fully reflects the main results of the dissertation work.

I. RELEVANCE AND FUNDAMENTAL IMPORTANCE

The dissertation is dedicated to an extremely topical and fundamentally important issue, positioned at the border between plant biotechnology and molecular pharmacology. The research addresses the acute clinical need for new, effective and targeted therapeutic strategies for the treatment of psoriasis - a chronic inflammatory disease. The integration of sustainable biotechnological production of secondary metabolites

with precise pharmacological screening is an innovative approach that gives the work high scientific-fundamental and applied value.

II. OBJECTIVES AND METHODOLOGY

The goal is clearly defined: to perform a comprehensive evaluation of the antipsoriatic activity of extracts from in vitro systems of *L. angustifolia* and *H. procumbens* and their biologically active metabolites, by applying a clinically relevant in vitro model of psoriasis (stimulated human keratinocytes).

The tasks set are fully adequate to achieve the goal. The methodology used is complex and multidisciplinary, including:

- ✓ **Phytochemical analysis:** Identification and quantification of metabolites by NMR spectroscopy and HPLC-MS;
- ✓ **Molecular-pharmacological screening:** Application of modern techniques such as RT-qPCR, immunoblot analysis (Western blotting) and immunofluorescence on a keratinocyte model (HaCaT) to clarify signaling pathways.
- ✓ ***In vivo* validation:** Testing of active compounds in an animal model of psoriasiform dermatitis.
- ✓

III. MAIN SCIENTIFIC CONTRIBUTIONS

The dissertation contains significant scientific and applied scientific contributions, which can be systematized as follows:

- ➢ **FUNDAMENTAL CONTRIBUTIONS:**
  - ✓ **Evidence for targeting the JAK/STAT transcriptional pathway:** For the first time, the inhibitory effect of rosmarinic acid and leukoseptoside A (LEU) on key elements of the inflammatory signaling pathway JAK2/STAT1 under conditions of psoriatic stimulation has been demonstrated and visually confirmed (by immunoblot and RT-qPCR). This result is of utmost importance, as it positions the natural compounds in the group of potential JAK inhibitors.
  - ✓ **Clarification of multi-purpose action:** It has been found that the anti-inflammatory effect is also due to modulation of the PI3K/AKT signaling pathway, which explains the antiproliferative and anti-inflammatory potential of the studied extracts.
  - ✓ **Efficiency *in vivo*:** Successful validation of the anti-inflammatory activity of rosmarinic acid in vivo has been performed, supporting preclinical data regarding the compound's potential for topical therapy.

- **APPLICATION CONTRIBUTIONS:**
  - ✓ **Development of a biotechnology protocol:** *In vitro* suspension cultures have been optimized as a standardized and sustainable source for induced production of high concentrations of active antipsoriatic metabolites (e.g. dry extract rosmarinic acid).
  - ✓ **Creation of a screening platform:** A modern screening platform for assessing antipsoriatic activity has been implemented and routinely applied, which can serve as a reference test for new natural products.

The dissertation is a large-scale and in-depth scientific study that demonstrates a high level of academic competence and research autonomy on the part of the doctoral student. The results are excellently illustrated, presented logically, correctly interpreted and argued against the literature data. The work contains significant original scientific achievements and provides a solid foundation for future developments of innovative phytopharmaceutical products with precisely targeted action.

OVERALL ASSESSMENT OF THE CANDIDATE'S COMPLIANCE WITH THE MANDATORY REQUIREMENTS OF THE ZASRB

The results of this dissertation have been summarized and published for the period 2021 - 2025 in 5 scientific articles in prestigious scientific journals with a total impact factor of 39.1, they have been cited 180 times by December 15, 2025. The total number of citations of Eng. Koycheva is 243, H index 8 (Scopus).

Based on the above, I believe that the dissertation work of Eng. Ivanka Koycheva fully meets and exceeds the requirements of the Law on the Protection of Biologically Active Substances, the Regulations for its implementation and the relevant Regulations of the Institute of Biotechnology - Bulgarian Academy of Sciences. I give my positive assessment and I confidently propose to the esteemed Scientific Jury to award Eng. Ivanka Kostadinova Koycheva the educational and scientific degree "Doctor" in the professional field 5.11. Biotechnology (Technology of biologically active substances).

15.12.2025 г. Prepared the opinion:
Sofia / Prof. Milka Mileva /