EXPERT OPINION

by **Assoc. Prof. Dr. Dilyana Petrova Nikolova**

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Member of the Scientific Jury, according to Order No. I-171/28.10.2025 of the Director of the Institute of Microbiology "Stephan Angeloff" – BAS
On a dissertation for the award of educational and scientific degree "Doctor"
in the field 5.11. Biotechnology (Technology of biologically active substances)

Author of the dissertation: **Ivanka Kostadinova Koycheva**, doctoral student at the Institute of Microbiology "Stephan Angeloff" – BAS

Thesis 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**, Institute of Microbiology "Stephan Angeloff" – BAS

REGARDING THE PROCEDURE:

The author of the dissertation is Eng. Ivanka Kostadinova Koycheva – PhD student at the Metabolomics Laboratory, Department of Biotechnology, Institute of Microbiology "Stephan Angeloff" – BAS.

The candidate's documents complie with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, its implementation regulations and Regulations of BAS, and the criteria for obtaining the educational and scientific degree of "Doctor" have been met.

The PhD student has attached five publications to her dissertation, all of which are referenced and indexed scientific journals in global databases, three of which are in quartile Q1 and two in Q2, with a total IF of 39.1. According to internal institutional requirements, Eng. Koycheva is the first author of three of the five scientific publications presented. According to Web of Science databases, there are 168 citations of the publications attached to the dissertation, excluding self-citations. Eng. Koycheva has participated in four national projects and one international project. The results of the research work in the dissertation have been presented at four international scientific forums.

EVALUATION OF THE DISSERTATION:

Relevance and significance of the scientific topic.

The main topic of the dissertation is focused on the study of extracts from medicinal plants and natural compounds with anti-inflammatory activity, which can be used to modulate the pathways of inflammation in keratinocytes and can be included as an effective therapeutic approach for the treatment of a socially significant diseases such as psoriasis. Modern "omics" approaches were applied to study extracts from *in vitro* systems of *Lavandula angustifolia* Mill. and *Harpagophytum procumbens* (Burch.) DC. ex Meisn., which are plant species with established anti-inflammatory activity. Such approaches have also been applied to study their biologically active metabolites in an

in vitro model of psoriasis in human keratinocytes and to investigate the molecular mechanisms of their action. On this basis, the topic of the dissertation is particularly relevant, as there is sustained research and applied interest in both the development of sustainable methods for obtaining valuable biologically active metabolites of plant origin and the selection and construction of appropriate model systems for demonstrating their activities.

The candidate's literary awareness and theoretical preparedness.

The literature review is structured in a total of five sections and includes information on the following aspects:

- A detailed review of psoriasis is provided, describing keratinocyte damage, the role of interleukins in the pathogenesis of the disease, molecular signalling pathways for manifestation, and anti-angiogenic mechanisms in psoriasis. Information is also presented on the use of certain biologically active metabolites of plant origin for the treatment of psoriasis.
- Information is included on *in vitro* and *in vivo* experimental models for studying psoriasis, as well as on the significance and application of various therapeutic approaches.
- The main stages and methods used in metabolomic analysis and profiling of biologically active metabolites of plant origin are discussed;
- Biotechnological approaches for obtaining biologically active metabolites of plant origin are presented;
- Specific information is included on studies of the phytochemical composition and biological activities of the two plant species, *L. angustifolia* and *H. procumbens*, which are the subject of the research work in the presented dissertation.

The information presented in the literature review is organised logically and consistently, comprising a total of 44 pages and illustrated with three complex figures. A total of 268 references are used for all sections of the dissertation, with those from the last 10 years predominating.

Compliance of the chosen research methodology with the objectives and tasks of the dissertation

The defined specific objective in the dissertation is to investigate the pharmacological potential of extracts from *in vitro* cultures of *L. angustifolia* and *H. procumbens*, as well as their biologically active metabolites in an *in vitro* model of psoriasis in human keratinocytes, as well as the effect of topical application of rosmarinic acid in an IMQ-induced model of psoriasis in mice.

To achieve the set objective, six tasks have been clearly and logically formulated:

1. Preparation and metabolic profiling of extracts from *in vitro* systems of *L. angustifolia* and *H. procumbens* using nuclear magnetic resonance (NMR).

2. Isolation of a biologically active compound from extracts H. procumbens extracts *in vitro* systems.

3. Adaptation of an *in vitro* model of psoriatic inflammation by combined cytokine stimulation of human epidermal keratinocyte cell line (HaCaT).

4. Research of anti-inflammatory activity and molecular mechanism of action of extracts

from cell suspensions of *L. angustifolia*, *H. procumbens* and their individual pure compounds in an *in vitro* model of psoriasis in human keratinocytes.

5. Adaptation of an imiquimod-induced model of psoriasis in mice (C57BL6).

6. Study of the antipsoriatic potential of rosmarinic acid at the organismal level using an *in vivo* model of psoriasis.

The selected and applied research methods are described in detail in the "Materials and Methods" section. These methods are modern and appropriately selected, and they are implemented precisely in accordance with the set objective and tasks. The main methods used include suspension culture cultivation and extraction, NMR metabolic profiling and chromatographic analyses. Other methods include the use of cell cultures as an in vitro model of psoriasis and treatment with the studied extracts and their active compounds, RT-qPCR, immunoblot analyses, creating an induced *in vivo* psoriasis model, assessing severity, analyzing mouse T cell proliferation, and evaluating psoriatic skin lesions histologically. Adequate statistical analysis methods were employed.

Assessment of the results achieved and contributions of the dissertation.

In order to accomplish the tasks set out in the dissertation, a generalised experimental approach was formulated to determine the mechanisms of inflammation modulation in psoriasis through the application of plant extracts and individual pure compounds.

All research tasks have been consistently implemented. The main results are related to:

- Establishing the phytochemical profile of extracts from cell suspensions of *L. angustifolia* and *H. procumbens* and quantitatively determining the content of key secondary metabolites - rosmarinic acid, verbascoside and leucoseptozide A in the studied plant extracts;

- Adaptation and application of an *in vitro* model of psoriasis in human epidermal keratinocytes and an *in vivo* model of psoriasiform dermatitis in mice;

- A reduction in psoriatic inflammation in human keratinocytes has been demonstrated upon treatment with biotechnologically produced extract from *L. angustifolia* and rosmarinic acid.

- An improvement in psoriasis-induced inflammation has been established through the suppression of signalling pathways in HaCaT cells when treated with Leucoseptoside A, isolated from an extract of *H. procumbens* cell suspension.

- The effect of topical application of rosmarinic acid in alleviating the skin symptoms of imiquimod-induced psoriatic inflammation at the organism level has been proven.

Seven specific conclusions have been formulated based on the obtained results. The formulated contributions demonstrate the importance and indisputable relevance of the scientific results and dissertation in both scientific and applied contexts.

ABSTRACT

The abstract of decertation fully corresponds to the dissertation and reflects the results achieved. It also meets the requirements of the Regulations for the Implementation of the Law on the Development of Academic Staff in the Republic of Bulgaria.

CONCLUSION

The presented dissertation is a comprehensive, original, and in-depth scientific study of theoretical and practical value. The results are convincing and well-reasoned, supported by experimental data. The dissertation meets the requirements for the educational and scientific degree of "Doctor."

Based on the above, I conclude that the presented dissertation meets all the criteria for the educational and scientific degree of "Doctor" in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria, its implementing regulations, and the regulations of the Institute of Microbiology "Stephan Angeloff" – BAS. I am confident in giving my positive assessment and propose that the distinguished members of the scientific jury award the educational and scientific degree of "Doctor" to Ivanka Kostadinova Koycheva in the field 5.11. Biotechnology (Technology of biologically active substances).

Date: 16 December 2025 (Assoc. Prof. Dilyana Nikolova, PhD)