To the Chairman of the Scientific Jury, determined by Order № I-105/ 29.07.2025 of the Director of the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences

I hereby submit: Opinion statement

on dissertation defense procedure

of Blagovesta Dimitrova Todorova – a PhD student

in the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences

on the topic "Anti-inflammatory effect of *Crocus sativus* extract and *astaxanthin* in a mouse model of collagenase-induced osteoarthritis"

Field of higher education: 4. Natural sciences, mathematics and informatics

Professional field: 4.3. Biological Sciences, Doctoral Program: Immunology

Scientific advisors: Prof. Dr Andrey Ivanov Chorbanov and

Assoc. Prof. Dr Nikolina Mihaylova Mihaylova

Member of scientific jury: Prof. Dr. Sevdalina Nikolova Lambova, MD, PhD

Field of higher education: 7. Health and Sports

Professional field: 7.1. Medicine

Scientific specialty: Rheumatology

Institution: Medical University – Plovdiv, Department of Propaedeutics of Internal Medicine, "Prof. Dr. Anton Mitov"

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OPINION STATEMENT

by Prof. Dr. Sevdalina Nikolova Lambova, MD, PhD
Department of Propaedeutics of Internal Medicine "Prof. Dr. Anton Mitov", Faculty of Medicine, Medical University - Plovdiv

External member of the scientific jury appointed by Order No. I-105/ 29.07.2025 of the Director of Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences

Regarding: dissertation defense procedure
of Blagovesta Dimitrova Todorova - full-time PhD student
in the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences
on the topic "Anti-inflammatory effect of *Crocus sativus* extract and *astaxanthin* in a mouse model of collagenase-induced osteoarthritis"
for awarding educational and scientific degree "Doctor of Philosophy"

Field of higher education: 4. Natural sciences, mathematics and informatics
Professional field: 4.3. Biological Sciences, Doctoral Programme: Immunology
Scientific advisors: Prof. Dr Andrey Ivanov Chorbanov and
Assoc. Prof. Dr Nikolina Mihaylova Mihaylova

The opinion is prepared in accordance with the requirements of the Act for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Conditions and Procedure for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences.

I. Description of the materials submitted under the procedure

In connection with the current procedure for the defense of a dissertation on the topic "Anti-inflammatory effect of *Crocus sativus* extract and *astaxanthin* in a mouse model of collagenase-induced osteoarthritis", the PhD student Blagovesta Dimitrova Todorova has submitted all necessary documents and evidence in accordance with the requirements of the Act for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Conditions and Procedure for Acquiring Academic

Degrees and Holding Academic Positions at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences.

II. Presentation of the PhD student

Blagovesta Dimitrova Todorova was born in 1994. She completed her secondary education at the St. Kliment Ohridski Secondary School - Montana. For the period 2018 - 2020, she studied at the Sofia University "St. Kliment Ohridski", Sofia and obtained a master's degree in biochemistry. Since 2021, she is a PhD student in immunology at the Bulgarian Academy of Sciences. For the purposes of her work in the field of experimental immunology, Blagovesta Todorova has completed training on the topic "Protection and humane treatment of experimental animals used for scientific and educational purposes" in 2023, as well as a course on the topic "Theoretical and practical foundations of classical and modern histological methods" (2022).

III. Evaluation of the dissertation

The dissertation is 142 pages long, including the literature review and references with 243 sources. The topic of the dissertation "Anti-inflammatory effect of *Crocus sativus* extract and *astaxanthin* in a mouse model of collagenase-induced osteoarthritis" is topic of current interest with high scientific and practical significance. The aim of the dissertation is to analyze the therapeutic effect of *Crocus sativus* extract and *astaxanthin* administered orally in an experimental mouse model of collagenase-induced osteoarthritis, by monitoring immunological parameters, tissue damage and the effect on symptoms during the disease course.

Despite the significant progress in the knowledge about the pathogenesis of osteoarthritis, there is currently no established disease-modifying therapy for the disease. The difficulties in establishing disease-modifying treatment in osteoarthritis in the contemporary rheumatology are associated with the clinical heterogeneity of the disease, damage of avascular structure that is articular cartilage, lack of established criteria for early diagnosis. In this regard, the role of scientific research related to experimental animal models of osteoarthritis is essential, in which it is possible to analyze the effects of potential disease-modifying therapeutic interventions in a controlled environment.

The literature review is well-structured and demonstrates good knowledge of the topic regarding the pathogenesis of osteoarthritis, as well as experimental animal models of the disease. Based on the review, goals and objectives have been formulated and hypotheses have been suggested for the potential pathogenetic effect of *Crocus sativus* extract and *astaxanthin*

in an experimental mouse model of osteoarthritis. An experimental animal model of osteoarthritis in mice, induced after intra-articular injection of collagenase, was used. Standardized methods were used, including measurement of cytokine levels in serum by ELISA, flow cytometry, MTT test, histological analysis, and statistical methods.The own results are presented in details and an analytical discussion is conducted, which analyzes the own results with those in the world literature. The dissertation is illustrated with 70 figures, of which 57 are original and included in the "Results" section.

At the end of the dissertation, conclusions are formulated regarding the established effects of the applied *Crocus sativus* extract and astaxanthin in an experimental mouse model of osteoarthritis. It was concluded that the *Crocus sativus* extract has a pathogenetic effect by reducing the bone and cartilage destruction that was confirmed by histological analysis of joint structures of treated mice with induced osteoarthritis. Regarding the application of *astaxanthin* in experimental animals, it was concluded that it stimulates the processes of joint remodelling. I accept the results, the discussion and the conclusions made by the PhD student in the course of the scientific research.

IV. Evaluation of the résumé of the dissertation

The presented résumé of the dissertation work of Blagovesta Dimitrova Todorova summarizes the methodology, main results and conclusions of the dissertation work. The abstract is well structured and illustrated.

V. Publications on the topic of the dissertation and overall assessment of the PhD student

In connection with the dissertation, two publications on the topic have been published in a journal with an impact factor (Life/mdpi, IF – 3.2), which fulfills the mandatory requirements for the defense of the educational and scientific degree "Doctor of Philosophy" according to the Regulations for the Conditions and Procedure for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences. In relation to the presentation of the results of the dissertation, the PhD student has participated with scientific reports in 9 scientific forums. A reference is presented for 13 citations of the two publications included in the current procedure.

VI. Conclusion

In conclusion, Blagovesta Todorova has presented a scientific paper with original results in the field of experimental immunology, which are also of scientific and practical interest in the field of clinical medicine. Blagovesta Todorova meets the minimum national requirements for the award of the educational and scientific degree "Doctor of Philosophy ", as well as the specific requirements according to the Regulations for the Conditions and Procedure for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Microbiology "Stefan Angelov". Based on the above, I give my positive vote for awarding the educational and scientific degree "Doctor of Philosophy" in the field of "Biological Sciences", in the scientific specialty "Immunology" to Blagovesta Dimitrova Todorova.

11.09.2025
Plovdiv

Prof. Dr. S. Lambova, MD