REVIEW

By

Prof. Milena Georgieva, PhD

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Member of the Scientific Jury according to Order No. I-63/28.04.2026

on

Dissertation thesis entitled:

"Modulating lifespan-extending mechanisms in *Caenorhabditis elegans* through the use of biologically active compounds", for the acquisition of the educational and scientific degree "DOCTOR", by **Monika Nikolaeva Todorova**, Bulgarian Academy of Sciences, Institute of Microbiology "Stefan Angelov", Department of Biotechnology, Laboratory of Metabolomics

Field of higher education: 5. Technical Sciences
Professional field: 5.11. Biotechnology
Doctoral program: Technology of biologically active substances
Scientific supervisor: Prof. Dr. Milen I. Georgiev

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1. General description of the submitted materials

By Order No. I-63/28.04.2026 of the Director of the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences, I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation entitled "Modulating lifespan-extending mechanisms in Caenorhabditis elegans through the use of biologically active compounds" for the acquisition of the educational and scientific degree "DOCTOR" in the field of higher education 5. Technical Sciences, professional field 5.11. Biotechnology, Doctoral Program "Technology of biologically active substances".

The submitted documentation package has been prepared in accordance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria and its implementing regulations. It includes the dissertation thesis, an abstract in Bulgarian and

English, scientific publications, documents confirming the fulfillment of the individual study plan, a scientific output report, declarations of originality, and all other materials required for the procedure. The documentation is complete, properly prepared, and allows for an objective evaluation of the dissertation and the PhD candidate's scientific achievements.

2. Brief biographical data of the PhD candidate

Monika Nikolaeva Todorova is a young researcher with well-defined scientific interests in biotechnology, phytochemistry, and aging biology. In 2018, she obtained a Master's degree in Molecular Biology and Biotechnology from Paisii Hilendarski University of Plovdiv. Since 2022, she has been a PhD candidate at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences, Laboratory of Metabolomics, within the doctoral program "Technology of biologically active substances," under the supervision of Prof. Dr. Milen I. Georgiev.

During her doctoral training, she has developed in-depth theoretical knowledge and practical skills in the molecular biology of aging, using the model organism *Caenorhabditis elegans* to investigate mechanisms regulating lifespan and healthspan. Her professional development is consistently and closely linked to scientific research activities. In the period 2021–2024, she worked as a molecular biologist and biotechnologist in the PlantaSYST project at the Bulgarian Academy of Sciences, and since 2024, she has held the academic position of "Assistant" at the Institute of Microbiology – BAS.

Her overall scientific output includes 13 publications in international peer-reviewed journals indexed in reputable scientific databases, with a total impact factor of 78.8 and an h-index of 4. She has participated as a researcher in six national and international scientific projects, demonstrating active engagement in research and the ability to work in interdisciplinary and international scientific environments. Her membership in the Bulgarian Phytochemical Society and the European Phytochemical Society further confirms her commitment to the scientific community.

The presented scientific results, publication activity, and project participation outline the profile of a well-prepared young researcher with clearly defined scientific interests, strong experimental competence, and consistent academic development. The accumulated experience and achieved results provide grounds for a highly positive evaluation of her research activity and indicate that she possesses the necessary qualities and potential for successful future development as an independent scientist.

3. Relevance of the topic and knowledge of the scientific problem

Aging is one of the most significant biological processes determining quality of life and the development of a range of socially important diseases. Modern gerontology focuses not only on lifespan extension but also on improving healthspan by targeting key molecular mechanisms responsible for cellular homeostasis, stress adaptation, and metabolic balance.

In this context, the PhD candidate Monika Todorova demonstrates in-depth knowledge of the principal regulatory processes underlying aging in eukaryotic organisms, including insulin/IGF signaling, cellular stress response mechanisms, autophagy, oxidative stress, and hormetic

adaptive responses. She shows a clear understanding of these processes as an interconnected regulatory network governing cellular survival and longevity.

Particularly noteworthy is her ability to integrate concepts and methodologies from multiple scientific disciplines such as molecular biology, biotechnology, phytochemistry, pharmacology, and gerontology, and to apply them within a unified conceptual framework for the analysis of biologically active compounds with potential geroprotective properties. This interdisciplinary approach reflects a high level of scientific competence, analytical thinking, and a deep understanding of the research problem.

4. Methodology of the study

The experimental approach is modern, well-structured, and fully consistent with the stated objectives. The study includes a range of plant-derived natural products, including the prenylated flavonoid icariin, a major secondary metabolite of *Epimedium brevicornum* Maxim. leaf and juice extracts from *Punica granatum* L., as well as a combined product containing a standardized mixture of adaptogenic plants *Rhodiola rosea*, *Schisandra chinensis*, and *Eleutherococcus senticosus*.

The research focuses on a detailed analysis of their biological activity, evaluated using modern genetic and physiological approaches in the model system *C. elegans*. Experiments include lifespan assays, resistance to oxidative and thermal stress, assessment of physiological markers of healthy aging, and studies on mutant *Caenorhabditis elegans* strains. This approach enables the investigation of signaling pathways such as DAF-2/DAF-16, HSF-1, SKN-1, and HLH-30.

The selected methods are appropriately applied and yield reliable, statistically robust results.

5. Characterization and evaluation of the dissertation

The dissertation of Monika Todorova is developed within the current and rapidly advancing field of aging biology and gerontology, which in recent years has established itself as one of the most promising areas in modern biomedical research. The study is dedicated to evaluating the potential of natural products and biologically active compounds to modulate aging and longevity processes through the use of the well-established model organism *Caenorhabditis elegans*.

The dissertation follows the classical academic structure and contains all necessary sections ensuring logical coherence and clarity of presentation. It comprises 163 pages. It is illustrated with 31 figures and 12 tables. A total of 271 literature sources have been cited.

The presented background demonstrates a deep understanding of current knowledge in the field. It covers molecular mechanisms of aging, the concept of healthy longevity, the role of evolutionarily conserved signaling pathways, and the use of *C. elegans* as a model for geroprotective interventions. The candidate shows strong ability to synthesize large volumes of information critically and to clearly identify unresolved scientific questions and the need for new approaches to modulate aging.

The research aim is clearly defined and logically developed through eight specific objectives that cover both methodological development and the comprehensive analysis of physiological,

metabolic, and molecular aging markers. Some tasks are of methodological importance, including the implementation of approaches for nuclear translocation tracking of transcription factors, analysis of mitochondrial dynamics, and development of a mitochondrial dysfunction model in *C. elegans*.

The methodological approach is highly complex and interdisciplinary. It includes phenotypic analyses, lifespan assays, oxidative and thermal stress models, locomotion and reproductive capacity assays, fluorescence and confocal microscopy, subcellular protein localization tracking, RT-qPCR gene expression analysis, immunoblotting, lipid and triglyceride quantification, and NMR-based metabolomic profiling. All methods are appropriately selected and yield robust, statistically valid results.

Particular value lies in integrating molecular, physiological, and metabolic parameters into a unified experimental framework, enabling a more comprehensive interpretation of the observed effects.

The first experimental part investigates icariin, demonstrating its ability to extend lifespan and enhance stress resistance in *C. elegans* without adverse effects on viability, neuro-sensory function, or reproduction. The data strongly support the involvement of the DAF-2/DAF-16 axis and HSF-1 in mediating these effects, indicating a hormetic mechanism of action.

The second part focuses on *Punica granatum* leaf extract. NMR-based metabolomic profiling contributes to product standardization and characterization. The extract improves stress resistance, extends lifespan, and positively affects lipid homeostasis and mitochondrial integrity. The involvement of DAF-16/FOXO, SKN-1/NRF2, and HLH-30/TFEB is convincingly demonstrated.

The third part examines *Punica granatum* juice as a functional food, showing improvements in locomotor activity and health-related aging markers, although lifespan extension is more modest.

The adaptogenic product ADAPT-232 is also investigated. A mitochondrial dysfunction model induced by a high-carbohydrate diet is developed and validated. ADAPT-232 improves mitochondrial function, restores lipid homeostasis, and positively affects aging-related parameters, offering translational research perspectives.

The scientific discussion is well-structured and demonstrates a critical interpretation of the results in the context of the current literature and regulatory mechanisms of aging.

6. Contributions and significance of the work

The dissertation contains original scientific results of both fundamental and applied significance.

A major contribution is the establishment of *Caenorhabditis elegans* as an experimental platform for functional screening of natural products with potential geroprotective effects.

The study demonstrates the hormetic mechanism of icariin and the involvement of DAF-2/DAF-16 and HSF-1 pathways in lifespan regulation and stress resistance.

A key finding is the identification of HLH-30/TFEB as a central regulator of *Punica granatum* leaf extract activity, along with coordinated involvement of HLH-30, SKN-1, and DAF-16.

The results related to ADAPT-232 and the mitochondrial dysfunction model represent an important methodological contribution with broad applicability.

Overall, the work provides a strong foundation for future preclinical and translational studies targeting metabolic health and healthy aging.

7. Evaluation of publications

The dissertation results are published in three Q1 journals: Food Frontiers, International Journal of Molecular Sciences, and Phytomedicine. These are high-impact international journals with strong visibility. The total impact factor exceeds 20, and 17 citations are currently registered. These indicators exceed the national requirements for the doctoral degree.

8. Personal contribution of the PhD candidate

The candidate is the first author in all publications related to the dissertation, indicating substantial personal contribution. She has actively participated in all stages of the research process, including experimental design, execution, data analysis, interpretation, publication preparation, and dissertation writing. The results presented are to a significant extent the product of her independent scientific work and initiative.

9. Dissertation synopsis

The dissertation synopsis is prepared in accordance with the requirements and accurately reflects the dissertation's structure, content, results, conclusions, and contributions.

10. Critical remarks, recommendations, and questions

No substantial critical remarks are noted.

The only recommendation is to expand future work toward translational validation using mammalian cell models or additional functional assays in higher organisms.

Two questions are addressed to the candidate for academic discussion:

1. Based on your results involving DAF-16/FOXO, SKN-1/NRF2, HLH-30/TFEB, and HSF-1, do you consider the existence of a common coordinating regulatory mechanism integrating the effects of natural products on aging, and which transcription factor appears central in this network?

2. Considering the evolutionary conservation of the signaling pathways studied, which would you identify as the most promising molecular target for future geroprotective interventions in mammals and humans, and why?

CONCLUSION

The dissertation contains original scientific results and contributions of fundamental and applied significance in biotechnology and aging biology. The study is highly relevant, methodologically sound, and demonstrates a high level of scientific competence.

The candidate possesses strong theoretical knowledge and practical skills in the professional field of 5.11 Biotechnology and demonstrates the ability to conduct independent research.

On this basis, I give my EXCELLENT evaluation of the dissertation and recommend to the Scientific Jury that Monika Nikolaeva Todorova be awarded the educational and scientific degree "DOCTOR" in the field of higher education 5. Technical Sciences, professional field 5.11 Biotechnology, Doctoral Program "Technology of biologically active substances".

I wish the candidate continued academic success and inspiration in her future scientific career.

Date: 06.06.2026

Sofia, Bulgaria

Reviewer:

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/Prof. Milena Georgieva, PhD/