Review

by Assoc. Prof. Dr. Nikolina Mihaylova Mihaylova,

Laboratory of Immunohistochemistry and Immunopathology, Department of Immunology,

Institute of Microbiology, Bulgarian Academy of Sciences

Selected as a member of the Scientific Jury by Order No. I-63/28.04.2026 of the Director of the Institute of Microbiology

on a PhD dissertation for the award of an educational and scientific degree "Doctor"

Author: Monika Nikolaeva Todorova

Topic: Modulation of longevity-associated mechanisms in Caenorhabditis elegans through biologically active compounds

Academic Supervisor: Prof. Milen Georgiev

Field of Higher Education: 5. Technical Sciences, **Direction:** 5.11 "Biotechnology," **Doctoral Program**: "Technology of Biologically Active Substances"

1. General description of the submitted materials

The author of the dissertation is Monika Nikolaeva Todorova - a full-time doctoral student at the Laboratory "Metabolomics" of the Department of Biotechnology, Institute of Microbiology, BAS, with scientific supervisor Prof. Dr. Milen Georgiev

The set of materials presented for consideration on paper and in electronic form is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB and the Regulations of BAS for the Implementation of LDASRB and meets the criteria of the Regulations on the Conditions and Procedure for Acquiring Scientific Degrees and Academic Positions at the Institute of Microbiology "Stefan Angelov" at BAS for obtaining the Educational and Scientific Degree "Doctor".

2. Brief biographical data

Monica Nikolaeva Todorova was born on May 4, 1994, in the city of Burgas. In 2018, Monika graduated from Plovdiv University "Paisiy Hilendarski" with a Master's degree in Molecular Biology and Biotechnology. In 2021, she was appointed to the position of Molecular Biologist and Biotechnologist in the Laboratory "Metabolomics" of the Department of Biotechnology at the Institute of Microbiology under project No. 739582 PlantaSYST, Plovdiv. In 2022, she was enrolled as a full-time doctoral student in the field 5.11. Biotechnology, Technology of

Biologically Active Substances at the Institute of Microbiology, BAS, Laboratory of Metabolomics.

3. Relevance and significance of the topic in the dissertation

The dissertation addresses one of the most significant demographic and biomedical problems of modern society - the progressive aging of the population and the fact that the duration of life in good health (healthspan) does not increase proportionally to the total duration of life (lifespan). Global life expectancy reached 73.3 years in 2024, an increase of 8.4 years since 1995. The number of people aged 60 years and over worldwide is projected to increase from 1.1 billion in 2023 to 1.4 billion by 2030. This trend is particularly evident and rapid in developing regions. This demographic shift has significant implications for public health. Developing strategies for lifelong health maintenance and disease prevention can significantly reduce the risk of developing noncommunicable and chronic diseases, including heart disease, stroke and cancer, as well as functional impairment. As global life expectancy increases, the incidence of age-related diseases is also increasing, making understanding the molecular mechanisms of aging critical for the development of new therapeutic strategies. The study of aging processes and the factors that influence them is a central issue in molecular biology, gerontology and biomedicine.

The model system used for the preparation of the dissertation is a widely established model in the study of aging due to its short life cycle, well-characterized genetics and a high degree of conservation of key signaling pathways, oxidative stress and autophagy. A particularly relevant aspect of the dissertation is the focus on natural products of plant origin that could modulate cellular and molecular processes associated with aging. This approach is in line with modern trends in pharmacology and nutrigenomics, which are aimed at identifying substances with the potential to extend healthy life, and not only the overall lifespan. The practical significance of the study is expressed in the potential for translation of the obtained results to higher organisms, including humans. Many of the discoveries made in Caenorhabditis elegans have served as the basis for further research in the field of age-related diseases such as neurodegenerative, metabolic and oncological conditions.

The dissertation submitted to me for review examines a topic that is not only scientifically sound and modern, but also has a high degree of interdisciplinarity and applicability, which makes it very relevant and significant in the contemporary context of biomedical and biotechnological research.

4. Relevance of the set goals and objectives

The goal of this work is to evaluate the geroprotective potential of selected natural products on key physiological and molecular markers of aging. To achieve the set goal, 8 tasks have been formulated. A figure is presented that summarizes the experimental strategy used in this work, which makes an extremely good impression and provides clarity about the level of knowledge of the problem and maturity of the scientific approach.

5. Knowledge of the problem

The literature review is very well structured with a logical sequence of the presentation. The impression is of a very good knowledge of the current state of the problems studied by the doctoral student. The literature review is divided into five subsections, each of which examines different aspects of the work. 1 table and 10 duly cited color images are included. Over 270 literary sources have been used in the literature review and discussion, of which more than 70% are from the last 5-6 years.

6. Research methodology

The methodology used in the development of the dissertation is clearly defined and justified. Standardized and validated methods were used - biotechnological methods for obtaining plant extracts, nuclear magnetic resonance for phytochemical analysis; approaches for growing and monitoring the phenotype of roundworms, methods for analyzing gene expression and for monitoring resistance to oxidative and thermal stress and changes in lipid metabolism, etc. The methods are described in detail and allow reproduction. The depth of the molecular studies is impressive. The work is not limited to monitoring the phenotypic survival of the model system. By using mutant strains (e.g. *daf-2, sir-2.1, aak-2*) and GFP-tagged transcription factors (*HLH-30, DAF-16, SKN-1*), specific signaling pathways have been localized and proven.

7. Characteristics and evaluation of the dissertation

The dissertation follows the structure outlined in the Regulations for the Development of the Academic Staff at the Institute of Microbiology, BAS, including the following chapters:

- Title
- Table of Contents
- Abbreviations and symbols used
- Introduction – 2 pages
- Literature review – 55 pages including 5 subsections examining Aging; Longevity; Caenorhabditis elegans as a model system; Approaches to delay aging and extend life; Natural products and biologically active substances for longevity; The review is illustrated with 1 table and 10 duly cited color images.
- Aim and objectives – 2 pages presenting a well-formulated aim with 8 tasks and visualization of the experimental strategy in the dissertation work.
- Materials and methods – 10 pages, with a description of the chemicals and reagents used, pure substances, antibodies and kits; description of the strains of the experimental model used; detailed description of methods used: Preparation of P. Granatum leaf extract; Extraction of P. Granatum fruit juice; Nuclear magnetic resonance (NMR) spectroscopy; Cultivation of C. elegans and treatment; Phenotypic parameters – locomotor activity; Phenotypic parameters – reproductive capacity; Morphological parameters; Vitality assessment; Life span monitoring; Chemotaxis assay; Stress models – heat and oxidative

stress; Lipid accumulation staining; Colorimetric quantification of triglycerides; Study of mitochondrial dynamics; Expression and cellular localization in transgenic strains; Confocal microscopy and data analysis; Real-time polymerase chain reaction (RT-qPCR) of mRNA; Immunoblot analysis to determine protein expression; Statistical analysis;

- Results – 37 pages, 10 tables and 18 complex figures containing graphs and photos of very good quality.
- Discussion – including 15 pages of synthesized and summarized information from the results obtained in the dissertation work, as well as two author's figures presenting molecular mechanisms of action.
- Conclusions – 1 page, 6 in number fully corresponding to the results obtained and in accordance with the set goals and objectives
- Contributions – 1 page – contributions of a scientific-fundamental nature and contributions of a scientific-applied nature are presented.
- Bibliography – over 270 literary sources are used.

8. Contributions and significance of the work for science and practice

A total of 8 contributions have been formulated, divided into Contributions of a scientific-fundamental nature (5 in number) and Contributions of a scientific-applied nature (3 in number).

Contributions with scientific and fundamental character:

1. The effectiveness of *C. elegans* as an integrative model system for investigating natural products with the potential to modulate ageing and longevity at the organismal level has been demonstrated.
2. The hormetic nature and mechanism of action of icariin have been established, involving modulation of insulin/insulin-like signaling and hsf-1.
3. For the first time, the role of the transcription factor HLH-30 as a key mediator of the geroprotective effects of pomegranate leaf extract has been identified, acting in coordination with SKN-1 and DAF-16.
4. The effects of pomegranate juice on ageing-related physiological parameters in *C. elegans* have been characterized.
5. The potential of ADAPT-232 to modulate ageing processes through regulation of mitochondrial function and metabolic homeostasis has been demonstrated.

Scientific and applied contributions:

1. An integrated experimental platform for the functional evaluation of natural products with potential effects on ageing and longevity in C. elegans has been developed and implemented.
2. The obtained data on the geroprotective effects of ADAPT-232 provide a basis for its potential repositioning from a product used for stress and physical performance management to an intervention with possible applications in maintaining metabolic balance and mitochondrial health.
3. A model of mitochondrial dysfunction based on the induction of metabolic stress through high-carbohydrate exposure in C. elegans has been adapted and validated, enabling its use for the evaluation of metabolically targeted interventions.

9. Evaluation of the Publications Related to the Dissertation

The topic of the dissertation has three articles published in refereed international scientific journals (all in Q1), with a total IF of 20.1. In all of the mentioned publications, Monika Todorova is the first author. Scopus finds 17 citations excluding self-citations. During her dissertation, Monika Todorova presented the results of her dissertation at 5 international scientific forums through 3 oral reports and 4 poster presentations.

10. Summary of the dissertation

The summary of Monika Todorova's dissertation reflects the main results achieved in the dissertation and meets all generally accepted requirements in the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria (RALDASRB) for its preparation.

11. Critical remarks and recommendations

I have no substantive remarks. All comments and suggestions made during the preliminary defense are reflected in the final version of the dissertation.

I have the following questions for the doctoral student:

1. Is there data on varietal diversity in terms of the phytochemical profile and the content of biologically active substances in the leaves/fruit of pomegranate? If such differences exist, how would they be reflected in a possible standardization of a product for human use?

2. The product ADAPT-232 is a commercially available product for humans. Based on your data on its protective effect against mitochondrial dysfunction induced by high glucose in C. elegans, what would be the theoretical equivalent doses for humans and do you see potential for its application as an adjuvant therapy in metabolic syndrome or type 2 diabetes mellitus?

CONCLUSION

The dissertation ***contains scientific and applied scientific results that represent an original contribution to science*** **and meet all the requirements** of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB and the Regulations for the Implementation of LDASRB of the Bulgarian Academy of Sciences. The development has high potential for subsequent applied research in the field of preventive medicine, the development of new nutritional supplements and strategies for slowing down age-related degenerative processes in higher organisms and humans.

The presented dissertation work categorically **proves** that Monika Nikolaeva Todorova **possesses** in-depth theoretical knowledge and practical skills for independent planning and conducting scientific research.

Based on the above, ***I confidently give my positive assessment*** of the conducted research, and ***I propose to the esteemed jury to award Monika Nikolaeva Todorova the educational and scientific degree "Doctor" in the field 5.11 Biotechnology***.

June 07, 2026

Reviewer:

[Assoc. Prof. Dr. Nikolina Mihaylova]