O P I N I O N

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Regarding the dissertation on the topic: "Modulation of lifespan extension mechanisms in Caenorhabditis elegans using biologically active substances" for the acquisition of the educational and scientific degree "doctor"

Scientific direction: 5.11. Biotechnology, Doctoral program Technology of biologically active substances

PhD student: Monika Nikolaeva Todorova

Scientific supervisor: Prof. Dr. Milen Ivanov Georgiev

This review was prepared in accordance with Order No. I-63/28.04.2026 of the Director of the Institute of Microbiology "Stefan Angelov" - BAS (IMikB) by decision of the Scientific, 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 Monika Nikolaeva Todorova, 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 Monika Nikolaeva Todorova is written on 136 pages and is structured within the framework of generally accepted standards. The bibliography includes over 270 literary sources. The results are illustrated with 30 figures and 12 tables. The abstract is 60 pages long, and fully reflects the main results of the dissertation work.

I. RELEVANCE AND FUNDAMENTAL IMPORTANCE

The topic of Monika Nikolaeva Todorova's dissertation is extremely relevant for several fundamental reasons, which the author justifies in detail in the chapter "Introduction" - progressive aging of the population, dissonance between life expectancy and health, a new paradigm in gerontology - a shift from the concept of "treatment of individual diseases" to modulation of the aging process itself, the potential of natural products to promote healthy longevity.

II. OBJECTIVES AND METHODOLOGY

The goal is clearly defined: to evaluate the geroprotective potential of selected natural products on key physiological and molecular markers of aging. The research is based on the development and validation of

an integrated preclinical experimental platform for assessing longevity, based on the model organism *C. elegans*, characterized by a high degree of genetic conservatism compared to humans and providing an opportunity to track the biological effects of the application of natural products. The illustration of the experimental strategy used in the dissertation work is presented through a detailed flowchart (Figure 11, p. 66), which traces the logical and chronological course of the study - from the selection of the products to the deciphering of the molecular mechanisms. This is evidence that the work goes through strict scientific filtration - from a basic assessment of safety and phenotypic changes, through stress adaptation, to precise transcriptional and subcellular mapping of the anti-aging effect.

The tasks set are fully adequate to achieve the goal. The methodology of the dissertation is impeccable, balanced and fully compliant with world standards. It is designed to filter the results at three levels: organic (phytochemistry), phenotypic (WormLab, vitality, stress) and molecular (RT-qPCR, confocal microscopy, mutant strains). This gives exceptional weight, credibility and high value to the scientific contributions made.

III. MAIN SCIENTIFIC CONTRIBUTIONS

The results are structured in four main directions, corresponding to the four studied natural products: Effect of icariin on the life cycle and stress; Metabolic profiling and effects of pomegranate leaf extract (PGL); Potential of pomegranate fruit juice (PGJ) as a functional food with a pronounced geroprotective effect; Effects of the adaptogenic complex ADAPT-232 as a modulator of the transcription factors of longevity by correcting disorders in lipid metabolism and cellular bioenergetics, restoring damaged mitochondrial integrity caused by metabolic stress.

This dissertation closes several pages with outdated understandings - about aging as "inevitable mechanical wear", and puts an end to the myth that plant polyphenols and flavonoids help the body simply by swimming in the cytoplasm and purely chemically capturing and neutralizing free radicals (direct "chemical cleaning").

This work opens a whole new page in which phytonutrients are viewed as intelligent signaling molecules capable of reprogramming the software of the cell, aging is viewed as a dynamic biological process subject to precise biotechnological modulation and control, and natural products are viewed as genetic switches. Bioactive substances act as ligands that press specific molecular buttons in cell signaling.

IV. APPLICATION CONTRIBUTIONS:

Monika Todorova's dissertation successfully builds an innovative, digitized preclinical platform, based on the model organism *C. elegans*, for quantitative and visual assessment of healthy longevity. In depth, the

study deciphers the precise molecular cascades of action of icariin and pomegranate leaf extract, proving that they reprogram the cellular software through evolutionarily conservative metabolic sensors and transcription factors. The scientific and applied scientific contributions open a completely new page in biotechnology, turning inedible plant waste and adaptogens into scientifically proven, mitochondrial-protective agents against aging and metabolic stress. 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. The scientific approach, experimental design and research philosophy of the dissertation bear all the distinctive features, methodological style and high standards imposed by the modern research school of the scientific supervisor Prof. Milen Georgiev.

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

The results of this dissertation were summarized and published in 2024 in 3 scientific articles in prestigious scientific journals in the quartile Q1, with a total impact factor of 20.1, they were cited 17 times. The total number of citations of doctoral student Monika Todorova is 23, H index 4 (Scopus).

Based on the above, I believe that the dissertation work of doctoral student Monika Nikolaeva Todorova 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 - BAS. I give my positive assessment and confidently propose to the esteemed Scientific Jury to award Monika Nikolaeva Todorova the educational and scientific degree "Doctor" in the professional field 5.11. Biotechnology, Doctoral program "Technology of Biologically Active Substances"

01.06.2026
Sofia

Prepared the opinion:
/ Prof. Milka Mileva /