OPINION

on a thesis for the acquisition of
educational and scientific degree "doctor"

Author of the doctoral thesis: **Monika Nikolaeva Todorova**
Dissertation topic: **Modulation of lifespan extension mechanisms in *Caenorhabditis elegans* using biologically active compounds**
Member of the scientific jury: **Petko Nedyalkov Denev, PhD, professor at Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences**

1. **Relevance of the studied in the thesis problem in scientific, applied scientific and applied terms**

Population aging is one of the most significant social, medical and economic challenges facing modern society. Therefore, investigating the molecular mechanisms of aging and the factors determining longevity is of considerable scientific importance. Contemporary theories of aging describe this process as a result of complex genetic, epigenetic, and metabolic alterations associated with oxidative stress, mitochondrial dysfunction, chronic inflammation, and impaired cellular homeostasis. In this context, increasing attention is being paid to the distinction between chronological and biological age, with a major focus on strategies aimed at delaying biological aging and extending healthspan. Particular interest has been directed toward the identification of natural compounds and dietary factors capable of modulating the molecular pathways involved in aging and longevity. The development of effective strategies for healthy aging includes both lifestyle and nutritional interventions, as well as biological and pharmacological approaches targeting key cellular signaling pathways. In this regard, the model organism *Caenorhabditis elegans* represents a highly valuable experimental system due to its short lifespan, well-characterized genome, and the high evolutionary conservation of fundamental mechanisms related to aging and stress response. *C. elegans* enables rapid and reliable evaluation of the potential of natural products to extend lifespan and improve physiological functions during aging. Therefore, research in this field has not only fundamental scientific significance but also substantial potential for the development of novel approaches for the prevention of age-related diseases and improvement of quality of life.

2. Knowledge about the state of the problem and the literature

The literature review is structured into several major thematic sections that consistently present current scientific knowledge related to aging, longevity and the potential of natural products to modulate these processes. The first part discusses the main theories of aging, epidemiological aspects and the key

molecular hallmarks characterizing age-related changes in the organism. The following section is devoted to longevity, addressing the differences between biological and chronological age, the factors influencing lifespan, and the principal molecular mechanisms associated with the maintenance of cellular homeostasis and stress resistance. Special attention is given to *C. elegans* as an established model for aging research and for evaluating the potential of natural products to extend lifespan. Its anatomical and physiological characteristics, age-related changes, and advantages as an experimental system are thoroughly discussed. A separate section analyzes contemporary approaches for delaying aging and extending lifespan, including the role of nutrition, biological therapies, and pharmacological strategies. The final part of the review focuses on natural products and biologically active compounds with potential geroprotective effects. Data are presented on icariin from *Epimedium brevicornum* and *Punica granatum*, as well as on a combined product based on plant adaptogens – *Rhodiola rosea, Schisandra chinensis* and *Eleutherococcus senticosus*. Their phytochemical composition, biological activity and the possible mechanisms through which they may influence aging and longevity are analyzed. Thus, the literature review comprehensively covers all major aspects of the problem addressed in the dissertation. It makes a very good impression that both the literature review and the dissertation as a whole are written in a clear and scientifically rigorous style. In addition, the review is well focused, the aim of the study is clearly defined, and the research objectives are specific and logically formulated. Altogether, this demonstrates that the PhD candidate possesses a thorough understanding of the scientific problem addressed in the dissertation.

3. Correspondence of the chosen research methodology with the set aim and tasks of the doctoral thesis.

A wide range of methodologies were employed in the present study, including approaches for the preparation and chemical characterization of plant extracts, as well as modern biological, molecular-biological, and microscopic techniques for evaluating their effects on aging and longevity in the model organism *C. elegans*. To assess the biological effects, phenotypic analyses were performed, including measurements of locomotor activity, reproductive capacity, viability, morphological parameters, lifespan, and chemotaxis. Heat and oxidative stress models were also applied, together with investigations on lipid metabolism. In addition, mitochondrial dynamics, cellular localization, and the expression of key proteins in transgenic *C. elegans* strains were examined using confocal microscopy. Molecular mechanisms were further analyzed by RT-qPCR for gene

expression assessment and immunoblot analysis for monitoring protein expression. The obtained results were processed using appropriate statistical methods. Overall, the selected methodologies are well chosen and fully adequate for accomplishing the individual research tasks and achieving the overall aim of the dissertation.

4. Contributions of the doctoral thesis

The dissertation presents results of both fundamental and applied scientific significance related to the investigation of the potential of natural products to modulate aging and longevity. The applicability of the model organism *C. elegans* as an effective integrative platform for evaluating the geroprotective effects of natural compounds has been convincingly demonstrated. Key molecular mechanisms associated with the action of icariin and pomegranate-derived products were identified, including the involvement of signaling pathways and transcription factors regulating stress response, metabolic homeostasis, and longevity. Furthermore, the effects of pomegranate juice on age-related physiological changes in *C. elegans* were characterized. The obtained results also demonstrate the potential of the combined adaptogenic product ADAPT-232 to influence aging processes through modulation of mitochondrial function and cellular metabolism. From an applied scientific perspective, an experimental platform for the functional evaluation of natural products in *C. elegans* was successfully established, and a model of mitochondrial dysfunction induced by high-carbohydrate intake was adapted and validated. The generated data provide a valuable basis for the future application of the investigated natural products and adaptogens in strategies aimed at maintaining metabolic and mitochondrial health and promoting healthy aging.

5. Evaluation of the publications based on the thesis

The PhD candidate has presented three scientific publications related to the topic of the dissertation, including two experimental papers and one review article. In all three publications, she is the first author, which clearly demonstrates her substantial contribution to the conception, execution, and preparation of the published work. All three papers have been published in journals with high impact factors (total IF above 20), which is indicative of both the high quality of the conducted research and the scientific relevance and timeliness of the dissertation topic.

6. Personal impression, opinion, recommendations and remarks

I have no critical remarks about the dissertation. It gives a good impression that it is written in a good scientific style.

7. Conclusion with a clear positive or negative assessment of the dissertation.

The dissertation meets the requirements in terms of both scope and quality set by the Academic Staff Development Act in the Republic of Bulgaria, its implementing regulations, and the regulations of the Institute of Microbiology, Bulgarian Academy of Sciences, for the application of the Act. The dissertation is devoted to a highly relevant and socially significant scientific problem, and the conducted research has been performed at a very high scientific level. The PhD candidate has mastered and successfully applied a wide range of modern methodologies, thereby fulfilling the educational objectives of the doctoral training process. Based on the presented analysis, I give a strongly positive evaluation of the dissertation and consider it fully justified to propose that Monika Todorova be awarded the educational and scientific degree "Doctor" in the scientific field 5. Technical Sciences, professional field 5.11. Biotechnology (Technology of Biologically Active Substances).

08.06.2026 Jury member:

/ prof. Petko Denev, PhD /