

## STATEMENT

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On a dissertation titled "MODULATION OF LIFE EXTENSION MECHANISMS IN CAENORHABDITIS ELEGANS USING BIOLOGICALLY ACTIVE SUBSTANCES" submitted by **Monika Nikolaeva Todorova** for the award of the educational and scientific degree "Doctor" in Field 5.11. Biotechnology (Technology of Biologically Active Substances)

The topic of the dissertation is exceptionally relevant – not only scientifically, but also socially and economically. I would say the dissertation hits the mark perfectly in terms of timing and societal needs. The topic lies at the heart of contemporary biomedical research, possesses clearly articulated scientific and applied aspects, and meets the scientific community's expectations for interdisciplinary approaches (biotechnology, pharmacology, nutrition, aging). Even after 5–10 years, this work will not sound outdated, because fundamental questions about aging remain.

The doctoral candidate is thoroughly familiar with the subject matter, as evidenced by the extremely detailed bibliography presented. The literature review and discussion are written professionally and comprehensively, allowing step-by-step immersion into the details of the topic. The title concisely reflects the aim and essence of the conducted research. The presentation clearly outlines the relevance and necessity of this study in light of the contemporary concept of healthy aging and longevity as a socially significant factor. The dissertation cites and discusses modern hypotheses and opinions on the issue. Additional focus is placed on the level of current molecular-genetic research in the field. Based on all the above, I consider that the doctoral candidate knows the problem in detail, which entitles me to define her as a specialist in the field.

The work is exceptionally rich in content. It immediately becomes evident that the PhD student did not take the easy path of testing one or two compounds, but rather built a comprehensive strategy. The work is well-structured, progressing from a pure substance (icariin), through a plant extract and a functional food (pomegranate), to a complex combined product (ADAPT-232). This demonstrates an in-depth reading of the literature and a sense of what is worthwhile to investigate. The problem of an aging population and the paradox – we live longer, but not necessarily healthier – is concisely and clearly outlined. The choice of *C. elegans* is well-justified, with its specific advantages noted (short life cycle, conserved signaling pathways).

The aims are formulated very specifically and clearly. Figure 1 is successful – it visually summarizes the entire experimental strategy. The tasks are logically ordered – from method selection and mastery, to specific experiments. It shows that an enormous amount of methodological work has been completed, which often remains invisible. I appreciate that the candidate does not try to fit everything into a single aim, but calmly lists the individual directions. Regarding the methodology – congratulations! Numerous complex techniques have been adapted and validated – from NMR profiling, through various stress models, to tracking nuclear translocation in transgenic strains. This is serious experimental work. The model using 2% glucose for mitochondrial dysfunction sounds particularly useful for future research. The presented material shows that executing the experimental work was a good school for the methodological training of the doctoral candidate, and in this sense, the purpose of the PhD as an educational degree has been fulfilled.

I must emphasize that the experimental part of the dissertation is a substantial, in-depth, and precisely executed scientific study with scientific and applied contributions. The obtained results and their skillful interpretation convincingly demonstrate that the doctoral candidate is a methodologically and informationally well-prepared researcher. Overall, the dissertation represents a well-substantiated, complex, and completed scientific investigation. I would highlight the research on pomegranate juice and leaf extract. This is the strongest part, in my opinion. The effects are consistent – extends lifespan, reduces lipids, improves stress response. The data on mitochondrial membrane potential and mass are technically challenging to obtain and are highly informative. In my view, the most valuable finding is the demonstration of cross-regulation between HLH-30/TFEB, DAF-16, and SKN-1. This is non-trivial and builds upon existing literature. The double mutant MIR13 is a good move to confirm the role of AMPK and SIRT1. Regarding pomegranate juice, it is more than interesting – the effect on lifespan is observed only at the highest dose, but functional parameters (movement, stress) improve even at lower doses. This is realistic – not every "functional food" must drastically extend lifespan to be meaningful. It improves the quality of aging. I regret that there are not more molecular data here (e.g., why exactly this dose works), but I understand the volume of work is large.

The dissertation is distinguished by its scientific-fundamental and applied contributions related to the study of natural products and their impact on aging processes and longevity. Within the study, the significance of *C. elegans* as a reliable and integrative experimental system for analyzing the biological activity of natural compounds with potential geroprotective effects has been confirmed. The mechanisms of action of icariin have been elucidated, establishing its hormetic nature and its involvement in the regulation of insulin/insulin-like signaling and hsf-1. For the first time, the key role of the transcription factor HLH-30 in mediating the geroprotective effect of pomegranate leaf extract, in coordination with the SKN-1 and DAF-16 signaling pathways, has been demonstrated. Furthermore, the effect of pomegranate juice on physiological parameters associated with aging in *C. elegans* has been analyzed, as well as the potential of ADAPT-232 to influence aging processes by modulating mitochondrial function and metabolic homeostasis.

The research also has substantial applied value, as an experimental platform for the functional evaluation of natural products with potential effects on aging and lifespan in *C. elegans* has been developed and integrated. The results regarding the geroprotective properties of ADAPT-232 create prerequisites for expanding its application beyond stress control and physical exertion, including as a possible intervention for maintaining metabolic balance and mitochondrial health. Additionally, a model of mitochondrial dysfunction induced by high-carbohydrate intake in *C. elegans* has been adapted and validated, which can be used to assess the effectiveness of various metabolically targeted interventions.

In conclusion, the dissertation contributes both by providing new mechanistic insights into the influence of natural products on aging processes and by creating an integrative experimental platform with broad possibilities for future research. The achieved results confirm the view that targeted intervention in the fundamental biological mechanisms of aging through natural compounds is a realistic and scientifically justified approach for extending the period of healthy life, while simultaneously laying the foundation for subsequent molecular and clinical studies in the fields of gerontology and phytotherapy. The dissertation does not remain solely in abstract science. ADAPT-232 is a commercial product, and pomegranate juice is a widely available functional food. The idea that something a person can buy from a store or pharmacy could have a real geroprotective effect is very attractive to both the general public and industry. Pomegranate leaf extract, on the other hand, is an example of upcycling – using a by-product (leaves that would otherwise go to waste) as a valuable raw material. This is also a modern and sustainable direction.

The **publication activity** of the doctoral candidate is one of the strongest aspects of the entire dissertation. This is not merely "fulfillment of a formal requirement" for a PhD, but a serious scientific output that would be a source of pride for many more experienced researchers. The publications are excellent. On their own, they would be sufficient to meet the requirements for a doctoral degree in most European universities. In the Bulgarian context, this is an indisputably strong indicator. The total impact factor of 20.1 for three articles is impressive for a PhD. In Bulgaria, where such indicators are still perceived as a high standard, this is undoubtedly a distinction. Even internationally – for a period of 2-3 years of work – this is very good productivity. The distribution is also fortunate: one review (showing breadth of knowledge), two experimental articles with different orientations (icariin, pomegranate extract). It is a pity that the experiments with pomegranate juice and ADAPT-232 have not been formatted as separate publications – judging by the volume of data, there is probably material for another one or two articles in preparation. But this is only a guess.

The **abstract** accurately reflects and reveals the main points and ideas of the dissertation, presents the most important results of the conducted research, and is prepared according to the relevant requirements.

I have two questions regarding future directions:

1. Which of the four interventions (icariin, PGL, PGJ, ADAPT-232) do you think has the greatest potential to progress to tests in mammals (e.g., mice) and why? What would be your next research steps?
2. Mitochondrial dysfunction: You restore mitochondrial potential in the glucose model with ADAPT-232. Does this mean the product would be useful in type 2 diabetes or metabolic syndrome? Or is it more prophylactic?

## CONCLUSION

The dissertation by Monika Nikolaeva Todorova is a truly substantial, in-depth, and original scientific study of fundamental and innovative character, possessing undeniable and significant merits for theory and science. It is distinguished by:

- Methodological depth – the combination of phenotypic analyses, gene expression, nuclear translocation, mitochondrial functional tests, and metabolic profiling is impressive for a PhD.
- Logical structure – from simple compound to complex mixtures, from effect to mechanism.
- Publication activity – three Q1 articles – this speaks for itself.
- Relevance – works with current concepts – hormesis, mitochondrial function, TFEB, adaptogens.

The scientific work possesses a very high scientific and cognitive value with undeniable contributions and meets all requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Implementation of the law, as well as the relevant Regulations of the Institute of Microbiology, BAS. Due to the above, as well as due to the research qualities of the doctoral candidate, I give my positive assessment of the conducted research and the presented dissertation. I confidently propose to the specialized scientific jury to award Ms. Monika Nikolaeva Todorova the educational and scientific degree "Doctor" in Field 5.11. Biotechnology (Technology of Biologically Active Substances).

Plovdiv  
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Signature: .....  
(Prof. Albert Krastanov)