OPINION

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On dissertation for awarding of the educational and scientific degree **„Doctor"**

In higher education field 4. "Natural sciences, Mathematics and Informatics"
professional classification *4.3. „Biological Sciences"*
PhD program *„Biochemistry"*

Author : *STEFKA MARINOVA STOEVA*

Topic : *"Comparative study of the biological effects of natural dietary sources of sulfur on human intestinal cells"*

Scientific supervisor : *Assoc. Prof. Milka Asparuhova Nashar, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Faculty of Pharmacy, Department of "Biochemistry, Molecular Medicine and Nutrigenomics"*

1. General presentation of the procedure and the PhD student

According to administrative act No. P-109-320/25.09.2026 by Prof. Dimitar Raykov, Rector of the Medical University "Prof. Dr. Paraskev Stoyanov", Varna (MU-Varna), I have been appointed as a member of the scientific jury in the PhD thesis defense procedure. The title of the thesis is "Comparative study of the biological effects of natural food sources of sulfur on human intestinal cells" for the acquisition of the educational and scientific degree "Doctor" in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional field 4.3. "Biochemical Sciences", PhD programme "Biochemistry". The author of the dissertation is **Stefka Marinova Stoeva** - PhD student in full-time studies at the department "Biochemistry, Molecular Medicine and Nutrigenomics", Faculty of Pharmacy at MU-Varna with scientific supervisor Assoc. Prof. Milka Nashar.

The set of paper materials submitted by PhD student Stefka Stoeva **is in accordance** with Article 69 of the Regulations for the Development of the Academic Staff of MU-Varna dated 27.07.2026. It includes the following documents:

- Application to the Rector of MU-Varna to initiate the procedure for the defense of a dissertation;
- CV up-tu-date;
- Minutes of the Departmental Council meeting regarding the report on readiness to initiate the procedure and the preliminary discussion of the dissertation;
- List of scientific publications related to the dissertation topic;
- Copies of scientific publications related to the dissertation topic;
- List of posters, and presentations at scientific conferences related to the dissertation;
- Declaration of Authenticity of the Submitted Documents;
- Report on the Similarity between the Dissertation and the Scientific Publications Submitted as Part of the Procedure for Awarding the "Doctor" Academic Degree;

- Documentation proving compliance with the minimum scientometric requirements for the award of the "Doctor" degree
- Dissertation;
- Abstract;

The PhD student has attached **2 publications** and evidence of participation in **6 scientific conferences** , two of which were abroad.

2. Topical relevance

In the era of the high technology and artificial intelligence, increasing importance is given to human nutrition and health - the accessibility and quality of food with high nutritional and health potential. We talk about "super" and "functional" foods. Foods and waters rich in sulfur have a proven impact on human health. The focus of this dissertation is the biological effects of sulfur-containing biologically active compounds from food sources and sulfur mineral water from the Varna region on human intestinal epithelial cells.

Expanding the knowledge about the health potential of foods rich in natural BAS, including S-containing compounds, is more than relevant. It is a challenge related to the food supply of the population and the preservation of human health. The scientific significance of the results obtained is indisputable: new insights into the effects of sulfur-containing mineral water from the Varna region—which also has an environmental aspect—as well as natural S-containing substances on the expression of genes associated with the inflammatory cellular response and biotransformation. A comparative cellular approach has been developed and applied for a preliminary assessment of the molecular effects of natural sulfur sources.

3. Subject knowing

The PhD-thesis has a separate section "Literature Review", that includes 21 pages of detailed introduction to the problem. 298 sources have been reviewed. The information is arranged in several sub-items, from the general one moving on to the subject of the research chosen by the PhD student, sulphur-containing organic and inorganic compounds from natural sources (food and water). The knowledge accumulated so far about their natural sources, benefits for human health, as well as methodologies for assessing their biological activity have been compared and summarized. As a result of the literature review, a clear scientific hypothesis has been formulated.

4. Research methodolog

To achieve the goal set in the PhD-thesis, the human epithelial cell line HIEC-6 was studied as a cell model for screening the biological effects of the studied natural substances and water, in conditions of lipopolysaccharide-induced inflammation. Several clearly defined tasks were accomplished:

- Cell viability was determined upon treatment with sulfuric mineral water (SMW), sulforaphane, taurine, and alliin;
- An assessment of the influence of the studied sulfur sources on the expression of target genes was made;
- The obtained data were integrated and a comparative analysis of the anti-inflammatory, antioxidant and adaptogenic profile of the studied sulfur sources was performed;
- An analysis of the potential of the studied sulfur sources as factors determining detoxification processes was performed.

PhD candidate Stefka Stoeva has mastered the methods and laboratory techniques used, not only in the field of Biochemistry, but also in the field of Molecular Biology and Genetics.

5. Characterization and evaluation of the dissertation work and contributions

PhD thesis values 154 printed pages, and includes 73 figures, 6 tables and 2 diagrams. The references includes 298 sources. The dissertation work is divided into eight main sections: Introduction, Literature review, Aim and objectives, Materials and methods, Results, Discussion, Summary, Conclusions. Each chapter is presented comprehensively, with the parts devoted to the author's own research predominating: over 100 pages out of a total of 133 pages of the main text. Eight conclusions have been made:

1. In the experimental model used, sulfur mineral water, sulforaphane, alliin, and taurine preserved cell viability in the studied concentration ranges, allowing subsequent analysis of their biological effects.

2. The natural sulfur sources studied differ in their ability to modulate the inflammatory response in human intestinal epithelial cells.

3. SMW exhibits a wide spectrum of effects on the expression of key genes for pro-inflammatory markers, which outlines its potential to influence molecular mechanisms associated with the cellular inflammatory response.

4. As expected, sulforaphane has the most significant impact on the expression of genes involved in antioxidant defense and cellular adaptation, while the effects of taurine and alliin on these signaling pathways are more limited and depend to a greater extent on the applied concentration.

5. SMW, along with its influence on pro-inflammatory mediators, also positively changes the expression of genes related to the antioxidant response.

6. The studied sulfur sources exhibit different selectivity with respect to the expression of sulfotransferases, with sulfur mineral water primarily affecting the expression of SULT1A1, while sulforaphane and alliin have a more pronounced effect on SULT1A3.

7. The most stable expression dependence is Nrf2/GPx, which is positive and changes in the same direction in all studied treatments, both in unstimulated cells and in conditions of LPS-induced inflammation.

8. The negative expression relationship NF-κB/Nrf2 is preserved in all treatments, both in unstimulated cells and in conditions of LPS-induced inflammation.

The contributions are summarized as follows:

<u>with an original character</u>

1. New experimental data have been obtained on the effects of sulfur-containing mineral water from the Varna region on the expression of genes associated with inflammatory and redox-related cellular response and biotransformation.

2. Knowledge of the molecular effects of the poorly studied organosulfide alliin has been expanded, establishing its potential to modulate the expression of genes associated with inflammatory and redox-related cellular responses and biotransformation.

<u>of an applied nature:</u>

1. A comparative cellular approach was developed and applied for preliminary evaluation of the molecular effects of natural sulfur sources in HIEC-6 cells under basal and LPS-stimulated conditions.

of a confirmatory nature

1. The results obtained for sulforaphane and taurine complement and support the available literature data on their influence on the expression of genes associated with inflammatory and redox-related cellular responses.

6. Assessment of the PhD student's publications and personal contribution

The experimental data in this Dissertation have been published in 2 peer-reviewed and indexed journals with a high rating - one in a journal with Q2 , and the other in a journal with Q4. According to Scopus and WoS data, Stefka Stoeva has long been recognizable and has an accumulated asset, which provides her with an h- index of 2. In addition, the results have been promoted at national forums and conferences abroad with four posters and two reports, one of which was awarded a First Place award.

7. Abstract

The abstract contains 65 pages and is compiled according to the requirements of the relevant regulations, focusing on the main results and scientific achievements of the PhD thesis.

8. Recommendations for future use of the dissertation contributions and results

The dissertation is written in a high academic language, with a very good style, concise and organized. The material is easy to read and understand. PhD candidate Stefka Stoeva is developing this work under a project funded by the Medical University of Varna. The work can be defined as interdisciplinary, since the data were obtained not only through techniques and methods of biochemistry, but also from the field of molecular biology and genetics.

There are several technical errors in the Dissertation and in the Abstract, which does not reduce the scientific research value of the work. Overall, I have no critical remarks. I would recommend that Stefka Stoeva continue her development in the chosen scientific field and use and build on her competencies and skills in the multidisciplinary field of biologically active compounds for the benefit of human health.

CONCLUSION

The PhD thesis contains results with an original contribution to science and meet all the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the low and the relevant Regulations of the Medical University "Prof. Dr. Paraskev Stoyanov", Varna.

The PhD thesis shows that **Stefka Marinova Stoeva** possesses in-depth theoretical knowledge and professional skills in the scientific field of the "Biochemistry", demonstrating qualities for independently conducting scientific research.

In conclusion, I confidently give my positive assessment of the conducted research, presented in the above-mentioned dissertation, abstract, achieved results and contributions. I kindly recommend scientific jury **to award the degree "Doctor" to Stefka Marinova Stoeva** in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics", professional field 4.3. "Biological Sciences", PhD program "Biochemistry".

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

20. 10. 2026

Prepared by: .....

/Assoc. Prof. Milena Tsanova, DSc /