

STATEMENT

From Professor Milen Ivanov Georgiev, PhD
Laboratory of Metabolomics, The Stephan Angeloff Institute of Microbiology,
Bulgarian Academy of Sciences

of a dissertation work for awarding the educational and scientific degree “doctor” in the doctoral program “Technology of biologically active substances”, in professional area 5.11. Biotechnology

Author: Kristiana Miroslavova Amirova

Type of PhD: Full-time doctoral student at the Stephan Angeloff Institute of Microbiology – Bulgarian Academy of Sciences (BAS)

Title: Isolation of natural compounds modulating the function of Nrf2 transcription factor

Supervisors: Assoc. Prof. Petya A. Dimitrova, PhD and Prof. Milen I. Georgiev, PhD

Short biographical data:

Doctoral student Kristiana Miroslavova Amirova was born on May 5, 1993. She graduated from Paisii Hilendarski University of Plovdiv in 2017 with a master’s degree in molecular biology and biotechnology. During the period 2017-2022, she held the position of biologist at the Center of Plant Systems Biology and Biotechnology in the city of Plovdiv.

General presentation of the procedure and the PhD student:

The presented set of materials of Kristiana Amirova is in accordance with the regulations of the Stephan Angeloff Institute of Microbiology – BAS and includes all necessary documents.

Kristiana M. Amirova, after winning a competition in 2018, was enrolled as a full-time doctoral student at the Stephan Angeloff Institute of Microbiology - BAS in the doctoral program “Technology of biologically active substances”, in professional area 5.11. Biotechnology with supervisors Assoc. Prof. Petya A. Dimitrova, PhD and Prof. Milen I. Georgiev, PhD.

Relevance of the topic:

The search for (drug) leads, supplementing or completely replacing the ordinary therapy of degenerative diseases is essential nowadays. Plants and the biologically active substances accumulated in them have been used for centuries to control inflammation in chronic/acute disorders. Although this knowledge is defined as empirical (the so-called “sense knowledge”), there is an increasing volume of scientific-based evidence for the enormous potential of medicinal plants (and the pharmaceutically relevant metabolites synthesized by them) in this direction. In Western societies, today, the topic of the lost connection between generations in the transmission of knowledge about the use of plants in the treatment of a number of diseases and pathological conditions in humans is particularly relevant.

The Nrf2 is a transcription factor, regulating the expression of a significant number of cytoprotective genes, and in this regard its activation/inhibition could be used as an effective strategy in the fight against a number of degenerative diseases.

Structure of the PhD thesis:

Kristiana M. Amirova's dissertation is written on 175 pages and is structured within the generally accepted standards. The bibliographic reference includes in excess of 200 literature sources. The results are illustrated with 36 figures, 4 pictures and 7 tables.

Evaluation of the contributions of the PhD thesis work:

The contributions from the dissertation work are divided into three main groups, such as contributions of a confirmatory nature (3 items), contributions of a fundamental nature (6 items) and those with an applied orientation (3 items).

Contributions of a confirmatory nature:

- ⇒ The metabolomics analyses of extracts from *B. nigra*, *C. vulgare*, *L. cardica*, and *H. rhodopensis* confirm the data from the literature for the presence of different groups of secondary metabolites, such as phenolic acids, flavonoids, phenylethanoid glycosides and diterpenes.
- ⇒ Local application of the Nrf2 activator, CDDO-Me, has a therapeutic effect in a model of degenerative joint disease.
- ⇒ Human granulocytes and mouse neutrophils have increased expression of Nrf2 at transcriptional and protein level upon Nrf2 activation by CDDO-Me

Contributions of a fundamental nature:

- ⇒ Evidence is presented for the first time that pharmacological activation of Nrf2 with CDDO-Me reduces sensitivity to aging - senescence of mouse neutrophils.
- ⇒ Osteoarthritis progression has been found to be accompanied by the acquisition of a senescent neutrophil phenotype.
- ⇒ The *C. vulgare* extract and the active metabolites – caffeic and chlorogenic acids – suppress COX-2 cyclooxygenase expression.
- ⇒ Fraction D, myconoside and calcelarioside E affect the expression of the *NFE2L2* gene and the percentage of Nrf2 positive neutrophils.
- ⇒ A different mechanism of action of the two pure compounds on Nrf2 has been established, which is due to the peculiarities of their structure, their selective action on Nrf2 signaling and PI3K expression.
- ⇒ Fraction D, myconoside and calcelarioside E administration improve symptoms and pathological changes in an arthritis model by modifying the expression of Nrf2 and Nrf2-target genes (*SOD-1* and *PRX1*) in synovial extracts by reducing the number of TNF- α + neutrophils in the circulation.

Applied contributions:

- ⇒ A utility model has been developed for the cultivation of *Haberlea rhodopensis* Friv. *in vitro*, in which the content of myconoside is several times higher compared to the wild-grown plants.
- ⇒ The herbal extract of *Haberlea rhodopensis* Friv. can serve to obtain fractions with a different ratio of myconoside and calcelarioside E.
- ⇒ Myconoside and calcelarioside E can be administered in combination for an effective pharmacological modification of the transcription factor Nrf2.

Thus presented, the contributions of the dissertation work reflect its essence and at the same time do not overestimate the results obtained.

Assessment of the PhD student’s publications and personal contribution:

The results of the present dissertation are summarized in 4 scientific publications, positioned in the first quartile (Q1) for the respective field. There are five participations at national and international conferences. The doctoral student participated in two scientific projects.

Abstract:

The abstract has 52 pages and is illustrated with 11 figures and one table. The content of the abstract is in accordance with the requirements and fully reflects the main results of the dissertation work.

CONCLUSION

The dissertation contains fundamental and applied results, which represent an original contribution to the field and fully meet the requirements of Act for the Development of the Academic Staff in the Republic of Bulgaria (ADAS), the Rules for ADAS application, as well as the specific Rules of BAS and the Stephan Angeloff Institute of Microbiology – BAS. The presented materials and results fully correspond to the specific requirements and quantitative criteria adopted in connection with the specific requirements the Stephan Angeloff Institute of Microbiology – BAS’s Rules. The dissertation shows that Kristiana M. Amirova possesses theoretical knowledge and professional skills in the scientific area.

Due to the above and in conclusion, I give my positive assessment of the conducted research (presented by the peer-reviewed dissertation work, abstract, achieved results and contributions) and **propose to the honorable scientific jury to award the educational and scientific degree “doctor” to Kristiana Miroslavova Amirova in a doctoral program “Technology of biologically active substances”, in professional area 5.11. Biotechnology.**

September 26, 2023
Plovdiv, Bulgaria

Prepared by:
/Prof. Milen I. Georgiev, PhD/