

REVIEW

by Prof. Milen I. Georgiev, PhD
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of a dissertation work for awarding the educational and scientific degree “Doctor” in the field of higher education 4. “**Natural Sciences, Mathematics and Informatics**”, in professional area 4.3. “**Biological sciences**”, scientific degree “**Application of the principles and methods of cybernetics to the study of the structure-activity dependence of biologically active substances**”

Author: Antonia Georgieva Diukendjieva-Todorova

Type of PhD: Full-time doctoral student

Department: QSAR and Molecular Modelling, Institute of Biophysics and Biomedical Engineering – BAS (IBPhBME-BAS)

Topic: Research on ADME/TOX properties and molecular interactions of flavonolignans from *Silybum marianum* L. by approaches *in silico* and *in vitro* approaches

Supervisors: Prof. Ivanka Tsakovska, PhD and Corresponding Member Prof. Ilza Pajeva, DSc

Short biographical data:

Doctoral student Antonia Georgieva Diukendjieva-Todorova was born on January 17th 1989. She graduated from Sofia University “St. Kliment Ohridski” in 2012 with a Bachelor's degree in biotechnology and later earned a Master's degree in biochemistry with a focus on molecular biology in 2014, again from the Sofia University “St. Kliment Ohridski”. Since 2010, she has been a member of ABI, research intern at IBPhBME – BAS, biologist, and head of scientific programs in various companies. Since 2018, she has been an assistant at IBPhBME – BAS, and since 2020, she is currently a biologist specialist at the same institute. Antonia Diukendjieva-Todorova speaks English (fluent) and German (basic).

General presentation of the procedure and the PhD student:

The presented set of materials is in accordance with the regulations of Institute of Biophysics and Biomedical Engineering – BAS and includes all necessary documents. The dissertation work was discussed during an extended scientific seminar of the “QSAR and molecular modeling” section at IBPhBME – BAS on July 28th 2023. Antonia G. Diukendjieva-Todorova, MSc, was enrolled as a doctoral student in the QSAR and molecular modeling section at IBPhBME - BAS, with scientific supervisors Prof. Ivanka Tsakovska, PhD and Corresponding Member Prof. Ilza Pajeva, DSc.

In 2017, she received the “Ivan Evstratiev Geshov” award of the Bulgarian Academy of Science in the youngest scientists contest.

Relevance of the topic:

The search of (drug) leads, supplementing or completely replacing conventional therapies for a variety of diseases and pathological conditions, is essential nowadays. Plants and the biologically active substances they produce have been used for centuries for therapy and prevention of a number of chronic/acute diseases and neoplasms. 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 today's Western societies, the topic of the lost link between generations in the transmission of knowledge about the use of plants in the treatment of various diseases and pathological conditions in humans is particularly relevant. In these same societies, however, another discussion is ongoing – about the excessive use of animals in laboratory and pre-clinical testing studies.

In this regard, I acknowledge that Antonia G. Diukendjieva-Todorova's dissertation represents a contemporary scientific approach of both fundamental and applied nature, specifically integrating *in silico* approaches with modern methods for assessing antineoplastic potential. It should be noted that the team formed by Prof. Pajeva and Prof. Tsakovska has significant achievements and possesses well-established scientific profile in the field of QSAR (quantitative structure-activity relationships) and molecular modeling.

Structure of the PhD thesis:

Antonia G. Diukendjieva-Todorova's dissertation is written on 144 pages and is appropriately structured within the generally accepted standards. The bibliographic reference includes in excess of 202 literature sources. A favorable impression is made by the fact that a

substantial portion of the cited literature is from recent years, serving as additional evidence of the dissertation's topicality and the doctoral student's expertise in the particular field.

The comprehensive understanding of the problem is most evident in the adept and intelligently structured literature review of the dissertation. The literature review clearly demonstrates the doctoral student's profound knowledge in the field. In this regard, I would recommend Antonia G. Diukendjieva-Todorova to consider the prospect of publishing a scientific review in an international journal, drawing upon the overview of the current state of research in the field presented in the dissertation.

The literature review concludes with an aim of the dissertation work and an appropriate formulation of the main tasks.

A favorable impression is made by the fact that Antonia G. Diukendjieva-Todorova utilizes modern methods with a diverse range of applications, encompassing techniques for quantitative structure-activity relationships, molecular docking, molecular similarity search, expert systems for ADME/Tox properties, PAMPA permeability, etc.

The results are both original and well-illustrated with 25 figures, 16 tables, as well as two appendixes labeled as Appendix 1 and 2.

Evaluation of the contributions of the PhD thesis work:

The contributions of the dissertation are as follows:

1. A QSAR model was developed, allowing reliable assessment of membrane permeability and gastrointestinal absorption of orally administered biologically active substances. The model is freely available through the Alternative Methods Database of the EU Reference Laboratory for Alternatives to Animal Experiments.
2. The applied combined *in silico/in vitro* analysis elucidates molecular mechanisms of action of flavonolignans from *Silybum marianum* L. and identifies them as suitable lead structures for the design of new biologically active substances with positive effects on human health.
 - 2.1. It was established that the main components of *Silybum marianum* L. as well as their derivatives can be considered to pass well through the gastrointestinal tract.
 - 2.2. Evidence is presented that enantiomeric forms of silybin undergo stereospecific interactions with the estrogen receptor alpha, offering a molecular-level explanation for the experimentally observed differences in their toxic effects.
 - 2.3. B-Raf kinase and the Smoothed receptor have been identified as novel pharmacological targets involved in the mechanisms of anti-tumor activity of

flavonolignans from *Silybum marianum* L. Dehydrosilybins have been outlined as promising lead structures for the development of anti-tumor drugs.

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 6 scientific publications, 3 of them were published in journals with an impact factor (Natural Product Communications, Q2; Phytomedicine, Q1 and Antioxidants, Q2), one was published in a journal with SJR (Computational Toxicology), and two were included in conference proceedings published in full text. The total number of participations in national and international congresses is 8.

One of the publications is in the prestigious journal Phytomedicine (published by Elsevier), which is positioned in the top journals (Q1) in the field of natural products.

The doctoral student meets and exceeds the specific requirements of the Regulations of the IBPhBME – BAS, as follows: out of the required 80 points for the Doctor of Philosophy degree, Antonia Diukendjieva-Todorova has collected 117 points, being the first author in all publications on the dissertation work.

My critical remarks towards the doctoral student are minor and primarily pertain to the presence of slight linguistic errors, which, in essence, do not diminish the overall quality of the dissertation work.

I firmly believe that the peer-reviewed dissertation, abstract, achieved results, and contributions would not have been possible without the expert guidance and professional supervision provided by Prof. Tsakovska and Prof. Pajeva.

Abstract:

The abstract has 56 pages and is illustrated with 11 figures and 6 tables. The content of the abstract is in accordance with the requirements and fully reflects the main results of the dissertation.

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 IBPhBME – BAS. The presented materials and results fully comply with

the specific requirements and quantitative criteria adopted in connection with the Rules of IBPhBME – BAS for application of ADAS.

The dissertation demonstrates that Antonia G. Diukendjieva-Todorova, MSc, possesses in-depth theoretical knowledge and professional skills in the scientific specialty “Application of the principles and methods of cybernetics in the field of structure-activity dependencies of biologically active substances” within the field of higher education 4. “Natural Sciences, Mathematics and Informatics”, in professional area 4.3. “Biological sciences”, demonstrating the essential qualities and skills for independent research. From my perspective, the dissertation work represents a comprehensive scientific development with an original character!

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 **confidently suggest to the honorable scientific jury to award the educational and scientific degree “Doctor of Philosophy” to Antonia G. Diukendjieva-Todorova**, in professional area 4.3 “Biological sciences”.

November 23, 2023
Plovdiv, Bulgaria

Prepared by:

/Prof. Milen I. Georgiev, PhD/