

STATEMENT

by Prof. **Albert Ivanov Krastanov**, DSc

Head of the Biotechnology department at the University of Food Technology – Plovdiv

of a dissertation for the award of the educational and scientific degree “Doctor of Philosophy” in the scientific direction 5.11. Biotechnology (Technology of biologically active substances)

Doctoral candidate:

Martina Stoyanova Savova, MPharm

Dissertation title:

“Mechanism of modulation of obesity in *in vitro* model of human adipocytes through application of bioactive compounds”

1. Overview of the procedure and the doctoral candidate

Martina Stoyanova Savova was born on August 10th, 1994, in Pazardzhik. In 2019, she completed a MSc degree in Pharmacy at the Medical University in Plovdiv. During the same year, she attained a position as a PhD student in the scientific direction 5.11. Biotechnology at the Bulgarian Academy of Sciences, Institute of Microbiology, Laboratory of Metabolomics, Plovdiv, under the supervision of Prof. Milen I. Georgiev. She has authored and co-authored 14 scientific papers. Citations: 176 (until June 6th, 2023; self-citations are excluded; source: Scopus). *H*-index: 7 (Scopus). Total impact factor: 84.71 (Scopus). **Impressive!** Martina Savova has submitted all the necessary documents and materials, fulfilling the requirements of the current Act for the Development of the Academic Staff in the Republic of Bulgaria and the and the Rules of the Institute of Microbiology, Bulgarian Academy of Sciences .

The dissertation work was approved and directed for defense by the Scientific Board at the Institute of Microbiology, BAS (Protocol №46/25.05.2023).

The research included in this dissertation has been carried out with the financial support of the PlantaSYST project (SGA No. 739582 and FPA No. 664620) under the Horizon 2020 programme of the European Union, and project BG05M2OP001-1.003-001-C01, funded by the European Regional Development Fund through the Operational Programme "Science and Education for Smart Growth".

2. Relevance of the dissertation topic

The dissertation presented for review is dedicated to a significant and compelling problem related to the implementation of an *in vitro* platform for evaluation of the impact of plant extracts and their secondary metabolites on the physiology and function of lipid cells, which play a crucial role as structural components within adipose tissue, along with exploring the modulation of lipid cells'

response to low-grade inflammation. The chosen topic of the dissertation holds immense importance from both scientific and practical perspectives, given the increasing demand for cell-based model systems in obesity research, which could provide valuable insights and opportunities for formulating hypotheses regarding specific molecular mechanisms involved in these processes.

Plants are a natural source of structurally diverse biologically-active molecules with insufficiently studied potential for obesity modulation. The development of an experimental approach that combines molecular pharmacology, ethnopharmacology, and phytochemical analysis could provide valuable insights and a detailed examination of the therapeutic potential of medicinal plant extracts and evaluation of pure compounds with anti-obesity activity.

3. Awareness of the subject matter

Based on the evaluated PhD thesis, my opinion on the awareness of the subject matter is excellent. The candidate has detailed knowledge of the problem, as I could discern from the comprehensive bibliography included. The discussion and review sections are academically and thoroughly written, gently introducing the dissertation details to the reader. The title of the thesis briefly presents the aim and essence of the research. The introduction highlights the relevance and crucial importance of conducting such research in light of the contemporary focus on anti-obesity therapy as a socially significant factor. In the dissertation, recently proposed hypotheses and opinions related to the matter have been thoroughly discussed and cited. Additional focus has been placed on molecular genetics research in the field. Based on all that has been said until now, I consider that the candidate has a detailed scope of knowledge over the topic, which gives me the right to assume that she is a specialist in this area.

4. Research methodology

Based on the precisely conducted experiments and the critical examination of the results, the anti-adipogenic activity of leaf extracts of *Z. jujuba* and aerial parts of *P. aviculare* and *P. hydropiper*, as well as their secondary metabolites, is evaluated for the first time in an *in vitro* model of obesity using human adipocytes. Furthermore, the most promising compounds have been validated in an *in vivo* model of obesity in nematodes of the *C. elegans* strain.

In the present thesis, a model of obesity in human adipocytes has been adapted for the first time in Bulgaria. Indeed, this result alone is sufficient for a high appraisal of the scientific and applied contributions of this doctoral thesis. Screening for the modulation of adipogenesis and lipolysis through medicinal plant extracts used in herbal combinations for the treatment of metabolic disorders, as well as natural compounds, has been conducted. The precisely structured experimental platform provides opportunities for evaluation of the anti-adipogenic activity and determination of the molecular mechanisms of action of natural compounds.

The presented material shows that the implementation of the experimental workflow was beneficial for the methodological preparation of the candidate. In this sense, the purpose of the PhD as an educational degree has been fulfilled.

5. Characteristic and evaluation of the dissertation and its contributions

The PhD candidate demonstrates a comprehensive understanding of the contemporary state of the problem, as well as a profound knowledge of the vast literature on the subject. In the introduction of the dissertation, she delves deeply into the complexities of the current research, highlighting their utmost importance and the compelling necessity for the development of this very dissertation. The text contains numerous tables and graphs, which enhance the clarity and informativeness of the research data. The literature review progresses from the fundamentals to the specific aspects of the scientific issues related to the subject of the dissertation. Questions, encompassing important information regarding the discussed problematics in the presented dissertation, have been competently addressed.

Regarding the candidate's formulated contributions, I would like to express my opinion that those with scientific and fundamental character demonstrate originality and also pave the way for further research in this area. Consequently, they offer an opportunity for this work to be continued and developed in various more specialized directions, which I consider an additional beneficial aspect of this dissertation. The results from the current doctoral thesis provide detailed mechanistic information for the targeted molecular pathways upon treatment with the chosen plant extracts and pure natural compounds. Following the comprehensive data analysis, future perspectives have been formulated regarding the continuation of work in the scientific field of molecular pharmacology of obesity.

I insist to emphasize that the experimental part of the presented thesis is voluminous, exhaustive, and meticulously conducted, providing valuable scientific contributions as well as practical applications. The accumulated results, along with their persuasive interpretation, indicate that the candidate is a methodically and informatively prepared scientist. Generally, the dissertation presents a well-justified, comprehensive, and completed scientific research with significant scientifically-applied contributions.

In regard to the formulated contributions with scientific and applied character, the methodological approaches used fit very accurately to the context of the scientific elaboration and the specificity of the examined models. The accumulated data on the molecular mechanism of action of betulinic acid could serve as a foundation for the development of products for obesity management.

I consider the contributions with a scientific and fundamental character to be significant as they practically reveal specific searches defined by the candidate as distinct objectives in the directions pursued by the research, as follows: the anti-adipogenic activity of leaf extracts of *Z. jujuba* and aerial

parts of *P. aviculare* and *P. hydropiper* in an *in vitro* model of obesity in human adipocytes have been established; the molecular pathways involved in the mechanism of anti-adipogenic action of apigenin, betulinic and maslinic acid in adipocytes have been determined; the effect of rosmarinic acid in SGBS adipocytes on adipogenesis and lipolysis as well as on the expression of inflammatory factors during adipocyte differentiation was characterized; the inhibitory effect on lipid depots by administration of betulinic acid (10 μ M) was shown to be associated with stimulation of *nhr-49* and *acs-2* expression, whereas at a concentration of 50 μ M betulinic acid affected genes related to lipid hydrolysis and the action of desaturases in *C. elegans* were proven; the effect of betulinic acid on the expression of microRNAs, whose target genes are involved in lipolysis and lipogenesis in *C. elegans*, has been evaluated.

The dissertation is well-constructed methodically, executed precisely at a high level, utilizing a diverse range of approaches, and presenting well-processed and concrete results. The writing style is distinctive and employs terminologically clear language.

The presented dissertation is written on 131 pages, which include 2 tables and 31 figures, with each figure containing several panels (ranging from 4 to 6, and in some cases up to 14). The bibliography mainly consists of titles from the past 10 years.

6. Assessment of the publications and personal contribution of the doctoral candidate

The publications presented for the current defence procedure for awarding the scientific degree “**Doctor of Philosophy**” include 6 publications in international journals. The quartiles of the publications are reported based on the metrics of scientific publications referenced in Scopus Scimago Journal Rank (SJR) <https://www.scimagojr.com/journalrank.php>., where 4 of the candidate publications included in the dissertations are in Q1. This is a result that a few doctoral candidates could be proud of, as it clearly demonstrates the high level of the conducted research and the accumulated results. I consider the candidate's personal contribution essential, especially given the fact that she is the first or second author of the publications presented. Consequently, the main part of the contributions reflects the personal merit of Martina Savova.

7. Summary

The summary of the dissertation, along with its content, accurately reflect and reveal the main points and ideas of the doctoral thesis. The most important results from the current research are presented in the summaries in both Bulgarian and English. The presented summary has been prepared according to the respective requirements.

CONCLUSION

The presented PhD thesis work of MPharm Martina Savova is indeed an extensive, thorough, and original scientific research with a fundamental and innovative character, which undoubtedly holds significant and substantial value for the theory of science. The dissertation has a very high scientific and knowledgeable impact with unquestionable contributions and meets all the requirements of the current Act for the Development of the Academic Staff in the Republic of Bulgaria and the Rules of the Institute of Microbiology, BAS. Based on the analysis made and the evident growth of the doctoral student, I provide my positive assessment for the research and the presented dissertation. I propose to the respected members of the scientific jury to award **Martina Stoyanova Savova** the educational and scientific degree of "**Doctor of Philosophy**" in the professional field 5.11. Biotechnology (Technology of biologically active substances).

17.07.2023

Plovdiv

Prepared by:

(Prof. **Albert Ivanov Krastanov**, DSc)