

SCIENTIFIC STATEMENT

on the dissertation work on the topic:

«Investigation of anti-obesogenic potential of extracts of *Alchemilla monticola* Opiz and *Ononis spinosa* L. for obesity control in vitro and in vivo models»

for awarding the educational and scientific degree "Doctor"

in the field 5.11. Biotechnologies

(Technology of biologically active substances)

to Eng. Saveta Georgieva Mladenova

with scientific supervisor: Prof. Dr. Milen I. Georgiev

By: Prof. Dr. Svetla Trifonova Danova, D.Sc.;

Institute of Microbiology "Stefan Angelov"/ BAS,

Scientific specialty 4.3. Biological sciences, Microbiology

1. REGARDING THE PROCEDURE:

Pursuant to a decision of the Scientific Council of the “Stefan Angelov” Institute of Microbiology—Bulgarian Academy of Sciences (Minutes No. 8/October 30, 2026) and an order (No. I-98/June 30, 2026) of the Director, I have been selected as a member of the Scientific Jury, in accordance with Article 4 of the Law on Scientific Research and Academic Degrees in the Republic of Bulgaria (ZRASRB) and Article 29 of the Regulations on the Conditions and Procedures for the Awarding of Academic Degrees and Titles at the Institute of Microbiology “Stefan Angelov”—BAS.

I hereby declare that I have no joint publications, projects, or conflicts of interest of any other nature within the meaning of paragraph 1, items 3 and 5 of the Law on the System of Scientific Degrees and Titles in the Republic of Bulgaria with the candidate being evaluated for the award of the “Doctor” degree.

2. EVALUATION OF THE DISSERTATION

2.1. Relevance of the Problem Addressed in the Dissertation

The PhD thesis submitted to me for review is a cutting-edge scientific study addressing the serious scientific challenge of obesity. It has been called the pandemic of the 21st century due to its rapidly increasing prevalence among adults and children, with severe social, economic, and health consequences. The doctoral candidate examines it as a multifactorial metabolic disease in which disturbances in energy balance and adipose tissue regulation are associated with chronic low-grade inflammation and an increased risk of type 2 diabetes, cardiovascular and metabolic diseases, fatty liver disease, and certain types of cancer. The complex and multifactorial pathogenesis of obesity underscores the need to seek new, multipurpose therapeutic approaches. In this context, medicinal plants and their biologically active compounds represent a promising natural resource, as their multicomponent

composition allows for simultaneous effects on various metabolic and molecular mechanisms. The doctoral candidate correctly notes that the application of modern “omics” approaches—metabolomics, transcriptomics, and proteomics—enables a more in-depth understanding of the mechanisms of action and the identification of new natural agents with anti-obesity potential. Therefore, the study of natural products as a source of effective and multipurpose agents for the prevention and control of obesity is scientifically relevant. All of this leads me to evaluate this research as a highly relevant fundamental scientific study with significant potential for practical application.

2.2. The candidate's literary awareness and theoretical preparation/

The literature review is well structured, concise and precisely focused on the topic of the dissertation. Current scientific data on biological activity, traditional use and phytochemical characteristics of *Alchemilla monticola* Opiz and *Ononis spinosa* L. (thunderthorn) as the main medicinal plants studied are presented in a balanced manner and data on the mechanisms of obesity are presented in a logical sequence. Special attention is paid to pathological growth of adipose tissue and its role in obesity, experimental models of obesity, connecting these two parts with a summary section dedicated to approaches to control and therapy of obesity. The literature review demonstrates the scientific and applied importance and prospects of new studies of medicinal plants.

Thus, the goal of the dissertation is logically derived: «Investigation of the influence of extracts of *A. monticola* and *O. spinosa* and their secondary metabolites on adipogenesis and lipid accumulation in an in vitro model of human adipocytes and subsequent in vivo validation of the most significant results in nematodes of the species *C. elegans*». To achieve this, 6 logically related tasks have been formulated.

The dissertation is clearly interdisciplinary in nature and requires serious theoretical and experimental training in the field of biochemical and metabolomic, cellular and molecular biological in vitro and in vivo research. This is evident both from the implementation of the 5 experimental tasks and from the way the results are presented. The number of cited literature sources is impressive - 237, with a predominance of those from the last 10-15 years.

The entire work is written in a concise scientific style, grammatically sound and very well technically designed on 109 standard pages. The PhD thesis is illustrated with 24 color figures and seven tables. An interdisciplinary work, in which current scientific terminology is correctly used in the fields of botany, biochemistry, and molecular biology phytochemistry and the results are interpreted.

2.3. Analysis of methodological approaches to achieving the goal of the dissertation work

The PhD student has employed an impressive array of diverse, modern methodological approaches, with proven reproducibility and relevance of the data obtained. For the selected medicinal plants, the collection site has been documented in accordance with the Nagoya Protocol. I highly commend the choice of subject matter, the experimental logic and consistency in the research design, and the

integration of well-documented modern methodological approaches into an innovative protocol. This original protocol combines NMR-based metabolic profiling, molecular docking analysis, and the identification of potentially bioactive plant metabolites, followed by in silico prediction of molecular interactions and in vitro validation in human adipocytes to elucidate the molecular mechanisms of anti-adipogenesis. Carefully designed in vivo experiments using the nematode *Caenorhabditis elegans* contribute to this. Here, I would like to highlight the inclusion of a logical sequence of in vivo test panels, such as the viability and motility assay in nematodes and the determination of glucose-induced lipid accumulation in *C. elegans*; protein detection via immunoblotting, and quantitative real-time polymerase chain reaction (RT-qPCR) for the analysis of RNA and microRNA in the in vivo model of the wild-type *C. elegans*. This is an original experimental platform with methodological significance because it can be successfully applied to the evaluation of other medicinal plants or their beneficial effects.

2.4. Evaluation of the achieved results and contributions of the dissertation work

The goal of the dissertation is ambitious, it was achieved by solving the panel of 6 tasks, and even the first task - the literature review on the problem has an important methodological contribution - presents an original way to integrate metabolomic, computational/in silico approaches and cellular models. The doctoral student obtained and characterized extracts from ethnopharmacologically applied medicinal plants *Alchemilla monticola* Opiz and *Ononis spinosa* L. (thunderthorn) and their specific metabolites. NMR - based metabolic profiling visualizes organic acids and flavonoid representatives - astragalin, catechin, quercetin, quercetin-3-O- β -glucuronide, quercitrin, isoquercetin, rutin, vanillic acid and vitexin in the extracts from the ground parts of alchemy.

Metabolic profiling of an extract of thunderthorn roots was also performed by analyzing one- and two-dimensional NMR spectra, proving ononine, sativanon, onogenine and maakiain. The doctoral student does not limit herself to empirical research on the composition of the two plant extracts, but builds on it with subsequent docking molecular analysis and in silico assessment of potential interactions with protein molecules. Thus, specific plant metabolites were selected, which allows us to move from correlation to scientifically proven disclosure of the effect. Thus, the scientifically based hypothesis about the anti-obesogenic mechanisms of action of these substances was objectively evaluated with the panel of in vitro, in silico and in vivo experiments. This is already a qualitative development of the study. I cannot fail to note the fact that the work presents an unexplored aspect of the potential of the two medicinal plants, by identifying specific bioactive candidates with an anti-obesogenic effect. Cytotoxicity, gene expression of genes from important metabolic pathways and the anti-adipogenic potential of *A. monticola* extracts and two metabolites astragalin (AST) and quercetin

(QUE) in an in vitro model of human adipocytes and of *O. spinosa* extract, ononin (ONON) and maackiain (MACK) were evaluated.

Important fundamental and applied scientific results have been obtained, including in vivo cellular experiments. For example, a dose-dependent and statistically significant reduction in lipid accumulation was observed in a glucose-induced model following treatment with MASK. Furthermore, the doctoral student included in vivo tests with *C. elegans*, which is a highly suitable model for verifying whether the observed cellular anti-adipogenic effects are confirmed as a reduction in lipid accumulation throughout the entire organism. The data obtained from computer-aided docking simulations, gene expression analysis, and changes in the levels of target proteins provide evidence for the potential mechanisms underlying the anti-adipogenic effect of the extract from the aerial parts of *Astragalus* and its secondary metabolite, astragalin.

A distinctive final stage consists of molecular transcriptomic analyses demonstrating that MACK and ONON influence the expression of numerous transcription factors during both the early and terminal phases of adipogenic differentiation. The results are very well illustrated with color figures. The doctoral student very successfully uses heat maps to visualize the effects of multivariate analyses, but I would recommend that she avoid summarizing gene expression using a minimum-maximum heat map scale. Given the abundance of results—particularly the data from the cellular, qPCR, and in vivo experiments—I would recommend a slightly more critically structured and analytical discussion that clearly highlights the significance and original contributions of the doctoral candidate and the team from the Metabolomics Laboratory.

A distinctive final stage consists of molecular transcriptomic analyses demonstrating that MACK and ONON influence the expression of numerous transcription factors during both the early and terminal phases of adipogenic differentiation. The results are very well illustrated with color figures. The doctoral student very successfully uses heat maps to visualize the effects of multivariate analyses, but I would recommend that she avoid summarizing gene expression using a minimum-maximum heat map scale. Given the abundance of results—particularly the data from the cellular, qPCR, and in vivo experiments—I would recommend a slightly more critically structured and analytical discussion that clearly highlights the significance and original contributions of the PhD student and the team from the Metabolomics Laboratory. This comment does not diminish the indisputable scientific merits of the overall study; on the contrary, it will clearly demonstrate the significance of the work. The indisputable evidence for this high assessment is the three publications in highly cited international journals, of which Eng. Saveta Mladenova is the first author. This demonstrates her erudition, theoretical and experimental expertise, and the doctoral candidate's personal involvement, supported by a division of labor protocol (proving a leading contribution of 50%). To date, the three articles have been cited a total of 63 times, which is the highest mark of recognition within the international scientific community.

The 6 conclusions drawn are a logical consequence of the presented results and prove a comprehensive, complex and very high scientific level doctoral dissertation. I fully accept the formulated 7 scientific-fundamental and scientific-applied contributions, and I would like to note that they modestly reflect only a part of the indisputable scientific achievements.

I have the following questions for the doctoral student:

1) Why is she studying the above-ground parts of the common thistle and only the root of the blackthorn?

2) To what extent does the analysis of the studied metabolites and plant extracts bring us closer to obtaining the much-desired anti-obesogenic natural compounds to help combat obesity and insulin resistance, and what is your opinion on a possible synergistic effect when combining the studied plant metabolites/extracts from these two medicinal plants?

3. CONCLUSION

In conclusion, I would like to summarize that, in terms of its objective, scope, results, and contributions, this dissertation fully meets and exceeds the requirements for the award of the academic degree of “Doctor.” The topic and objective of the dissertation are original and innovative. The methods are among the most modern, appropriately selected, and methodologically adapted to the study of two ethnopharmacologically used medicinal plants: *Alchemilla monticola* Opiz (lady’s mantle) and *Ononis spinosa* L. (thorny restharrow). The material is suitable for a dissertation and is the doctoral candidate’s own work. Based on the foregoing, I can confidently state that the reviewed dissertation represents an original scientific study with both theoretical and practical significance. It meets all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation, and the Regulations of the Institute of Medicinal Plants—Bulgarian Academy of Sciences (IMicb-BAS).

All of this gives me grounds for an overall high evaluation of the dissertation, on the basis of which I propose that the esteemed scientific jury award Eng. Saveta Georgieva Mladenova the educational and scientific degree of “DOCTOR” in scientific specialty 4, field 5.11. Biotechnology (Doctoral Program “Technology of Biologically Active Substances”).

Sofia, 21.08.2026 r.

.....(Prof. S. T. Danova, DSc)