

OPINION

From prof. Magdalena Spasova Kondeva-Burdina, PhD, ERT

Department “Pharmacology, pharmacotherapy and toxicology“,

Faculty of Pharmacy, Medical University-Sofia

RE: Dissertation on the topic: “Investigation of the anti-obesity potential of extracts from *Alchemilla monticola* Opiz and *Ononis spinosa* L. for the control of obesity in *in vitro* and *in vivo* models,” submitted for defense toward the award of the educational and scientific degree of “Doctor” in Field 5.11. Biotechnology (Technology of Biologically Active Substances), by eng. Saveta Georgieva Mladenova, under the supervision of prof. Milen Georgiev, PhD.

Pursuant to Order No. I-98 of June 30, 2026, I have been designated to prepare an opinion in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations on the Conditions and Procedures for Awarding Academic Degrees and Holding Academic Positions at the Institute of Microbiology – Bulgarian Academy of Sciences.

Brief Biographical Information

Education

Saveta Georgieva Mladenova earned a Master’s degree in Biotechnology Engineering in 2003 from the University of Chemical Technology and Metallurgy in Sofia.

Scientific Output

The author has published 3 scientific papers related to the dissertation in Q1-quartile journals. There have been 63 citations recorded (as of June 18, 2026).

The author has participated in 7 scientific conferences and congresses.

The scientific research included in this dissertation was carried out with the financial support of the PlantaSYST project (SGA No. 739582 and FPA No. 664620) under the European Union’s Horizon 2020 program, and project BG05M2OP001-1. 003-001-C01, funded by the

European Regional Development Fund through the “Science and Education for Smart Growth” Operational Program.

h-Index = 4 – IMPRESSIVE FOR A DOCTORAL STUDENT

Work Experience

March 2026 – present: Quality, Research, and Development Manager at “C7 Commercial” EOOD.

September 2022 – February 2026: Worked as a Quality Management System Specialist at “Komak Medical” EOOD, Business Sector: Phase I–IV Clinical Trials.

From May 2018 to September 2022: Worked as Head of the “Development and Innovation” Division at “BUL BIO-NCZPB” EOOD, Business Sector: Pharmacy. Prepared reports on studies related to biotechnological medicinal products. Participated in the preparation of annual quality reports for the Bulgarian Drug Agency (IAL) and the World Health Organization (WHO). Validated methods. Performed statistical analysis of test results to ensure processes remained under statistical control.

Relevance of the Topic

The prevalence of obesity has reached epidemic proportions in recent decades, leading to an increased incidence of associated diseases such as type 2 diabetes, atherosclerosis, hypertension, and dyslipidemia, as well as a corresponding rise in healthcare costs.

Current recommendations for managing obesity focus primarily on changes in diet and physical activity. However, diets that severely restrict calorie intake often pose a major challenge for patients in the long term, and once they return to a normal calorie intake, the body regains the lost weight. Combining lifestyle changes with drug therapy supports the weight-loss process. The approved anorexigenic agents currently available to the medical community are limited both in number and in mechanism of action. A revolutionary development in terms of weight loss achieved was the introduction in 2020 of incretin mimetics (originally developed for type 2 diabetes) and, in particular, the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide. Despite the high efficacy that semaglutide demonstrates in patients with obesity, as measured by metabolic profile and body mass index (BMI), it turns out that a significant percentage of patients do not respond to this biological therapy. Therefore, the discovery of a safe and effective treatment for obesity is of the utmost importance, given the serious limitations and adverse side effects associated with currently approved medications.

Structure of the dissertation

The dissertation submitted to me for review is structured in accordance with the requirements of the Law on the Development of Academic Staff and possesses all the qualities – AND EVEN EXCEEDS THEM—of a complete scientific product.

This **literature review** examines in detail the issues related to the key role and modulation of major signaling pathways involved in adipogenesis, energy metabolism, and intercellular and intracellular communication as a potential new approach to preventing overweight and obesity. Among the potential molecular targets for influencing obesity are the major adipogenic transcription factors – CCAAT/ enhancer-binding protein alpha (C/EBP α) and peroxisome proliferator-activated receptor gamma (PPAR γ), as well as the molecular components of lipogenesis regulated by them, such as sterol regulatory binding protein 1 (SREBP1), fatty acid synthase (FASN), and acetyl-CoA carboxylase. In addition, the stimulation of lipolysis is a parallel mechanism through which excess weight can be controlled. Another key signaling pathway is that of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) cascade, which is involved in numerous cellular functions, including differentiation and insulin-stimulated glucose uptake in adipocytes. The biological activity, traditional use, and phytochemical characteristics of *Alchemilla monticola* (lady's mantle) and *Ononia spinosa* (thunder thorn) are also discussed in detail.

The objective of this study is clearly and specifically formulated and stems from the conclusions of the literature review. Six specific tasks have been formulated to achieve this objective.

The methodological approach includes a wide variety of modern in vitro/in vivo methods, such as:

- NMR-based metabolic profiling;
 - Real-time polymerase chain reaction (PCR) of mRNA and microRNAs;
 - Western blot analysis of key transcription factors involved in lipolysis;
 - Nile red staining of accumulated lipid droplets in nematodes;
 - An *in vivo* model for studying the phenotype and lipid accumulation associated with obesity in *Caenorhabditis elegans* nematodes;
- which ensures a high degree of reliability for the results obtained.

The development of this *in vivo* model of obesity in *Caenorhabditis elegans* nematodes is in accordance with the requirements for reducing the number of experimental animals, known as the 3R principle (reduce, replace, refine).

The results are original in nature and are properly, accurately, and comprehensively illustrated. The objective has been successfully achieved at an exceptionally high scientific level.

The results obtained establish in detail the effect of plant extracts and their metabolites from *Alchemilla monticola* (lady's mantle) and *Ononia spinosa* (thunder thorn) on the function and physiology of fat cells – the primary structural units of adipose tissue – and on the modulation of their inflammatory response. A constellation of methods and models has been developed that allows for the elucidation of the possible molecular mechanism of action of the studied plants, their extracts, and metabolites. Inhibition of the PI3K/AKT signaling pathway has been identified as the primary molecular mechanism underlying the effect of blackthorn extract on adipogenesis. Quercetin significantly suppresses lipid accumulation, while lipolysis is moderately inhibited. The anti-adipogenic activity of thunder thorn extract is due to an indirect influence on the molecular pathways responsible for adipogenic differentiation. It has been established that ononin exhibits anti-adipogenic activity by regulating SIRT1 and inhibiting PPAR γ , while maakiacin inhibits adipogenesis via PPAR γ signaling and suppresses lipogenesis by downregulating SREPB1 and the lipogenic genes it regulates. For the first time, the potential of maakiacin to inhibit lipid metabolism has been confirmed at the organismal level in *C. elegans*.

In her **discussion** of the results, the author has successfully summarized the data obtained and compared them with those found in the literature.

The conclusions are presented concisely and are consistent with the stated objectives and tasks. They fully reflect the results of the studies.

I agree with **the contributions** made, namely:

Of a fundamental scientific nature:

1. The potential of *Alchemilla monticola* Opiz extract to inhibit differentiation and lipid accumulation in human adipocytes has been demonstrated, as well as the role of its active components, astragalin and quercitrin.

2. The effect of *A. monticola* extract in suppressing the early stage of adipogenesis in human adipocytes by inhibiting PI3K activity has been determined.

3. The signaling pathway responsible for the observed anti-adipogenic effect of *Alchemilla monticola* extract was identified, namely the PI3K/AKT pathway and PPAR γ protein levels.

4. The bidirectional effect of astragalin on the regulation of the PI3K/AKT signaling pathway in human adipocytes has been established, with the critical concentration for this bidirectional regulation ranging between 10-25 μ M.

5. The potential of maakiacin to inhibit adipogenesis via PPAR γ /C/EBP α signaling and to inhibit lipogenesis by downregulating SREBP1 and its lipogenic genes has been demonstrated.

6. A proposed mechanism of action for the anti-adipogenic effect of ononin involves increasing SIRT1 and inhibiting PPAR γ .

7. The potential of maakiin to activate molecular signaling pathways associated with caloric restriction (aak-2/sir2.1) and energy expenditure and lipid metabolism (nhr-49/mdt-15) has been determined in an in vivo model.

Scientific and Applied Implications:

The data obtained suggest that maakiain could be used as a natural compound capable of mimicking calorie restriction.

I would like to express my high regard for the detailed design of the study and the overall presentation of the work, which confirms the author's personal contribution to this dissertation. The dissertation represents a logically planned and competently executed scientific study.

It is noteworthy that the doctoral candidate has included three scientific publications related to the dissertation, all of which appear in journals with an impact factor and Q1 ranking; in one case, the impact factor exceeds 7. This far exceeds the quantitative criteria for the award of the academic and scientific degree of "DOCTOR."

CONCLUSION

The research conducted by eng. Saveta Georgieva Mladenova is a completed dissertation that EXCEEDS the requirements of the Law on the Development of

Academic Staff in the Republic of Bulgaria. The research topic is highly relevant, scientifically sound, and suitable for a dissertation. The findings and conclusions drawn from the research make a significant scientific contribution.

Based on the foregoing, I award an “Excellent” grade to this work and vote in FAVOR of conferring the academic and scientific degree of “DOCTOR” in Field 5.11. Biotechnology (Technology of Biologically Active Substances) upon eng. Saveta Georgieva Mladenova.

07.08.2026

Sofia

Prepared the opinion:

/prof. Magdalena Spasova Kondeva-Burdina, PhD, ERT/