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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	PharmDr. Jakub Treml, Ph.D.
Habilitation thesis	Natural phenolics in alleviation of symptoms and complications of type 2 diabetes mellitus
Reviewer	Doc. MUDr. Katarína Šebeková, DrSc.
Reviewer's home unit, institution	Ústav molekulárnej biomedicíny, Lekárska fakulta Univerzity Komenského v Bratislave, SR

1. The relevance and importance of the chosen topic

The habilitation thesis focuses on the *in vitro* antioxidant, anti-inflammatory, and antidiabetic effects of natural phenolic compounds, with a focus on their potential to modulate selected pathomechanisms, symptoms, and complications of type 2 diabetes mellitus (T2DM). This represents a highly relevant and socially significant topic. DM is one of the most prevalent chronic non-communicable diseases. The search for new therapeutic strategies, especially those based on natural substances with multitarget effects, is fully in line with current trends in pharmaceutical and biomedical research.

The chosen topic has an interdisciplinary focus, integrating knowledge from molecular pharmacy, biochemistry, cell biology, and pharmacology. From the perspective of the habilitation procedure, the topic meets the requirements for scientific independence and documents the author’s long-term, systematic research focus.

2. Objectives and hypotheses

The objectives of the habilitation thesis are formulated in the last sentence of the introduction. The author focuses on verifying the concept of the so-called "one compound with a combination of effects“, specifically the antioxidant, anti-inflammatory, and antidiabetic activities of natural phenolics. The hypotheses directly build on the author’s previous publication activities. The stated objectives are consistent with the methodological approaches employed and the presented results.

3. Structure of the thesis

The habilitation thesis comprises **217 numbered pages**. The structure meets the requirements for habilitation theses and allows the reader to follow the logical sequence from the theoretical background to the interpretation of the results. **The first 44 pages include** the *title page, author's statement, abstract in English, acknowledgements, list of abbreviations, table of contents, Introduction* (including the listing of articles forming the core of the habilitation thesis, with the candidate’s percentage contribution in the individual assessed indicators), then *the chapters: Molecular pharmacy* (with a curricular focus and a list of the candidate’s published papers that are not directly related to the topic of the habilitation thesis), *Complications and treatment of T2DM,*

Natural phenolics (including a description of the antioxidant, anti-inflammatory and antidiabetic activities of natural phenolics, encapsulation of natural products, with listed results obtained by the author), *Conclusions, and References*. **From p. 45 onwards**, there are **12 *ad extenso* articles**, forming the core of the scientific part of the habilitation thesis, published in peer-reviewed journals. These are published papers **that have undergone rigorous peer review in renowned professional journals, confirming scientific rigor and novelty of the findings**. I have no comments or questions about this part of the thesis. However, as the given data is strictly experimental, I miss the discussion and proposals on how these potentially beneficial effects could be translated into in vivo effects in animal models, regarding dosage/toxicity/potential side effects, bioavailabilty, pharmacokinetics, intercations, etc.

4. Comments

Regarding the Conclusions, given that the candidate is the Head of the Institute of Molecular Pharmacy, I consider the formulation of perspectives—presented in four points over ten lines, without any specific details, visions, or discussion of potential problems—to be insufficient.

A serious **problem with the thesis is the fact that** the habilitant explicitly links the investigated antioxidant, anti-inflammatory, and antidiabetic effects of natural phenolics to T2DM. I realize that the habilitant is not a physician, physiologist, or pathophysiologist, but the framing of the work into the pathophysiological context of T2DM had to be considered in advance, and profoundly studied to adhere to the basic and relevant facts, or the accuracy of the statements had to be consulted with an expert in the given field. **The section** on the pathophysiology, diagnostics, chronic complications, and therapy of T2DM in the submitted thesis **exhibits serious professional shortcomings, significantly reducing the overall scholarly level of the habilitation thesis**.

E.g., the passages on T2DM read like popularizing texts that do not meet the standards expected of a habilitation thesis, nor the level of professional expertise and pedagogical practice required in the field. The complex pathophysiological mechanisms of T2DM are reduced to simplified schemes that do not reflect current scientific knowledge of the disease's heterogeneity, the dynamics of insulin resistance, the role of β-cell dysfunction, or the complex interrelationship between metabolic, oxidative, and inflammatory status. Some statements are factually incorrect or misleading, and, in some cases, historical concepts are mixed with current diagnostic and therapeutic recommendations without a clear distinction, which may lead to misinterpretation of the clinical reality of T2DM. Selected issues are highlighted in the appendix to this review.

The aforementioned shortcomings represent **a fundamental weakness of the thesis**, which contrasts significantly with the high quality of the experimental part and the author's published outputs. From the perspective of the habilitation procedure, these sections **must be regarded as inadequately developed and require substantial revision** for the work to be considered to meet the pedagogical standards expected of a habilitated academic. Thus, **I do not recommend accepting the thesis** of *PharmDr. Jakub Treml, PhD.* **for defense** in its given form.

Conclusion

In the current form, the habilitation thesis entitled *"Natural phenolics in alleviation of symptoms and complications of type 2 diabetes mellitus*" by *PharmDr. Jakub Treml, Ph.D.*, **does not fulfil** the requirements expected of a habilitation thesis in the field of Molekulární biologie a genetika.

Date: December 25, 2025 Signature

Appendix to the assessment of the habilitation thesis of PharmDr. Jakub Treml, PhD.:

The description of DM diagnostics does not align with current guidelines (Good diagnostic procedures) and therefore does not reflect clinical practice. Nowadays, the diagnosis is not necessarily limited solely to glycemia. However, even if glycemia is considered, the recommendations (guidelines) on cut-offs are internationally proposed and accepted, and differ from those listed by the author. The author does not provide references for the threshold values he reports. Descriptions of diabetic syndrome, its diagnostics, complications, and treatment recommendations can be found on the website of the Czech Diabetologic Society (ČDS).

The thesis lacks an overview of the pathogenetic mechanisms. Although numerous mechanisms contribute to the pathogenesis of T2DM, the author could mention at least those in which he anticipates a beneficial effect of natural phenolics. Probably the most important being the metabolic pathways (glycation products, polyol and hexokinase pathways, altered structure and function of hexosamines), and minor ones such as impaired oxidative status and low-grade inflammation.

The description of chronic complications of T2DM is oversimplified and sometimes inaccurate, as it fails to address their pathogenetic diversity (for example, advanced glycation end products, AGEs, whose pathogenetic role among non-communicable chronic diseases is arguably most important in DM. Description of their pathomechanisms is missing: direct toxicity, i.e., modification of protein structure and function; and indirect toxicity (the author mentions only macrophages), namely the interaction of AGEs with the ubiquitous cell-surface receptor RAGE, leading to activation of NF-κB, MAPK, and other pathways, followed by the production of ROS, activation of inflammatory and pro-fibrotic metabolic pathways, and ultimately to the manifestation of microvascular complications).

The section on T2DM therapy is neither aligned with the current recommendations of the ČDS. The author presents a limited and outdated view of non-pharmacological treatment, predominantly focusing on diet therapy, whereas, as a first-line approach, it must be comprehensive. The description of pharmacological treatment does not accurately reflect current therapeutic algorithms, individualized treatment, the role of modern antidiabetics with different modes of action, proven cardiovascular and renal benefits, or drugs with dual effects. Moreover, I miss an explicit statement that the goals are to maintain optimal metabolic control, minimize the risk of hypo- and hyperglycemia, slow the development of microvascular complications, and prolong and improve the quality of life, as well as a description of the role (or vision) of natural phenolics in this context. In this section, the habilitant could envision the place of natural phenolics under investigation.

"...deterioration …in the kidney…may end in a need for renal dialysis" - it would be more appropriate to refer to the need for renal replacement therapy, which includes dialysis and transplantation.

3.2. Anti-inflammatory "…inflammation can lead to a harmful dysregulated response and produce systemic damage that results in chronic inflammatory disorders, such as obesity, insulin resistance, and T2DM." This formulation states that inflammation is a causative factor in systemic damage that leads to chronic inflammatory disorders. However, obesity and T2DM are diseases, and insulin resistance is a metabolic state characterized by chronic low-grade inflammation. They are not directly inducible by inflammation per se.

In addition to the metabolic endotoxemia mentioned by the author (the LPS–TLR4 mechanism linked to gut microbiota dysbiosis and disruption of the intestinal barrier), activation of RAGE receptors by their ligands—AGEs—as a direct consequence of chronic hyperglycemia, plays a crucial role in the induction of chronic low-grade inflammation in T2DM. This mechanism is probably activated during the manifestation of T2DM earlier than the aforementioned LPS-TLR4.

3.3. Antidiabetic activity. The liver is an insulin-independent tissue; in hepatocytes, insulin regulates the metabolic processing of already present glucose. (Glucose transport via GLUT1,2,3 is not dependent on insulin; therefore, under chronic hyperglycemia, insulin-independent tissues are primarily and most affected).

"Fragile equilibrium" – under conditions of homeostasis, this equilibrium is in fact extremely robust (regulated not only by glucagon but also by cortisol, adrenalin, growth hormone, etc.), in T2DM it is not a matter of "tipping the scales", but of a complex collapse of the system, when the beta cells of the pancreas are unable to produce an excessive amount of insulin to maintain glycemia.

"...insulin does not function properly" - more appropriate: reduced biological response of target tissues to insulin.