

## HABILITATION THESIS REVIEWER'S REPORT

### Masaryk University

#### Applicant

Mgr. Danuše Tarkowská, Ph.D.

#### Habilitation thesis

Trace and ultra-trace analysis of natural substances of terpenoid character

#### Reviewer

doc. Mgr. Jan Lochman, Ph.D.

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The habilitation thesis of Mgr. Danuše Tarkowská, Ph.D. is structured in a short description of objectives focused on the analysis of trace and ultra-trace amounts of substances of plant origin (p. 7), a part introducing terpenoids as a huge and structurally diverse group of substances (p. 9-10), followed by the problematics of plant hormones and their analysis with further focus on gibberelins (p. 11-23), brassinosteroids (p. 24-37) and other substances of terpenoid character (p. 38-51), and problems of matrix in trace analysis (p. 52-55). The author presents various sample preparation and pre-concentration strategies used in the analysis of different plant biological matrices and potential problems associated with ultra-low levels of selected analytes in plant material. The author discusses the potential challenges of different methodological approaches and aims to identify the most efficient methods for routine analysis of plant hormones across different matrices and concentration levels, based on her previous experience, followed by conclusions and future perspectives.

The topic of the thesis is highly relevant to plant research as many signaling compounds play a crucial role in developmental processes and responses to environmental changes. These compounds are often present in very low concentrations, and some have only recently been discovered due to advances in analytical approaches for their detection. Thus, the new methods can help reveal important aspects of these compounds and aid in the synthesis of new pesticides, insecticides, and substances to promote plant growth to combat global climate change.

Overall, the habilitation thesis presented is well-written and easy to follow. The text presents the author's role and contributions to different projects in an informed manner, while also clearly presenting the objectives and goals within the broader context of the studied field. This indicates a high level of professional scientific maturity. The five review papers and eleven research papers were published in high-impact (Q1 or Q2), international, peer-reviewed journals in the fields of plant science or analytical chemistry. Dr Tarkowská has made a significant contribution to these papers. This is evidenced by her role as corresponding/first author in seven of them or by the application of introduced analytical methods to other papers related mainly to plant physiology.

Although the overall impression of the work is positive, there are a few criticisms. The work contains several terminological and physiological inaccuracies, e.g. some forms of plant

hormones can act also between plants (methyl salicylate or methyl jasmonate) when salicylic and jasmonic acid were first described in the 1970s but were not classified as plant hormones due to a lack of definition (p10). Low molecular weight substances act as a chemical defense against both biotic and abiotic stresses. Conversely, proteins also act against herbivores or insects (p38). Sometimes incomplete abbreviation explanations make it difficult for uninitiated readers to navigate. In addition, to avoid pixelation when zooming in, the quality of some figures should be improved (Figs. 7, 10, or 16). As the author's paper primarily focuses on introducing new analytical approaches for trace amount analysis, it may be beneficial to provide a general description of the methods used for sample preparation and analysis in plant research, including their potential pitfalls. Chapter II.III could be placed at the beginning of the thesis to provide this context before proceeding to the analysis of individual terpenoid compounds.

**Reviewer's questions for the habilitation thesis defence** (number of questions up to the reviewer)

- 1) In practice, we usually analyze the concentration of a substance relative to fresh weight (FW) or dry weight (DW). However, from a physiological perspective, the most important factor is the actual concentration of a given plant hormone in a cell or intracellular space. Is there a way to calculate this concentration?
- 2) What are the approx.  $K_d$  values of receptors for studied phytohormones?
- 3) Would be 2D-LC a useful technique for separating gibberellins?
- 4) What are the current limitations and challenges in single-cell metabolomics regarding plant hormones?

**Conclusion**

The habilitation thesis entitled "Trace and ultra-trace analysis of natural substances of terpenoid character" by Danuše Tarkowská **fulfils** requirements expected of a habilitation thesis in the field of Analytical Biochemistry.

Date: 30.12.2023

Signature: