

CONCLUSION

of the Dissertation Council for the group of specialties/educational programs 6D060600, 8D05301—Chemistry; 6D072000, 8D07104—Chemical Technology of Inorganic Substances; 8D07102—Chemical Engineering; 6D072100, 8D07105—Chemical Technology of Organic Substances; and 6D73900, 8D07101—Petrochemistry at Al-Farabi Kazakh National University on the dissertation of Izaz Ahmad titled “Using advanced spectroscopic, and chromatographic techniques to study the biotransformation of steroidal drugs, and phytochemical constituents of *Tussilago Farfara* L” submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D05301—“Chemistry” (Protocol No. 79 dated June 10, 2026).

1. Based on the results of the defense and secret voting, the Dissertation Council decided to send Izaz Ahmad dissertation titled “Using advanced spectroscopic, and chromatographic techniques to study the biotransformation of steroidal drugs, and phytochemical constituents of *Tussilago Farfara* L” for revision to address the following comments:

1. Mass spectra and HPLC chromatograms of isolated compounds, which are very important for confirming the purity of isolated compounds and proving main provisions, are not provided in the dissertation. According to mass spectra provided in Supplementary Material to the article “Caffeoylquinic acid derivatives isolated from *Tussilago farfara* L. as potential anti-leishmanial agents; In vitro and in silico studies” published in Natural Products Research (2026), several isolated compounds can have major impurities.

2. Values of purities of the isolated compounds are not provided and not discussed in the dissertation.

3. For most isolated compounds (#1, 2, 4-17), provided m/z values are equal or very close to average molecular masses (MW) reported in the literature or (MW+H), not to monoisotopic masses of molecular ions ($[M]^+$ or $[M+H]^+$) determined by mass spectrometry. For example, 198.17 is the average molecular mass of syringic acid ($C_9H_{10}O_5$), while m/z of molecular ion $[M]^+$ obtained using EI-MS is 198 (exact mass after EI-HRMS is 198.0528). This contradicts the answer of Mr. Ahmad during the defense where he said that these m/z values were experimentally determined. Mass spectra provided in Supplementary Material to the article “Caffeoylquinic acid derivatives isolated from *Tussilago farfara* L. as potential anti-leishmanial agents; In vitro and in silico studies” published in Natural Products Research (2026) do not contain m/z values provided in the dissertation and the article for isolated compounds (#1, 2, 4-17). This also means that some statements in the dissertation are misleading, for example: “Compound 2 was obtained as a white powder; EI-MS: m/z 198.17 $[M]^+$, corresponding with the molecular formula $C_9H_{10}O_5$.” - m/z 198.17 most probably was not measured, and it does not correspond to the formula $C_9H_{10}O_5$.

4. The ^1H NMR spectra and the other spectra provided are insufficient to confirm the structures of compounds 6, 8-10, and 12-19. Establishing their regiochemistry and - for 14 and 15 - stereochemistry, as well as the site of glycosylation, requires ^{13}C NMR spectra and two-dimensional HMBC experiments; the relative configuration of compounds 14 and 15 requires NOESY (or ROESY). Correct interpretation additionally requires HSQC and COSY.

5. Section 2.7.1 reports spectroscopic methods, but mass spectrometry is not a spectroscopic method. In addition, mass spectrometry does not determine the mass, it provides mass spectra.

6. Detailed parameters of mass spectrometric analyses (ionization mode and energy, m/z range, temperatures and others) are not provided.

7. NMR and MS spectra must be presented in the thesis also in order to avoid any possibility of unreliability of the results: the NMR and MS data are correct for the proposed compounds but without these spectra, the correlation between these data and the experimental work can not be fully evaluated.

8. A deeper attention must be also given to the possibility that some compounds are actually artifacts of the separation procedure.

9. The presentation of the biological results and the related methodology must be detailed and clarified.

10. In the introduction the biological context and therapeutic rationale could be strengthened. In particular, a more focused state-of-the-art discussion on the biological assays employed, the medical relevance of leishmaniasis and aromatase-related pathologies, the cultural/traditional context supporting the selection of *T. farfara*, and the justification for the chosen therapeutic targets (PTR-1 and aromatase) would improve the scientific framing of the study.

11. The description of methodology and discussion of the preliminary attempt of extracting steroid compounds from *Tussilago farfara* is needed.

12. Collaborative work for the establishment and production of recombinant PTR-1 enzyme must be stated.

13. The methodology of the enzymatic inhibition assays would benefit from a more detailed description of the experimental controls. In particular, the composition and role of the negative controls should be explicitly reported (e.g., assay mixture lacking enzyme or substrate, solvent controls, etc.) to improve methodological transparency.

14. The procedure used to generate the dose-response curves and IC_{50} plots presented in Figure 16 should be described more clearly. The thesis should specify the software or mathematical model used for curve fitting and explain the concentration scale reported on the x-axis, which is not immediately intuitive for the reader.

15. The preliminary screening used to select the two microbial strains used for biotransformation must be described and discussed in the thesis

16. The source of the aromatase enzyme employed in the inhibition assays is not clearly indicated. The thesis should specify whether the enzyme was commercially obtained or produced in-house and, if commercial, provide the supplier and relevant specifications.

17. Please verify all reported m/z values for isolated compounds and metabolites against the original acquired mass spectra. Several reported values appear to resemble average molecular masses rather than experimentally observed m/z values. The dissertation should clearly distinguish observed m/z , nominal mass, monoisotopic exact mass, and average molecular mass.

18. For all compounds 1-20 from *Tussilago farfara* L., please provide a corrected mass-spectral table including molecular formula, ionization mode, ion type, calculated monoisotopic mass, experimentally observed m/z , mass error if HRMS was used, and corresponding spectrum/figure number.

19. Please specifically recheck the reported EI-MS values for simple phenolic compounds, such as vanillic acid, syringic acid, esculetin, *p*-hydroxybenzoic acid, quercetin, and kaempferol. If the values are average molecular masses or literature molecular weights, they should not be reported as experimentally observed EI-MS m/z values.

20. Please clarify whether HRMS data were actually recorded for each compound/metabolite. If HRMS was used, the dissertation should report calculated exact mass, observed exact mass, ion species, and mass error in ppm. If only low-resolution MS was recorded, the data should be reported as low-resolution MS without exact-mass claims.

21. Please provide chromatographic purity evidence for all isolated compounds/metabolites before biological testing. TLC visualization alone is not sufficient to confirm purity for enzyme inhibition studies. HPLC/UPLC chromatograms, retention times, peak purity, or other suitable purity evidence should be provided.

22. For closely related caffeoylquinic acid isomers, especially compounds 14-20, please provide clearer structural assignment evidence. The dissertation should specify the diagnostic NMR shifts, coupling patterns, HMBC/COSY correlations, and/or MS fragmentation evidence used to distinguish positional and stereochemical isomers.

23. Please verify the IC₅₀ value of compound 15 in the LmPTR-1 assay. The reported active range and the supplementary table appear inconsistent. This discrepancy should be corrected or clearly explained, and the number of biological/technical replicates used for IC₅₀ determination should be stated.

24. For the fungal biotransformation experiment, please clearly state the control experiments used to confirm that metabolites 2-9 are true biotransformation products. Substrate-only, organism-only, medium blank, and solvent-control experiments should be mentioned and discussed.

25. For the new steroidal metabolite 2, provide complete and traceable structural confirmation, including molecular formula, observed MS/HRMS ion, key ¹H- and ¹³C-NMR signals, DEPT evidence, and key HMBC/COSY/NOESY correlations supporting the dioxolane-containing structure and stereochemical assignment.

26. Interpret docking, MD, and MM-GBSA results as supportive mechanistic evidence only, not as direct proof of biological potency. The relationship between experimental IC₅₀ values, docking scores, and binding

energies should be discussed carefully wherever the correlation is weak or inconsistent.

2. The revised dissertation must be submitted to the Dissertation Council within three months, with the possibility of an extension for no more than an additional three months. The decision to extend the deadline is made by the Dissertation Council based on a request from the doctoral candidate. If the revised dissertation is not submitted within the established timeframe, the doctoral candidate must undergo a repeated defense.

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