

REVIEW
of the official reviewer

for the dissertation of Izaz Ahmad on the topic “Using Advanced Spectroscopic, and Chromatographic Techniques to Study the Biotransformation of Steroidal Drugs, and Phytochemical Investigation of Tussilago farfara L.” presented for the degree of Doctor of Philosophy (PhD) in the specialty “8D05301 – Chemistry”

No.	Criteria	Eligibility (one of the options must be checked)	Justification of the position of the official reviewer
1.	The topic of the thesis (as of the date of its approval) corresponds to the directions of development of science and/or state programs	1.1 Compliance with priority areas of science development or government programs: 1) The thesis was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program) 2) The thesis was completed within the framework of another state program (indicate the name of the program) 3) The dissertation corresponds to the priority direction of the development of science, approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction)	The dissertation does not clearly indicate a specific state-funded project or target program in the submitted abstract and introductory sections. Therefore, option 3 is the most appropriate selection. The thesis directly addresses medicinal chemistry, natural products chemistry, pharmaceutical analysis, biologically active substances, enzyme inhibition, and microbial biotransformation of steroidal drug substrates. These subjects correspond well to the priority direction “Science of life and health”, particularly the specialized field of pharmacy and biologically active substances. The chemistry-based methodology also supports its relevance to fundamental and applied research in natural sciences.
2.	Importance for science	The work makes /does not make a significant contribution to science, and its importance is well disclosed /not disclosed	The dissertation makes a scientifically meaningful contribution by integrating two complementary research directions: phytochemical investigation of Tussilago farfara L. and fungal-mediated biotransformation of methylprednisolone acetate. The work combines advanced chromatographic

			separation, spectroscopic/spectrometric structural elucidation, in vitro enzyme inhibition, and computational modelling. The reported isolation of twenty plant metabolites, identification of key caffeoylquinic acid derivatives, generation of steroidal biotransformation products including one new compound, and biological evaluation against LmPTR-1 and aromatase provide new knowledge relevant to natural product chemistry, medicinal chemistry, biocatalysis, and early drug discovery. The scientific importance is sufficiently disclosed, although the translational implications should be interpreted as lead-discovery rather than therapeutic validation.
3.	The principle of independence	Self-reliance level: 1) High ; 2) Medium; 3) Low; 4) No independence	The submitted dissertation states that the doctoral candidate independently conducted the literature analysis, optimization of chromatographic profiling, extraction, isolation, purification, structural interpretation, design and optimization of the microbial transformation protocol, preparation of figures/tables, interpretation of data, and preparation of the scientific narrative. The breadth of experimental work, including plant collection/authentication, fractionation, compound purification, enzyme assays and computational studies, indicates a high level of research independence under scientific supervision.
4.	The principle of inner unity	4.1 Justification of the relevance of the thesis:	The relevance is adequately justified

		1) Justified ; 2) Partially justified; 3) Not justified.	through the continuing importance of natural products, the medicinal value and safety concerns of <i>Tussilago farfara</i> L., the need for new antileishmanial leads, and the use of microbial biotransformation as a selective and green strategy for modification of complex steroidal scaffolds. The introduction, literature review, experimental design, results, and conclusions are coherent with the stated aim: investigation of phytochemical constituents, microbial biotransformation of a steroidal substrate, structural characterization, and biological evaluation. The two main provisions for defense are logically linked to the two experimental parts of the thesis. The author compares the biological and structural findings with known phytochemical, enzymatic, and steroid-biotransformation literature. The internal unity is therefore sufficient for a PhD dissertation.
		4.2 The content of the thesis reflects the topic of the thesis: 1) Reflects ; 2) Partially reflects; 3) Does not reflect	
		4.3. The purpose and objectives correspond to the topic of the thesis: 1) correspond ; 2) partially correspond; 3) do not correspond	
		4.4 All sections and provisions of the thesis are logically interconnected: 1) completely interconnected ; 2) the interconnection is partial; 3) there is no interconnection	
		4.5 The new solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with the known solutions: 1) there is a critical analysis ; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors 4) the analysis is absent.	
5.	Scientific novelty principle	5.1 Are the scientific results and provisions new? 1) completely new; 2) partially new (25-75% are new) ; 3) not new (less than 25% are new)	The novelty is scientifically sufficient and clearly present, but it is most accurately categorized as partially new because the dissertation includes both known compounds/metabolites and new findings. In the phytochemical part, twenty known phenolic and flavonoid-related compounds were isolated;
		5.2 Are the dissertation findings new? 1) completely new; 2) partially new (25-75% are new) ; 3) not new (less than 25% are new)	

		5.3 Technical, technological, economic or management decisions are new and reasonable: 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new)	however, compounds 1, 14, and 20 are reported for the first time from <i>Tussilago farfara</i> L., which expands the species-specific phytochemical profile. In the biotransformation part, the use of <i>Fusarium oxysporum</i> and <i>Penicillium chrysogenum</i> for methylprednisolone acetate generated one new steroidal metabolite and seven known metabolites, providing new structure-activity insights. The technical approach is based on established chromatographic, spectroscopic, in vitro and computational methods, but their combined application to the selected plant material, substrate and biological endpoints is novel enough for the PhD level.
6.	The validity of the main findings	All main conclusions are/are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities)	The main conclusions are supported by scientifically significant experimental evidence. The phytochemical conclusions are based on authenticated plant material, systematic extraction/fractionation, chromatographic purification, and spectroscopic/spectrometric characterization using NMR, MS, FT-IR and related techniques. The biotransformation conclusions are supported by controlled fungal fermentation, isolation of metabolites, structural elucidation, and comparison with known steroidal metabolites. The biological conclusions are based on in vitro enzyme inhibition assays and further supported by molecular docking,

			molecular dynamics and MM-GBSA analyses. Importantly, the dissertation recognizes that the current findings are mainly at the in vitro and in silico stage; additional in vivo, pharmacokinetic, selectivity and safety studies would be required for therapeutic translation. This limitation does not undermine the validity of the main PhD-level findings.
7.	The main provisions for the defense	Provision 1 The plant material of <i>Tussilago farfara</i> L. contains structurally diverse compounds (1–20), including simple phenolic acids, flavonoids, and caffeoylquinic acid derivatives, such as vanillic acid (1), (1S,3R,4R,5R)-3,5-di-O-caffeoylquinic acid (14), and 3,4,5-tri-O-caffeoylquinic acid methyl ester (20). 7.1 Is the provision proven? 1) proven; 2) rather proven; 3) rather not proven; 4) not proven 5) in its current wording, it is impossible to verify whether the statement has been proven. 7.2 Is it trivial? 1) yes; 2) no 3) in its current wording, it is impossible to verify whether the statement is trivial. 7.3 Is it new? 1) yes; 2) no 3) in its current wording, it is impossible to verify the novelty of the statement. 7.4 Application level: 1) narrow;	Provision 1 is proven by the experimental phytochemical workflow and by the corresponding peer-reviewed publication. The article by Ahmad I. et al., “Caffeoylquinic acid derivatives isolated from <i>Tussilago farfara</i> L. as potential anti-leishmanial agents; in vitro and in silico studies”, Natural Product Research, 2026, DOI: 10.1080/14786419.2026.2613766, reports the isolation of twenty known phenolic compounds (1-20) from <i>Tussilago farfara</i> L. and specifically states that vanillic acid (1), (1S,3R,4R,5R)-3,5-di-O-caffeoylquinic acid (14), and 3,4,5-tri-O-caffeoylquinic acid methyl ester (20) are reported from this species for the first time. It also reports LmPTR-1 inhibition for compounds 15-18 with IC50 values in the range of 81.9-189.7 micromolar and supportive docking/MD analysis. Therefore, the provision is proven, not trivial, and new at the level of species-specific phytochemical contribution and biological lead identification. The application level is medium because the

	2) medium; 3) wide 4) in its current wording, it is impossible to determine the scope of application of the statement. 7.5 Is it proven in the article? 1) yes; 2) no 3) in its current wording, it is impossible to verify whether the statement has been proven in the article. Provision 2 Microbial biotransformation of the selected steroidal substrate using the fungal strains <i>Fusarium oxysporum</i> and <i>Penicillium chrysogenum</i> affords regio- and stereoselective structural transformations, yielding one new compound, 20-(4',5'-dimethyl-1',3'-dioxolan-2-yl)-11β-hydroxypregn-4-ene-3,20-dione, and seven known metabolites. 7.1 Is the provision proven? 1) proven; 2) rather proven; 3) rather not proven; 4) not proven 5) in its current wording, it is impossible to verify whether the statement has been proven. 7.2 Is it trivial? 1) yes; 2) no 3) in its current wording, it is impossible to verify whether the statement is trivial. 7.3 Is it new? 1) yes; 2) no 3) in its current wording, it is impossible to verify the novelty of	findings are relevant to natural products chemistry, pharmacognosy, phytochemical standardization and early-stage antileishmanial lead discovery. Provision 2 is proven by the dissertation data and by the corresponding peer-reviewed publication. The article by Ahmad I. et al., "Fungal-mediated transformation of methylprednisolone acetate into potent aromatase inhibitors: In vitro and in silico studies", Journal of Molecular Structure, 2026, 1364, 145890, DOI: 10.1016/j.molstruc.2026.145890, reports incubation of methylprednisolone acetate (MPA; 1) with <i>Fusarium oxysporum</i> and <i>Penicillium chrysogenum</i>. The <i>Fusarium oxysporum</i> experiment yielded one new metabolite, 20-(4',5'-dimethyl-1',3'-dioxolan-2'-yl)-11β-hydroxypregn-4-ene-3,20-dione (2), together with six known metabolites, while <i>Penicillium chrysogenum</i> yielded metabolites 3, 5, 8 and 9. The publication further reports structural elucidation by 1H-, 13C- and 2D-NMR and FAB-, ESI- and EI-MS, as well as aromatase inhibitory activity with metabolite IC50 values of 69.32-177.50 nM, which were more potent than the parent compound and reference exemestane under the stated in vitro assay conditions. Therefore, the provision is proven, not trivial, and new
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		the statement. 7.4 Application level: 1) narrow; 2) medium; 3) wide 4) in its current wording, it is impossible to determine the scope of application of the statement. 7.5 Is it proven in the article? 1) yes; 2) no 3) in its current wording, it is impossible to verify whether the statement has been proven in the article.	because it combines a specific steroidal substrate, selected fungal strains, a newly identified metabolite, and biological evaluation. The application level is medium because the results are important for steroid biotransformation, biocatalytic scaffold diversification and aromatase-inhibitor lead discovery, although practical therapeutic application would require further validation.
8.	The principle of reliability Reliability of sources and information provided	8.1 Choice of methodology - is justified or the methodology is described in sufficient detail 1) yes; 2) no	The selected methodology is appropriate for the specialty 8D05301 - Chemistry and for the stated scientific objectives. For the phytochemical part, extraction, fractionation, chromatographic isolation and UPLC/HPLC profiling are suitable methods for separating secondary metabolites from <i>Tussilago farfara</i> L. For structural elucidation, the use of 1D/2D NMR, MS/HRMS, FT-IR and UV/Vis spectroscopy is scientifically appropriate and sufficient for assigning the structures of phenolic acids, flavonoids and caffeoylquinic acid derivatives. For the biotransformation part, fungal fermentation using <i>Fusarium oxysporum</i> and <i>Penicillium chrysogenum</i>, followed by isolation and spectroscopic characterization of metabolites, is a valid strategy for studying regio- and stereoselective steroidal transformations. The in vitro enzyme assays and in silico analyses are

			aligned with the biological objectives. Therefore, the methodological framework is justified.
		8.2 The results of the thesis were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies: 1) yes; 2) no	The dissertation applies modern analytical and computational tools, including UPLC/HPLC, advanced NMR spectroscopy, mass spectrometry, enzyme inhibition assays, molecular docking, molecular dynamics simulations and MM-GBSA binding energy calculations. These methods are current and widely accepted in natural product chemistry, medicinal chemistry, pharmaceutical analysis and biotransformation studies. Computer-assisted interpretation was used appropriately for molecular modelling, ligand-enzyme interaction analysis and binding energy estimation. The use of computational methods supports the mechanistic interpretation of the biological findings, while the primary experimental results remain based on chromatographic, spectroscopic and in vitro assay data.
		8.3 Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by experimental research (for areas of training in pedagogical sciences, the results have been proven on the basis of a pedagogical experiment): 1) yes; 2) no	The major conclusions are supported by experimental evidence. The phytochemical conclusions are supported by isolation and structural identification of twenty compounds from <i>T. farfara</i> L., including compounds reported for the first time from this species. The steroid biotransformation conclusions are supported by fungal

			incubation experiments, purification of metabolites and spectroscopic/spectrometric confirmation of structural modifications such as hydroxylation, ester cleavage and keto reduction. The biological conclusions are supported by in vitro LmPTR-1 and aromatase inhibition results. The computational models provide supportive evidence for binding interactions and stability; however, they should be interpreted as mechanistic support rather than independent proof. Overall, the experimental evidence sufficiently confirms the principal conclusions.
		8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature	The dissertation contains extensive literature support for the chemistry, pharmacology and traditional use of <i>T. farfara</i> L., the role of natural products in drug discovery, microbial biotransformation, steroidal substrate transformation and enzyme-based biological evaluation. The references include relevant scientific articles and established methodological sources. The important statements regarding the phytochemical diversity of <i>T. farfara</i> L., biological relevance of caffeoylquinic acid derivatives, microbial transformation of steroids, and use of docking/MD/MM-GBSA in structure-activity interpretation are sufficiently supported by the cited literature. A minor recommendation is to ensure that the final archived version contains

			complete and consistently formatted references, especially for the most recent publications and all cited figures/tables.
		8.5 Used literature sources are sufficient /not sufficient for a literature review	The literature review is broad and adequate for a PhD thesis in chemistry. It covers natural products, traditional medicine, T. farfara L. phytochemistry, pharmacological activities, safety issues, biotransformation concepts, microbial agents, steroid biotransformation and modern analytical approaches. The dissertation lists 258 references, which is sufficient for the scope of the research. The review provides a suitable scientific basis for the experimental chapters and demonstrates awareness of both historical and recent developments. Some improvement in organization and language polishing would strengthen readability, but the quantity, scope and relevance of the literature sources are sufficient.
9	Practical value principle	9.1 The thesis has theoretical value: 1) yes ; 2) no	The thesis has clear theoretical value. It expands the phytochemical knowledge of T. farfara L. by documenting structurally diverse secondary metabolites, including selected compounds reported for the first time from this species. It also contributes to the understanding of how fungal strains can modify a complex steroidal drug substrate and generate structurally altered metabolites. The work therefore adds to natural products chemistry, medicinal chemistry, steroid chemistry, biotransformation science and enzyme-

			targeted lead discovery. The structure-activity observations for caffeoylquinic acid derivatives and steroidal metabolites also provide a theoretical basis for future analogue design.
		9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice: 1) yes; 2) no	The dissertation has practical importance because it demonstrates experimental workflows that can be applied in phytochemical standardization, natural product lead identification, microbial transformation of steroidal scaffolds and early-stage enzyme inhibitor discovery. The LmPTR-1 and aromatase inhibition results provide promising starting points for further medicinal chemistry optimization. The fungal biotransformation approach may be practically useful for generating structurally diversified steroid analogues under mild and comparatively green conditions. However, practical pharmaceutical application is still at an early stage and will require further validation, including compound purity confirmation, selectivity profiling, cytotoxicity, microsomal stability, pharmacokinetics, in vivo efficacy/safety studies and process scale-up.
		9.3 Are the practice suggestions new? 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The practice-oriented suggestions are partially new. The general techniques of natural product extraction, chromatographic isolation, fungal biotransformation and enzyme inhibition screening are established; therefore, the

			practical approach is not completely new in a universal methodological sense. Nevertheless, their application to the specific plant <i>T. farfara</i> L., the specific fungal transformation of methylprednisolone acetate, the reported new steroidal metabolite, and the biological evaluation of the resulting compounds provide a meaningful new practical contribution. The novelty is therefore best classified as partially new rather than completely new.
10.	The quality of writing and design	Academic writing quality: 1) high; 2) average; 3) below average; 4) low.	The dissertation is scientifically understandable, logically structured and adequately illustrated with figures and tables. However, the manuscript contains a number of typographical, grammatical, nomenclature and formatting errors that should be corrected before final archiving. Examples include “Using Advance” instead of “Using Advanced”, “Strctural”, “ultra-perpormance”, inconsistent spelling of <i>Fusarium oxysporum</i>/oxysporium, “Erurasian”, “Examestane”, missing figure numbers in the steroid section, and some sentences that require grammatical polishing. These issues do not invalidate the scientific conclusions but reduce the formal academic presentation quality. Therefore, the writing quality is assessed as average rather than high.
11.	Comments on the thesis	1. The scientific novelty section should be corrected for consistency: the thesis at one point refers to compounds 1, 4 and 20 as first reports from <i>T. farfara</i> L., whereas the abstract, main results and conclusions indicate compounds 1, 14 and 20. This should be harmonized.	

		2. Typographical, grammatical, and formatting errors should be corrected throughout the thesis. Examples include “Using Advance” instead of “Using Advanced,” “Strctural elucidation,” “ultra-performance,” “dertermine,” inconsistent spelling of <i>Fusarium oxysporum</i>, “Examestane” instead of “Exemestane,” “MMGBSA” instead of “MMGBSA.” 3. Missing or incomplete figure references in the biotransformation section should be corrected, especially the sentence indicating that structures are presented in “Figure , and Figure .” (Page 92) 4. Compound numbering and naming in the steroidal biotransformation section should be checked carefully to ensure that the parent substrate, new metabolite and metabolites 3-9 are consistently numbered throughout the text, tables and figures. 5. The biological assay sections should clearly state the number of experimental replicates, statistical model for IC50 fitting, confidence intervals where available, and the composition of positive/negative controls. 6. The dissertation should ensure that complete spectral and chromatographic evidence is available for all isolated compounds/metabolites, including NMR spectra, MS/HRMS evidence, chromatographic purity and UPLC/HPLC traces, preferably as appendices or supplementary files. 7. The computational sections are useful and scientifically relevant; however, the interpretation should consistently state that docking, MD and MM-GBSA are supportive tools and not substitutes for experimental biological validation. The thesis appropriately notes a weak correlation between IC50 values and docking scores; this limitation should be emphasized in the final discussion. 8. Because <i>T. farfara</i> L. is known to contain pyrrolizidine alkaloids with safety concerns, the thesis should more explicitly discuss whether the isolated fractions/compounds are free from such alkaloids or how this issue affects translational claims. 9. The antileishmanial activity should be described as moderate lead-level activity because compound 18 is less potent than trimethoprim. Similarly, aromatase inhibition findings are promising but should not be extrapolated to therapeutic claims without CYP selectivity, cytotoxicity, microsomal stability, pharmacokinetic data, and in vivo efficacy/safety data. 10. These comments are mainly editorial, clarificatory, and translational in nature. They do not change the overall positive assessment of the dissertation's scientific merit.
12.	Scientific level of the doctoral	The publication record is directly aligned with the dissertation topic and is adequate for PhD defense. The doctoral

	student's articles on the topic of research (in case of defense of the dissertation in the form of a series of articles, the official reviewers comment on the scientific level of each article of the doctoral student on the topic of research)	candidate has published three original research articles and one review article in peer-reviewed journals indexed in Scopus according to the submitted dissertation/publication data. 1. Ahmad I. et al., “Caffeoylquinic acid derivatives isolated from <i>Tussilago farfara</i> L. as potential antileishmanial agents: in vitro and in silico studies”, <i>Natural Product Research</i>, 2026, DOI: 10.1080/14786419.2026.2613766. This article directly supports Provision 1 and presents isolation of twenty phenolic compounds, LmPTR-1 inhibition and computational support. 2. Ahmad I. et al., “Fungal-mediated transformation of methylprednisolone acetate into potent aromatase inhibitors: In vitro and in silico studies”, <i>Journal of Molecular Structure</i>, 2026, 1364, 145890, DOI: 10.1016/j.molstruc.2026.145890. This is a strong research article that directly supports Provision 2 by reporting fungal biotransformation, structural elucidation and aromatase inhibition. 3. Ahmad I. et al., “Aromatase (CYP19A1) Inhibitory Activity of Coltsfoot (<i>Tussilago farfara</i> L.) Phytochemicals: In Vitro and in Silico Evaluation”, <i>Eurasian Chemico-Technological Journal</i>, 2025, 27(4), 315-321, DOI: 10.18321/ectj1678. This article is relevant to the biological direction of the dissertation and supports the interest in natural products as aromatase-inhibitory scaffolds. 4. Ahmad I. et al., “Coltsfoot (<i>Tussilago farfara</i> L.; Asteraceae): modern methods of extraction, phytochemistry, nanoparticles synthesis, ethnopharmacology, and biological activities”, <i>Natural Product Research</i>, 2025, DOI: 10.1080/14786419.2025.2548616. This review supports the literature foundation of the dissertation and demonstrates scholarly command of the plant’s phytochemistry and pharmacology. Overall, the publications are thematically coherent, recent, relevant to the dissertation content, and of a level suitable for supporting the award of a PhD degree. The publication set also meets the general regulatory expectation that main results should be published before defense in international peer-reviewed scientific journals with appropriate database coverage/percentile performance.	
13.	Decision of the official reviewer (pursuant to paragraph 28 of the present Model Regulations)	Choose one of the following decisions: 1) to award the degree of the Doctor of Philosophy (PhD); 2) to send the dissertation for revision; 3) refuse to award the degree of the Doctor of Philosophy (PhD).	Based on the review of the dissertation, the main scientific results, and the corresponding peer-reviewed publications, I conclude that the dissertation has scientific merit and the main provisions are generally supported by experimental evidence. However, the dissertation contains several errors and inconsistencies that require correction

			before final acceptance and publication, including inconsistency in the novelty statement, typographical and nomenclature errors, incomplete figure references, and points requiring clarification in the biological assay and computational interpretation sections. Therefore, in accordance with the rules and the need to correct these issues before final publication, I recommend sending the dissertation for revision.
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Copies of the reviews of the official reviewers are handed over to the doctoral student no later than 5 (five) working days before the defense of the thesis.

Official Reviewer:

Assistant Professor,

Department of Pharmaceutical Analysis,

GITAM School of Pharmacy,

GITAM Deemed to be University, Visakhapatnam,

India-530045

(place of work, academic title)



(signature)

Dr. Siva Nageswararao Gajula

(FULL NAME)