ABSTRACT

of the dissertation of Izaz Ahmad entitled "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"

General description of the research. This dissertation investigates the phytochemical composition of *Tussilago farfara* L., using modern spectroscopic and chromatographic techniques and explores microbial biotransformation of selected steroidal compounds via fungal systems. It also evaluates the biological activity of both the isolated phytochemicals and the biotransformation products.

Relevance of the research work. *Tussilago farfara* or coltsfoot, is a medicinal plant that provides a sustainable, and environmentally friendly pathway for therapeutic research. *T. farfara* has more than 180 identified compounds which include phenolic acids, alkaloids, flavonoids, terpenoids, chromones, and volatile oils, many of which have been demonstrated to have anti-inflammatory, antioxidant, antiviral activities, and neuroprotective *etc*.

Therefore, the study of *T. farfara* provides a natural, renewable source of bioactive compounds, while microbial biotransformation of selected steroidal substrates represents a complementary strategy. Both strategies work together to promote the worldwide movement toward low-impact pharmaceutical manufacturing, sustainable drug discovery, and green chemistry.

Microbial biotransformation is becoming an increasingly important approach in current biotechnology, and drug development. It employs living organisms such as fungi, bacteria, and plant cell cultures to structurally modify complex organic compounds, including multifunctional, chiral, and inert compounds, typically in an aqueous medium, making the process cost-effective, and scalable.

Furthermore, this approach provides excellent enantio-, chemo-, stereo-, and regio-selectivity, enabling accurate molecular modifications. In addition, it adheres to the principle of green chemistry, resulting in minimal or non-toxic waste. Consequently, this approach has an important in various industries, including pharmaceuticals, agriculture, food, and environmental management.

As the demand for eco-friendly, sustainable medication development rises, microbial transformation emerges as a crucial technique to develop biologically active compounds.

The aim of this study is to employ advanced spectroscopic and chromatographic techniques to investigate the phytochemical constituents of *Tussilago farfara* L., to explore the microbial biotransformation of selected steroidal

substrate using fungal systems in a cost-effective and environmentally sustainable manner, and to evaluate the biological activities of the isolated phytochemicals and the resulting biotransformed derivatives.

To achieve this goal, the following tasks were formulated:

1. to investigate and select the medicinal plant *Tussilago farfara* L., and the fungal strains *Fusarium oxysporum* and *Penicillium chrysogenum* relevant for sustainable drug discovery;
2. to perform systematic extraction, fractionation, isolation, and purification of secondary metabolites from *Tussilago farfara* L., and to generate and purify fungal biotransformed steroidal metabolites using chromatographic techniques;
3. to elucidate the chemical structures of isolated compounds from *Tussilago farfara* L., and fungal biotransformed steroidal derivatives using advanced spectroscopic and spectrometric approaches;
4. to evaluate the *in vitro* antileishmanial activity (PTR-1 inhibition) of plant-derived compounds and the anti-aromatase activity of isolated biotransformed metabolites;
5. to support and validate experimental biological findings of plant-derived compounds and biotransformed steroids through *in silico* approaches, including molecular docking, molecular dynamics simulations, and binding energy calculations.

The objective of the research. Isolation and characterization of secondary metabolites from the aerial parts of the medicinal plant *Tussilago farfara* L., and Microbial biotransformation of methylprednisolone acetate using *Fusarium oxysporum* and *Penicillium chrysogenum*.

Research methods. A comprehensive methodological approach was employed including, advanced chromatographic such as, high performance liquid chromatography (HPLC), and ultra-performance liquid chromatography (UPLC) for the isolation and purification of bioactive compounds from *T. farfara*, and biotransformed metabolites. Structural elucidation was carried out using spectroscopic techniques, including 1D (^{1}H, and ^{13}C), and 2D-NMR, and mass spectrometry (EI-MS, ESI-MS, and FAB-MS), fourier transform infrared (FT-IR) and UV-Vis spectroscopy. Furthermore, *in vitro*, and *in silico* studies were performed to assess the antileishmanial, and anti-aromatase activities of bioactive compounds of *T. farfara*, and biotransformed derivatives, respectively.

Research hypotheses.

1. *Tussilago farfara* L., serves as a source of structurally diverse bioactive secondary metabolites with potential antileishmanial activity.
2. Fungal-mediated biotransformation of a selected steroidal substrate enables the generation of new, structurally modified derivatives with enhanced aromatase inhibitory activity.

Main statement to be Defended

1. The plant material of *Tussilago farfara* 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).

2. Microbial biotransformation of the selected steroidal substrate using the fungal strains *Fusarium oxysporum* and *Penicillium chrysogenum* 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.

Main Results of the Study:

The main findings of the study based on the phytochemical analysis of *Tussilago farfara* L., and the biotransformation of methylprednisolone acetate, are as follows:

1. The use of *Tussilago farfara* L., and fungal strains *Fusarium oxysporum* and *Penicillium chrysogenum* proved effective, leading to the successful isolation of diverse phytochemicals and the efficient biotransformation of methylprednisolone acetate into structurally modified metabolites.

2. Advanced chromatographic techniques, including TLC, column chromatography, HPLC, and UPLC, enabled the successful extraction, fractionation, and isolation of twenty secondary metabolites (1–20) from *Tussilago farfara* L. Additionally, microbial transformation of methylprednisolone acetate yielded eight metabolites (2–9), including one new compound (2) and seven known compounds (3–9).

3. The structures of all isolated compounds and metabolites were elucidated using comprehensive spectroscopic and spectrometric techniques, including ^{1}H NMR, ^{13}C NMR, 2D NMR, HRMS, EI-MS, FAB-MS, FT-IR, and UV/Vis spectroscopy. Among them, compounds 1, 14, and 20 were reported for the first time from this species. Biotransformation resulted in key structural modifications such as hydroxylation, ester cleavage, and keto reduction.

4. Biological evaluation revealed that compounds 15–18 exhibited moderate inhibition of *Lm*PTR-1, with compound 18 showing the highest activity (IC_{50} = 81.90 ± 1.12 µM). Furthermore, in the biotransformation study the parent drug and its metabolites demonstrated potent anti-aromatase activity, where compound 4 showed superior inhibition (IC_{50} = 69.32 ± 0.17 nM), outperforming both the parent compound and the standard drug exemestane.

5. Computational studies, including molecular docking, molecular dynamics simulations, and MM-GBSA analysis, confirmed strong and stable binding interactions of compounds with target enzymes. Caffeoylquinic acid derivatives

(15–18) showed stable interactions with PTR-1, while steroidal metabolites (2–9), particularly compounds 2 and 4, exhibited strong binding affinities toward aromatase, supporting their high inhibitory potential.

Scientific novelty of the research

This study contributes new knowledge such as, the isolation of three secondary metabolites (1, 4 and 20) reported for the first time from *Tussilago farfara* L., expanding the phytochemical profile of the plant. The study further investigates the microbial transformation of methylprednisolone acetate for the first with selected fungal strains of *Fusarium oxysporum*, and *Penicillium chrysogenum* yielding a new compound. The biological potential of purified compounds was elevated against antileishmanial, and anti-aromatase activities, which were further supported by *in silico* studied.

Theoretical and practical significance. This study merges two important disciplines of chemistry: medicinal plant phytochemistry, and selected steroidal substrate biotransformation which are both evaluated using sophisticated spectroscopic, chromatographic, and computational approaches. Furthermore, this study discovered new anti-leishmanial inhibitors that displayed lower potency than the standard drug, trimethoprim.

In parallel, microbial biotransformation of steroidal drugs demonstrates the ability of microorganisms to generate structurally modified and biologically enhanced derivatives from existing drug, exemestane.

Together, these complementary approaches encouraging the development of new drugs in two important fields: neglected tropical diseases through PTR-1 inhibition, and hormone-related cancers *via* aromatase inhibition.

The author's personal contribution. During the course of the research conducted for this dissertation, four scientific papers were published. These include three original research articles and one review article.

The first review article was published in *Natural Product Research* (Q2, percentile 78, CiteScore 5.2), second original article in *Natural Product Research* (Q2, percentile 78, CiteScore 5.2), third article in *Eurasian Chemico-Technological Journal* (Q3, percentile 33, CiteScore 1.7), and fourth article was published in *Journal of Molecular Structure* (Q2, percentile 84, CiteScore 8.0).

1. **I. Ahmad**, B. Kudaibergenova, M. Ahmad, M. Sadia, M.I. Choudhary, S. Hafez-Ghoran, Coltsfoot (*Tussilago farfara* L.; Asteraceae): modern methods of extraction, phytochemistry, nanoparticles synthesis, ethnopharmacology, and biological activities, *Natural Product Research*, (2025) 1-20, DOI: https://10.1080/14786419.2025.2548616. Ahmad Izaz is the first author of the paper and was responsible for writing–original draft, methodology, investigation, formal analysis, and conceptualization.

2. **I. Ahmad**, S. Hafez-Ghoran, M. Yousuf, H. Zafar, Atia-ul. W, S. Yousuf, M. Ahmad, I. Gul, B. Kudaibergenova, M.I. Choudhary, Caffeoylquinic acid derivatives isolated from *Tussilago farfara* L. as potential anti-leishmanial agents; *in vitro* and *in silico* studies, *Natural Product Research*, (2026) 1-9, DOI: https://10.1080/14786419.2026.2613766. Ahmad Izaz is the first author of the paper and was responsible for writing–original draft, methodology, investigation, formal analysis, and conceptualization.

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