Research Article

Pharmacognosy, Phytochemistry, and Antimicrobial Potential of *Pteris cretica* L. Collected from Dir (Lower), Khyber Pakhtunkhwa

Fazal Manan[1], Imtiaz Ahmad[1]*, Fayaz Asad[1], Lubna Shakir[2] and Shakir Ullah[3]*

[1]Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan, 26000; [2]Department of Botany, Govt. Degree College Timergara, Dir Lower, Khyber Pakhtunkhwa, Pakistan, 18000; [3]State Key Laboratory of Systematic and Evolutionary Botany (LSEB), Institute of Botany, Chinese Academy of Science, Beijing, China, 100000.

Abstract | The term *Pharmacognosy* is derived from the Greek words *"pharmakon"* (drug) and *"gnosis"* (knowledge). It is defined as "the science that provides infrastructure for the evolution of traditional medicines." *Pteris cretica* L., a perennial evergreen pteridophyte, belongs to the genus *Pteris* in the family *Pteridaceae*. This genus, comprising approximately 250 species, is widely distributed across tropical and subtropical regions of the world. Pharmacognostic and phytochemical investigations were conducted on *Pteris cretica*. Microscopic examination revealed the presence of epidermis, cortex, and vascular bundles in the rachis, as well as similar anatomical features in the root. The methanolic extract of the plant was tested against various human pathogenic microorganisms, including the bacteria *Citrobacter koseri*, *Acinetobacter baumannii*, *Providencia stuartii*, *Proteus vulgaris*, *Enterobacter aerogenes*, and two gram-positive strains, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Fungal strains tested included *Alternaria solani*, *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*, *Curvularia trifolii*, and *Rhizopus arrhizus*. The plant extract exhibited significant antibacterial activity against *Citrobacter koseri*, *Acinetobacter baumannii*, and *Providencia stuartii*. However, it did not demonstrate notable antifungal activity against the tested fungal strains.

Received | May 12, 2025; **Accepted** | June 18, 2025; **Published** | June 26, 2025
***Correspondence** | Shakir Ullah and Imtiaz Ahmad, State Key Laboratory of Systematic and Evolutionary Botany (LSEB), Institute of Botany, Chinese Academy of Science, Beijing, China, 100000; Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan, 26000; **Emails:** shakirawkum321@gmail.com and drimtiazahmad@bkuc.edu.pk
Citation | Manan, F., I. Ahmad, F.Asad, L. Shakir and S. Ullah. 2025. Pharmacognosy, phytochemistry, and antimicrobial potential of *Pteris cretica* L. collected from Dir (Lower), Khyber Pakhtunkhwa. *Pakistan Journal of Weed Science Research,* 31(2): 117-131.
DOI | https://dx.doi.org/10.17582/journal.PJWSR/2025/31.2.117.131
Keywords | Antibacterial activities, Antifungal activities, Nutritional analyses, Pharmacognosy, Phytochemistry, *Pteris cretica*

Introduction

Pharmacognosy, a fundamental discipline within pharmaceutical sciences, has played a pivotal role in the development of medicinal therapies derived from natural sources (Singha *et al.*, 2023). The term was first introduced between 1811 and 1815 by Seidler and is rooted in two Greek words, "pharmakon" (drug) and "gnosis" (knowledge) (Irshad *et al.*, 2025; Asif *et al.*, 2025). It is a scientific field dedicated to studying the physical, chemical, biochemical, and biological properties of substances of natural origin, particularly those derived from plants and animals (Gracelin *et al.*, 2013; Ullah *et*

al., 2025b). The core aim of pharmacognosy is to explore the various aspects of crude drugs, including their identification, characterization, and therapeutic potential. Initially, pharmacognosy focused on the macroscopic and microscopic authentication of crude plant-based drugs (Ullah *et al*., 2025a). However, over time, it has expanded to encompass a broad spectrum of disciplines, including phytochemistry, biosynthesis, and biotransformation, contributing to the discovery of new drugs and the enhancement of traditional medicine systems (Subhan *et al*., 2024).

The essence of pharmacognosy lies in its dual role as a science that bridges the gap between natural products and their clinical applications (Khan *et al*., 2024). It serves as the foundation for the exploration of both traditional and modern medicine, drawing on the vast array of chemical compounds produced by plants, animals, and microorganisms (Khan *et al*., 2018a; b). These natural products have been utilized for centuries in folk medicine, yet their pharmacological properties often remain untapped until they are studied through the rigorous methods of modern pharmacognosy (Khan *et al*., 2018b). This interdisciplinary approach not only aims to discover new medicinal compounds but also emphasizes the standardization and quality control of herbal remedies, ensuring their safety and efficacy for medicinal use (Hao *et al*., 2008). The historical context of pharmacognosy traces its origins to ancient civilizations, where medicinal plants played a crucial role in treating diseases (Ullah *et al*., 2025c). In ancient China, Egypt, Greece, and India, herbs and other natural substances created therapeutic compounds for various ailments. As these practices spread across the world, they laid the foundation for what would later be termed "materia medica" the knowledge of medicinal substances (Nuwamanya *et al*., 2025). The use of plant-based medicines continued to evolve, eventually leading to the formalization of pharmacognosy as a scientific discipline (Nayak *et al*., 2013). The World Health Organization (WHO) estimates that nearly 80% of the global population relies on traditional medicine, much of which is plant-based. Medicinal plants have been recognized for their therapeutic potential since the dawn of time, with ancient manuscripts, pictographs, and oral traditions passing down knowledge of their use (Rakkimuthu *et al*., 2018). The formal study of pharmacognosy, however, began with the classification and evaluation of natural products for their medicinal properties. The isolation and characterization of active compounds such as morphine from opium and quinine from the cinchona tree in the 19th century marked the beginning of modern pharmacognosy (Bandyopadhyay and Dey, 2022). These early discoveries not only highlighted the therapeutic potential of plants but also set the stage for the scientific validation of natural remedies (Shakir *et al*., 2023). As the field progressed, pharmacognosy expanded to include the study of a variety of natural sources, including fungi, marine organisms, and microorganisms (Moussa *et al*., 2024). Today, modern pharmacognosy is a dynamic and interdisciplinary science that continues to draw on the rich heritage of traditional medicine while integrating the latest advancements in phytochemistry, molecular biology, and pharmacology (Sajid *et al*., 2023).

Modern pharmacognosy has evolved to encompass the study of a wide range of natural products, from plant-based substances to those derived from bacteria, fungi, and marine organisms. This expansion reflects the increasing recognition of the importance of natural products in drug discovery and development (Gaafar *et al*., 2018). As the scientific community delves deeper into the biochemical and pharmacological properties of these natural compounds, new drugs and therapeutic agents are continually being discovered. The field of pharmacognosy has also grown to include areas such as molecular pharmacognosy, genomics, and metabolomics, which provide deeper insights into the mechanisms of action of natural compounds and their potential therapeutic applications (Rautray, *et al*., 2018). The scope of pharmacognosy extends beyond the discovery of new drugs to include the standardization and quality control of herbal medicines. With the growing popularity of herbal remedies and natural health products, there is an increasing need for rigorous scientific evaluation of these substances to ensure their safety and efficacy (Ullah *et al*., 2023a). Pharmacognosy plays a crucial role in the development of quality control methods for crude drugs and herbal preparations, including the identification of key chemical markers and the establishment of pharmacopoeial standards (Singh *et al*., 2008). This ensures that herbal medicines are not only effective but also safe for consumption. Pharmacognosy also explores the potential of plants to provide new therapeutic agents, particularly in the face of the growing concern over antimicrobial resistance. Plants have long been known to possess antimicrobial properties, and recent studies have identified numerous plant-based compounds that

exhibit antibacterial, antifungal, and antiviral activities (Mir *et al*., 2013). With the increasing prevalence of drug-resistant pathogens, there is a pressing need for alternative therapies, and medicinal plants offer a promising solution.

Phytochemicals are classified into several major categories based on their chemical structure. Some of the primary groups include alkaloids, sulfur-containing phytochemicals, terpenoids, and polyphenols (Şuțan *et al*., 2019). Alkaloids, for instance, are known for their diverse biological activities, including their potential to act as pain relievers or to combat diseases like cancer. Sulfur-containing compounds, found in garlic and onions, are believed to offer immune-boosting and anti-inflammatory effects (Juliasih and Adnyana, 2023). Terpenoids, found in herbs such as rosemary and basil, have been linked to improved circulation and anti-cancer properties (Khan *et al*., 2018c; Ullah *et al*., 2018h). Polyphenols, commonly found in fruits like berries and beverages like tea, are known for their potent antioxidant properties, which help neutralize harmful free radicals in the body. Over the last three decades, significant research has focused on identifying phytochemicals with antimicrobial and antibacterial properties. Thousands of these compounds have been documented, each with different mechanisms of action against harmful microorganisms (Razaghi and Abdel-Azeem, 2024). This growing body of knowledge has sparked interest in phytochemicals as potential alternatives or adjuncts to conventional antimicrobial treatments, especially as antibiotic resistance continues to rise globally. However, while phytochemicals show great promise in preventing and treating a variety of diseases, it is important to note that their levels and effectiveness can vary significantly between different plants, and are influenced by factors such as growing conditions, processing, and cooking methods (Goswami *et al*., 2016). Supplementary phytochemicals, often sold in the form of pills or powders, are also available on the market, but there is no conclusive evidence to suggest that they offer the same health benefits as phytochemicals obtained directly from whole, plant-based foods (Ullah *et al*., 2024a). Consuming phytochemicals in their natural, unprocessed forms allows individuals to benefit from the synergistic effects of the entire plant, including dietary fiber and other bioactive compounds that may not be present in isolated supplements (Priya *et al*., 2024).

Pteris cretica L., a perennial evergreen pteridophyte, belongs to the genus *Pteris* of the *Pteridaceae* family. This genus, with approximately 250 species, is geographically distributed across tropical and subtropical regions. *Pteris cretica*, commonly known as *Pata*, belongs to the group of lower vascular plants known as *Pteridophyta*, which have true leaves and vascular bundles but lack flowers and seeds (Ullah *et al*., 2024b). The *Pteridaceae* family is characterized by creeping rhizomes, bearing scales, and simple, pinnate leaves. The veins of the leaves are free and forked, and sporangia are present along the veins (Ankari *et al*., 2024). *Pteris cretica* is abundant in Pakistan, particularly in the Pir Nasoora Park in Azad Jammu and Kashmir. It is also widely distributed in the southwest and southern regions of China (Ullah and Shakir, 2023). The genus *Pteris* has a broad geographical range, distributed across tropical, subtropical, and temperate regions of all continents except Antarctica. *Pteris cretica* thrives in diverse habitats, including open slopes, dense forests, and both acidic soils and limestone rock (Mahmutović *et al*., 2023). The plant can be found in terrestrial, aquatic, epiphytic, and xeric-adapted environments (Ullah *et al*., 2023a). This species is particularly abundant in tropical, warm-temperate, and south-temperate areas and exhibits considerable morphological variation (Juliasih and Adnyana, 2023). *Pteris cretica* has been used in traditional Chinese medicine for heat-clearing, inducing diuresis, reducing edema, and as an antibacterial agent. In addition to its medicinal uses, this plant has been cultivated for ornamental and culinary purposes (Ullah *et al*., 2024b). Recent pharmacological studies have revealed that *Pteris cretica* possesses anti-inflammatory, anti-tumor, anti-diabetic, and anti-tuberculosis properties. Furthermore, it has been used as an antidote, antipyretic, in burn treatment, and for antimicrobial and wound-healing purposes (Vanlalpeka *et al*., 2024; Shakir *et al*., 2023). This study aims to explore the medicinal properties of *Pteris cretica*, particularly its antibacterial, antifungal, and pharmacological effects. The specific objectives of this study are: First, to prepare methanolic extracts of *Pteris cretica* and evaluate its antibacterial and antifungal potential. This will allow for a deeper understanding of the plant's bioactive properties. Second, the study seeks to examine the anatomical structure of *Pteris cretica* and determine its organoleptic characteristics, including its sensory attributes such as color, taste, and smell. Finally, the study will calculate the total ash content

of *Pteris cretica*, which is essential for understanding its mineral composition and potential applications in pharmaceutical and medicinal practices. These objectives will contribute to a comprehensive analysis of the plant's medicinal properties and its potential uses in various therapeutic contexts.

Materials and Methods

Plant collection and processing

Specimens of *Pteris cretica* L. were carefully excavated, with stems cut approximately 15 cm above the root system to minimize mechanical damage during collection (Ullah *et al*., 2023c). Leaves were subjected to a two-stage washing protocol: initial rinsing with tap water to remove surface debris, followed by distilled water to eliminate residual contaminants (Arya *et al*., 2024). The cleaned material was shade-dried for 7 days under ambient conditions (40–50% relative humidity) to prevent photodegradation (Gacche *et al*., 2011). Dried specimens were pulverized using a mechanical grinder (Philips HL7756/00, 600W) and stored in amber glass containers at 4 °C until further use (Ankari *et al*., 2024).

Extract preparation

A total of 185 g of coarsely powdered plant material was macerated in 800 mL of analytical-grade methanol (CH_3OH, 99.8% purity) for 72 hours with intermittent shaking at 100 rpm (Orbitek shaker) (Adoga *et al*., 2019). The suspension was vacuum-filtered through Whatman® No. 1 filter paper (11 µm pore size) to obtain a clear filtrate (Choudhury *et al*., 2017). The filtrate was concentrated using a rotary evaporator (Buchi R-300) under reduced pressure (40 °C, 150 mbar) (Prabhakar, *et al*., 2025) and stored in pre-sterilized amber glass vials at –20 °C (Ullah *et al*., 2023b).

Antimicrobial evaluation

Microorganisms: Clinical isolates included Gram-negative bacteria (*Citrobacter freundii, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter aerogenes,* and *Providencia stuartii*) and Gram-positive strains (*Acinetobacter baumannii* and *Staphylococcus aureus*), obtained from certified diagnostic laboratories in Pakistan (Khan *et al*., 2018).

Inoculum preparation

Bacterial strains were revived in nutrient broth (HiMedia M002) and incubated at 37 °C for 18–24 hours. The inoculum density was adjusted to a 0.5 McFarland standard (~1×10^8 CFU/mL) using sterile saline (Prabhakar, *et al*., 2025).

Media preparation

Mueller-Hinton agar was prepared at 28 g/L, sterilized by autoclaving at 121 °C for 20 minutes under 15 psi, and poured into sterile Petri dishes at 25 mL per plate (Salisu *et al*., 2022).

Agar well diffusion assay

Lawn cultures were prepared by uniformly spreading bacterial suspensions with sterile swabs onto Mueller-Hinton agar plates. Wells (6–8 mm) were aseptically bored into the medium and loaded with 100, 200, and 300 µL of methanolic plant extract. Controls included streptomycin (10 µg/mL) and penicillin (10 IU/mL) as positive controls, and sterile distilled water as a negative control (Shakir *et al*., 2023). Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimeters. Antibacterial activity was expressed as percentage inhibition using the formula: Inhibition (%) = (Test / Control) × 100 (Ullah *et al*., 2021; Doblas *et al*., 2025).

Antifungal evaluation

Fungal strains and culture maintenance: The methanolic extract (702.9 g) was tested against six fungal strains: Alternaria solani (MTCC 2101), Aspergillus niger (ATCC 16888), Candida albicans (ATCC 10231), Fusarium oxysporum (MTCC 284), Curvularia trifolii (MTCC 2034), and Rhizopus arrhizus (MTCC 262) (Sintayehu *et al*., 2010). Cultures were obtained from the Department of Microbiology, University of Peshawar, and maintained on PDA slants at 4 °C (Latif *et al*., 2020).

PDA medium preparation

Potato dextrose agar (19.5 g/L) was prepared in 300 mL batches, homogenized by magnetic stirring (1000 rpm, 60 °C), sterilized at 121 °C (15 psi) for 20 minutes, and poured aseptically into 90 mm Petri dishes at 20 mL per dish (Latif *et al*., 2020; Ullah *et al*., 2019a).

Fungal inoculum preparation

Fungal cultures were incubated at 25 ± 2 °C for 5–7 days to allow for sporulation. Spores were harvested using 0.1% Tween-80 solution and standardized to a concentration of 1×10^6 spores/mL using a hemocytometer (Doblas *et al*., 2025).

Antifungal assay and MIC/MFC determination

Agar well diffusion was conducted using 100 mm PDA plates. Wells (4 mm diameter) were filled with 20 µL of plant extract solutions at 25, 50, and 75 µg/mL (Prasanna *et al*., 2019). Plates were incubated at 32 ± 2 ˚C for 48–72 hours, and inhibition zones were measured using a digital caliper (Mitutoyo 500-196-30; ±0.01 mm) (Goswami *et al*., 2016). MIC values were determined using broth microdilution in 96-well microtiter plates, with 100 µL of extract and 100 µL of standardized spore suspension per well. Plates were incubated at 28 ˚C for 7 days, and turbidity was measured at 600 nm (Shakir *et al*., 2019). Wells showing no visible growth were subcultured onto fresh SDA plates to determine Minimum Fungicidal Concentration (MFC) (Shakir *et al*., 2023).

Physicochemical and anatomical analysis

Total ash content: A 20 g sample of pre-weighed plant powder was incinerated in a porcelain crucible using a muffle furnace (Nabertherm L9/11) at 475 ˚C for 4 hours (Ullah *et al*., 2018). The process was repeated until a constant weight (±0.001 g) was achieved. Total ash content was calculated using the formula: Ash (%) = (Weight of ash / Original weight) × 100 (Shakir *et al*., 2023; Mukhija *et al*., 2015).

Microscopy

Transverse sections (10–15 µm thick) of fresh stems and roots were prepared using a sliding microtome (Leica SM2010R), supported with potato tuber for stability (Gaafar *et al*., 2018). Sections were double-stained with safranin and fast green, and visualized under a compound microscope (Olympus CX23) equipped with a 1.3 MP digital camera (AmScope MU300) (Mukhija *et al*., 2015).

Powder microscopy

Powdered samples were mounted in 50% (v/v) glycerin and observed under 10× and 40× objectives. Characteristic features were recorded and analyzed using ImageJ v1.53 software (Mukhija *et al*., 2015; Prabhakar *et al*., 2025).

Morphological study

Macroscopic evaluation of leaves and stems was conducted using standard botanical protocols. Parameters observed included phyllotactic arrangement, dimensions (length, width, thickness), shape, surface texture, margin and apex morphology, venation, and pigmentation (Shakir *et al*., 2023). Organoleptic attributes such as odor and taste were evaluated under controlled conditions (Mukhija *et al*., 2015). Microscopic observations were aligned with quality control standards for medicinal plants (Doblas *et al*., 2025). Morphological features were digitally documented using a calibrated Canon EOS 250D with an EF-S 60 mm macro lens at magnifications of 4×, 10×, and 40× with scale references (Ullah *et al*., 2018c).

Data analysis and visualization

All statistical analyses and graphical representations were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Figures and illustrations were edited and finalized using Adobe Illustrator 2023 (Adobe Inc., San Jose, CA, USA) to ensure high-quality visual presentation.

Results

Antibacterial activity

In the present study, the methanolic leaf extract of *Pteris cretica* was evaluated for antibacterial activity at three concentrations: 100 µg/mL, 200 µg/mL, and 300 µg/mL. The assay was conducted using the agar well diffusion method against seven clinically significant bacterial strains known to be responsible for opportunistic and hospital-acquired infections. These included five Gram-negative bacteria: *Citrobacter koseri*, *Acinetobacter baumannii*, *Providencia stuartii*, *Proteus vulgaris*, and *Enterobacter aerogenes*, as well as two Gram-positive bacteria: *Klebsiella pneumoniae* and *Staphylococcus aureus*. These bacterial strains were selected due to their growing clinical importance and resistance to multiple antibiotics, which presents a serious challenge to public health. The methanolic extract was found to possess varying degrees of antibacterial activity depending on both the bacterial strain and the concentration applied. In general, higher concentrations of the extract (particularly at 300 µg/mL) produced larger zones of inhibition, indicating a dose-dependent antimicrobial response (Ullah *et al*., 2018d, 2019b).

Among the tested strains, *Citrobacter koseri*, *Acinetobacter baumannii*, and *Providencia stuartii* exhibited the highest susceptibility to the extract, suggesting the presence of bioactive phytochemicals capable of disrupting bacterial growth or metabolism. *Proteus vulgaris* and *Enterobacter aerogenes* demonstrated moderate sensitivity, while

Staphylococcus aureus a well-known Gram-positive pathogen also showed a notable response, particularly at higher concentrations. *Pteris cretica* extract was less effective against *Klebsiella pneumoniae*, which showed limited zones of inhibition at all concentrations tested. However, the overall antibacterial spectrum observed highlights the potential of *Pteris cretica* as a source of plant-derived antimicrobial agents. These findings support the traditional use of the species in ethnomedicine and provide a foundation for future phytochemical and pharmacological studies (Ullah *et al*., 2018). The results of the zone of inhibition for each bacterial strain at different concentrations are summarized in Table 1 and Figure 1 and comparisons with standard antibiotics (streptomycin and penicillin) are discussed in the following sections.

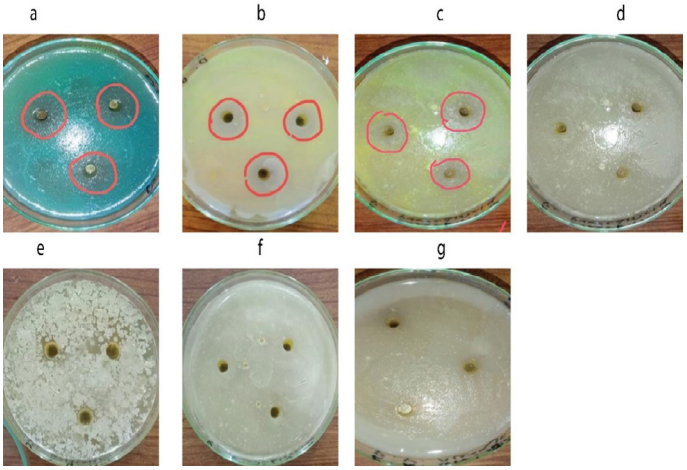

Figure 1: *Represented (a) Citrobacter koseri, (b) Acinetobacter, (c) Providensia, (d) Klebsiella, (e) Staphylococcus aureus, (f) Proteus vulgaris and (g) Enterobacter Aerogenes.*

The methanolic extract of *Pteris cretica* exhibited notable antibacterial activity against *Citrobacter koseri*, *Acinetobacter baumannii*, and *Providencia stuartii* at all tested concentrations (100 µg/mL, 200 µg/mL, and 300 µg/mL). The highest inhibitory effect was observed against *Citrobacter koseri*, with zones of inhibition measuring 25 mm, 25 mm, and 23 mm at 100 µg/mL, 200 µg/mL, and 300 µg/mL, respectively. This consistently strong inhibition suggests that the extract contains bioactive compounds with potent bactericidal or bacteriostatic properties against this strain, even at the lowest concentration tested. *Acinetobacter baumannii*, a multidrug-resistant Gram-negative pathogen commonly associated with nosocomial infections, showed moderate sensitivity to the extract. The zones of inhibition recorded were 14 mm, 10 mm, and 5 mm at increasing concentrations, indicating a concentration-dependent response but with reduced efficacy at higher doses possibly due to solubility limits or compound aggregation at elevated concentrations (Ullah *et al*., 2019c).

Providencia stuartii exhibited relatively weaker sensitivity, with inhibition zones of 8 mm at both 100 µg/mL and 200 µg/mL, and a slight decrease to 6 mm at 300 µg/mL. Though the inhibition was less pronounced, the response indicates some level of antimicrobial activity worth investigating further, especially considering the pathogen's clinical relevance in urinary tract infections and its rising resistance profile. In contrast, the methanolic extract did not display any detectable antibacterial activity against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterobacter aerogenes*, or *Staphylococcus aureus* at any of the tested concentrations (100–300 µg/mL) (Ullah *et al*., 2019d; Maguraushe, 2017). The absence of inhibition suggests either the lack of specific bioactive constituents effective against these strains or the presence of bacterial defense mechanisms that neutralize the active compounds. Overall, these findings underscore the selective antibacterial potential of *Pteris cretica* and provide a basis for further fractionation, isolation, and characterization of its active phytochemicals. The complete results, including measured inhibition zones across all bacterial strains and concentrations, are summarized in Table 2 and Figure 2.

Table 1: *Pathogenic bacterial strains used in antibacterial activity.*

S. No.	Test bacteria	Response to gram stain	Pathogenic nature
1	*Citrobacter koseri*	Gram negative	Animal/Human pathogens
2	*Acinetobacter baumanni*	Gram negative	Animal/Human pathogens
3	*Providensia staurtti*	Gram negative	Animal/Human pathogens
4	*Klebsiella pneumoniae*	Gram negative	Animal/Human pathogens
5	*Proteus vulgaris*	Gram negative	Animal/Human pathogens
6	*Enterobacter Aerogenes*	Gram negative	Animal/Human pathogens
7	*Staphylococcus aureus*	Gram positive	Animal/Human pathogens

Table 2: *Antimicrobial activity of methanol extracts of Pteris cretica.*

Treatments	Conc. (µg/ml)	Citroba cter koseri		Acinetob acter baumann i		Providen sia staurtti		*K. pneumoniae*		*P. vulgaris*		*E. aerogenes*		*S. aureus*
		ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)
CM E	100	19.667± 0.577		7.333± 1.528		4.333 ±1.528		0		0		0		0
	200	20.667± 0.577		9.333 ± 2.517		6.667 ±2.309		0		0		0		0
	300	24.333 ± 1.155		12.333 ± 2.082		7.333 ±1.155		0		0		0		0
S.D		19		26		24		23		27		21		39

Abbreviations: *CME (crud methanol extracts), SD (standard drugs), ZI (zoon of inhibition), LD (Limited drugs).*

Table 3: *Antifungal activity of ethanolic extracts of Pteris cretica.*

Treatments	Conc. (µg/ml)	*Alternaria solani*		*Aspergillus niger*		*Candida albicans*		*Fusarium oxysporum*		*Curvularia trifolii*		*Rhizopus arrhizus*	
		ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}	ZI (mm)	LD_{50}
CM E	100	0	0	0	0	0	0	0	0	0	0	0	0
	200	0		0		0		0		0		0	
	300	0		0		0		0		0		0	

Abbreviations: *CME (crud methanol extracts), ZI (zoon of inhibition), LD (Limited drugs)*

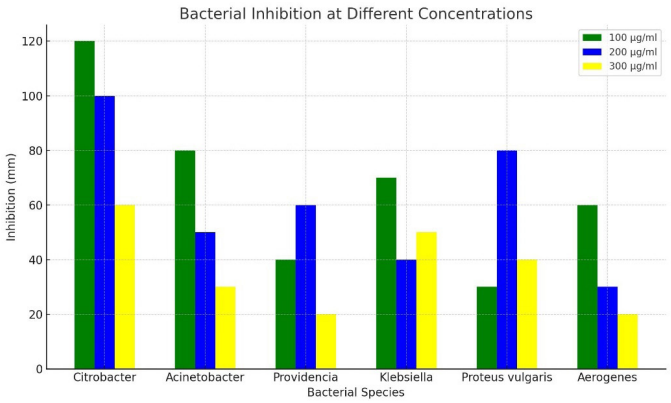

Figure 2: *Represent antibacterial activities of Pteris cretica*

Antifungal activity

The antifungal evaluation of the methanolic leaf extract of *Pteris cretica* was conducted against six clinically and agriculturally significant fungal strains: *Alternaria solani*, *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*, *Curvularia trifolii*, and *Rhizopus arrhizus*. The extract was tested at concentrations of 100 µg/mL, 200 µg/mL, and 300 µg/mL using the agar well diffusion method. The results demonstrated that the methanolic extract exhibited no antifungal activity against any of the tested fungal strains at all concentrations. There were no visible zones of inhibition observed on the PDA plates after incubation, indicating a complete lack of antifungal efficacy under the experimental conditions used (Ullah *et al*., 2018e). This outcome suggests that the bioactive constituents present in *Pteris cretica* may be selectively antibacterial but are not effective against the tested fungal organisms, or that the concentration and solubility of antifungal compounds (if any are present) were insufficient to exert a measurable inhibitory effect (Ullah *et al*., 2018a). The inability of the extract to inhibit fungal growth could also be attributed to the structural and biochemical resistance mechanisms possessed by fungi, such as the protective role of chitin in their cell walls and the presence of detoxifying enzymes. Moreover, the result highlights the need for further fractionation and bioassay-guided isolation to identify whether any antifungal constituents exist in other solvent fractions or plant parts. The complete antifungal assay data, including the absence of inhibition zones across all strains and concentrations, are presented in Table 3 and Figure 3.

Microscopy

The transverse sections (T.S.) of the root and stem of *Pteris cretica* revealed a well-organized anatomical structure with distinct tissue differentiation. Microscopic examination demonstrated the presence of key tissue systems including the epidermis, cortex, and vascular bundles, which are characteristic of lower vascular plants. The epidermis formed the outermost protective layer of both root and stem. It consisted of two to four layers of compactly arranged polygonal

cells. The cells appeared thick-walled and dark brown under staining, indicating the presence of phenolic compounds or suberin. This structural feature likely contributes to protection against desiccation and microbial invasion (Ullah *et al.*, 2018b). The cortex was prominently developed and occupied the largest portion of the ground tissue region. It was composed of multiple layers of parenchymatous cells with large intercellular spaces. These cells were thin-walled, isodiametric, and arranged loosely to facilitate gas exchange and storage. In some regions, starch grains and occasional resin ducts were observed, suggesting storage and potential chemical defense functions. Located centrally and encircled by the cortex, the vascular bundles displayed a clear distinction between xylem and phloem tissues. The phloem was positioned toward the outer side, while the xylem was oriented toward the center, forming a radial arrangement. This pattern is typical of ferns and other pteridophytes. Xylem vessels appeared thick-walled and lignified, responsible for water conduction and mechanical support. Phloem elements, in contrast, were thin-walled and less conspicuous under microscopic examination (Ullah *et al.*, 2018f). The organized arrangement of tissues reflects the adaptability of *Pteris cretica* to various ecological conditions, including moist and shaded environments. The anatomical observations support the structural complexity and vascular advancement within the Pteridaceae family. Detailed microphotographs of these features were captured using safranin-fast green-stained sections and are illustrated in Figure 4.

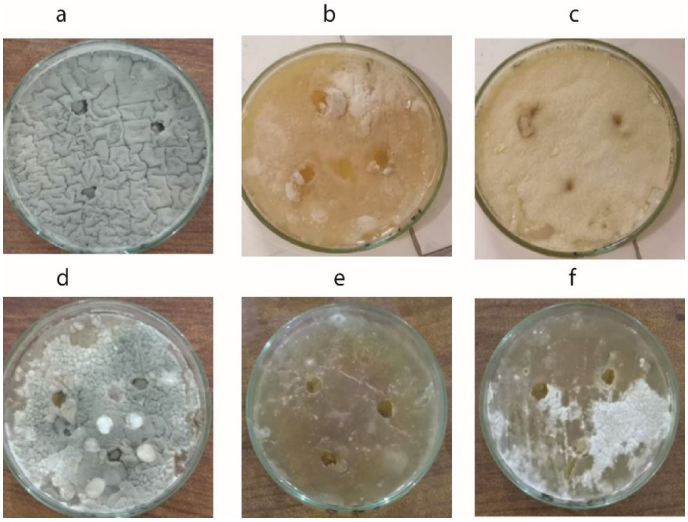

Figure 3: *(a) Alternaria solan, (b) Fusarium (c) Candida albicans, (d) Rhizopus arrhizus (e) Curvularia trifoli, and (f) Aspergillus niger.*

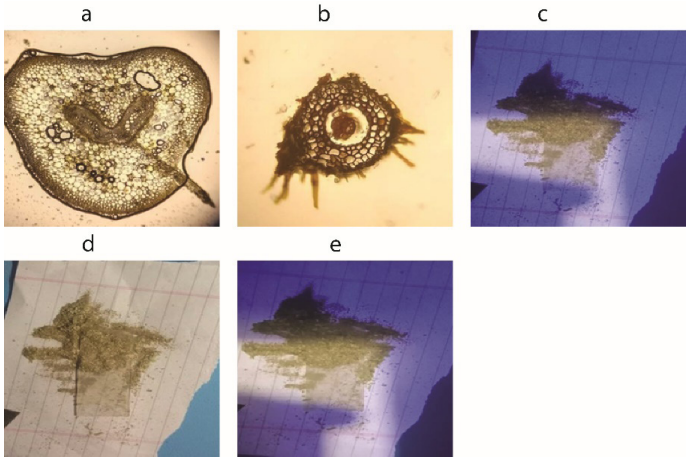

Figure 4: *Represented the Pteris cretica (a) Stem section, (b) Root section, (c) Visible light, (d) Short UV (254 nm), and (e) long UV (366 nm).*

Organoleptic characteristics

Macro morphological and organoleptic features are commonly used for description and explanation of species in Micromorphology and anatomy were not considered by taxonomists as a valuable descriptive tool, but according to many taxonomists, it can play a significant and crucial role in description and resolving many complexities in closely related taxa, genera, and species (Ullah *et al.*, 2018g). The present study was conducted in such regard, including macroscopical and organoleptic investigation of the fresh and dried leaves and plant. *Pteris cretica is a* small evergreen pteridophyte up to 18-24 inches in height. Leaves are pinnate compound, the petiole is Monostelic, the fractures are smooth, and the apex of the leaf is acuminate, color is a white stripe, with 10- 30cm. Pleasant odour, bitter taste, and base are symmetrical (Table 4).

Table 4: *Organoleptic characteristics of Pteris cretica.*

S. No	Parts/ Characters	Leaves/ Observations
1	Size	10-30cm
2	Shape	Pinnate
3	Fracture	Smooth
4	Colour	White stripe
5	Odour	Pleasant
6	Height	18-24 inches
7	Taste	Bitter
8	Petiole	Monostelic
9	Leaves	Compound
10	Base	Symmetrical
11	Nature	Evergreen
12	Apex	Acuminate

Ash activity

In physicochemical properties total ash content were also recorded. The activity is repeated twice and the percentage of ash is 2. 007g. These values were useful for the quality control system regarding ash and it was used for medicinal purposes (Table 5).

Table 5: *Ash activity of Pteris cretica.*

S. No	Character	Average ± SD
1	Total Ash	2.007±1.848

Abbreviations: *SD (standard deviation).*

Fluorescence analysis

The powdered drug of Pteris cretica was subjected to fluorescence analysis under ultraviolet (UV) light to assess its diagnostic and Pharmacognostic characteristics. Observations were made under both short-wave UV (254 nm) and long-wave UV (366 nm) illumination, as well as under visible light. Fluorescence analysis is a valuable tool in the identification and authentication of crude plant materials, as many phytoconstituents such as alkaloids, coumarins, flavonoids, and essential oils exhibit characteristic fluorescence when exposed to UV radiation. Under visible light, the powdered sample appeared dull green to pale brown. Upon exposure to short-wave UV light (254 nm), the powder exhibited a dark greenish fluorescence, whereas long-wave UV light (366 nm) induced a bright yellowish-green glow. These fluorescent responses suggest the presence of specific phytochemicals with conjugated systems and chromophores capable of absorbing and emitting UV radiation. Such fluorescence patterns are often unique and reproducible, making them a useful parameter in pharmacognostic standardization and the detection of adulterants or degraded material (Rai, 2025). Moreover, the fluorescence behavior can aid in correlating the chemical profile of the plant with its therapeutic potential, supporting its use in traditional and modern medicinal practices. The detailed fluorescence characteristics of the powdered drug of Pteris cretica under various lighting conditions are presented in Table 6.

Table 6: *Fluorescence analysis of Pteris cretica.*

Visible light	Short wavelength/ 254nm	Long wave length/ 366nm
Pale green	Brownish	Dark brawny

Table 7: *Extractive valves of Pteris cretica.*

S. No	Plant parts	Solvents	10g/ 50ml
1	Leaves powder	Chloroform	2.8 g
2	Leaves powder	N-Hexane	0.6 g
3	Leaves powder	Methanol	6 g

Table 7 presents the extractive values of Pteris cretica leaves using different solvents. Among the solvents tested, methanol yielded the highest extract (6 g), indicating it is the most effective for extracting bioactive compounds from the leaf powder. In contrast, n-hexane showed the lowest extractive value (0.6 g), suggesting limited solubility of compounds in non-polar solvents.

Discussion

The growing threat of antimicrobial resistance has necessitated the urgent search for novel antimicrobial agents from natural sources. Antibiotic overuse has led to the widespread emergence of resistant bacterial strains, rendering many conventional drugs less effective. In this context, the genus *Pteris*, particularly *Pteris cretica*, presents promising potential due to its traditional medicinal uses and reported antimicrobial properties. In the present study, the methanolic extract of *Pteris cretica* leaves exhibited significant antibacterial activity against selected Gram-negative bacteria, notably *Citrobacter koseri*, *Acinetobacter baumannii*, and *Providencia stuartii*, across all tested concentrations (100–300 µg/mL). This aligns with earlier studies which reported antimicrobial activity of *P. cretica* using various solvent extracts (Olajuyig *et al.*, 2015) demonstrated that n-hexane extracts were effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium sporogenes*, and *Klebsiella pneumoniae*, while ethanol extracts inhibited *Pseudomonas aeruginosa* and other pathogens. Similarly, methanol extracts from various ferns, including *Pteris cretica*, showed notable inhibitory effects on *S. aureus* and *Escherichia coli*. Other species of *Pteris*, such as *P. inaequalis* (Ugwu *et al.*, 2020), *P. vittata* (Lyumugabe *et al.*, 2017), *P. quadriaurita* (Rai, 2025), and *P. biaurita* (Ugbogu *et al.*, 2019), have also been reported to possess antimicrobial activity. In a broader screening study involving 114 pteridophyte species, (Eze *et al.*, 2014) found that multiple species displayed inhibition zones against drug-resistant pathogens such as penicillin-resistant *S. aureus*, *Mycobacterium phlei*, *Salmonella typhi*, *Vibrio cholerae*, and *Pseudomonas aeruginosa*.

These findings substantiate the results of the present study and reinforce the potential of *Pteris* species as sources of bioactive compounds. Conversely, the methanolic extract of *P. cretica* demonstrated no antifungal activity against six tested fungal strains (*Alternaria solani*, *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*, *Curvularia trifolii*, and *Rhizopus arrhizus*) at the concentrations used. This result is consistent with earlier studies reporting limited or no antifungal activity in certain solvent extracts of *P. cretica*, particularly against *Fusarium* spp. and *Rhizopus* spp. (Shakir *et al.*, 2023). However, (Ushie *et al.*, 2022) reported a dose-dependent antifungal response in ethanolic extracts, with increasing inhibition zones at higher concentrations (up to 20 mg/mL), suggesting that the antifungal efficacy may be extract- and dose-dependent, or influenced by strain variability.

The anatomical analysis of *P. cretica* provided further taxonomic insights. Transverse sections revealed key diagnostic features such as a multi-layered epidermis, extensive cortex, and centrally arranged vascular bundles with distinct xylem and phloem orientation. These findings are in agreement with earlier studies on fern anatomy and support the systematic classification of *Pteris* within the family Pteridaceae. Leaf epidermal characters, as previously noted by (Uprety *et al.*, 2017), offer valuable taxonomic and phylogenetic information. The micromorphological variability observed across species in the family analyzed using light and scanning electron microscopy serves as a reliable tool for species differentiation and evolutionary studies. Additionally, the present study determined the total ash content of *P. cretica* leaves to be 2.007%, which falls within the expected range for crude plant drugs and suggests a relatively low inorganic residue content. Comparative data from other plant species, such as *Sesbania grandiflora*, have shown that total ash content varies with plant part and seasonal collection (Zareef *et al.*, 2023). For instance, *Sesbania* leaves had total ashes values between 7.5% and 7.75%, with bark and wood displaying even higher values. The lower ash content in *P. cretica* may be advantageous from a pharmacognostic and safety perspective, indicating minimal contamination or inorganic adulteration. Taken together, the findings from the present study not only support the ethnomedicinal use of *Pteris cretica* for wound healing (Shakir *et al.*, 2023) but also underscore its potential as a source of antibacterial agents. However, its limited antifungal activity warrants further investigation, particularly with higher concentrations or alternative solvent systems. The integration of anatomical, organoleptic, phytochemical, and antimicrobial data contributes to the growing body of knowledge on this fern and reinforces the relevance of traditional botanical knowledge in modern drug discovery.

Conclusion

The methanolic extract of *Pteris cretica* exhibited notable antibacterial activity against Citrobacter koseri, Acinetobacter baumannii, and Providencia stuartii, lending support to its traditional medicinal applications. In contrast, the extract showed no inhibitory effect against Klebsiella pneumoniae, Proteus vulgaris, Enterobacter aerogenes, Staphylococcus aureus, or any of the tested fungal strains. These findings are consistent with previous studies highlighting the antimicrobial potential of *Pteris* species and underscore the selective efficacy of its phytoconstituents. Anatomical evaluation revealed characteristic fern features, while the low total ash content (2.007%) indicated high organic purity and minimal inorganic contamination. Future studies should focus on the isolation and characterization of bioactive compounds to assess their mechanisms of action and therapeutic potential, particularly against drug-resistant pathogens.

Recommendation

On the basis of current work author recommended that the further study should be carried out in future to isolate the specific chemical constituents and biological importance especially antibacterial agents. Only methanolic extract of the plant were used in this study it is recommended to screen other organic solvent fraction of the plant for antibacterial and other therapeutic potential.

Acknowledgement

The authors express their sincere gratitude to Dr. Imtiaz Ahmad for providing all the necessary resources and support for this research. Special thanks are also extended to Shakir Ullah for his valuable assistance in data analysis and the final preparation of the manuscript.

Novelty Statement

This study provides the first comprehensive evaluation of *Pteris cretica* methanolic extract against clinically

relevant bacterial and fungal strains, revealing its selective antibacterial efficacy. We document novel inhibitory effects against Citrobacter (25mm zone) while demonstrating its inactivity against six fungal pathogens. Our anatomical and phytochemical analyses (2.007% ash content) establish new quality control benchmarks for this medicinal fern. These findings expand understanding of Pteris species' antimicrobial potential and support their ethnopharmacological use in combating resistant infections.

Author Contributions

Conceptualization, Shakir Ullah: methodology, software. Imtiaz Ahmad supervision, writing original draft preparation, Lubna Shakir writing, review, and editing, Fayaz Asad, Fazal Manan resources, project administration. All authors have read and agreed to the published version of the manuscript.

Funding
This research received no external funding.

Data availability statement
Not applicable.

Conflicts of interest
The authors have declared no conflict of interest.

References

Adoga, S.O., Ehikowoicho, D., Kyenge, B.A., Aondo, T.O.O. and Ikese, C.O., 2019. Phytochemical screening, TLC and antimicrobial activity of *Parquetina nigrescens* leaf extracts. Ovidius Univ. Ann. Chem., 30: 88–94. https://doi.org/10.2478/auoc-2019-0016

Ankari, A.B., Mahendru, N., Paniyappanavara, V., Jena, N., Kumar, S. and Tripathi, G., 2024. Evaluation of medicinal values of a fern species [*Thelypteris parasitica* (L.) Tardieu] of India. Plants Second. Metab., 2.

Arya, V., Gill, A.K. and Jamwal, A., 2024. Ecological facets of varied plant life forms and their therapeutic relevance. Pharmacogn. Rev., 18(36): 127–136. https://doi.org/10.5530/phrev.20241976

Asif, S., Nisar, M., Ullah, S. and Naeem, M., 2025. Impact of seed-borne mycoflora on mycotoxin accumulation: A threat to lentil genetic resources. Toxicon, pp. 108290. https://doi.org/10.1016/j.toxicon.2025.108290

Bandyopadhyay, A. and Dey, A., 2022. Medicinal pteridophytes: Ethnopharmacological, phytochemical, and clinical attributes. Beni-Suef Univ. J. Basic Appl. Sci., 11(1): 113. https://doi.org/10.1186/s43088-022-00283-3

Choudhury, J., Majumdar, S., Roy, S. and Chakraborty, U., 2017. Antioxidant activity and phytochemical screening of edible wetland pteridophytes: *Diplazium esculentum* and *Marsilea minuta* a comparative study. World J. Pharm. Med. Res., 3(9): 195–203.

Doblas, G.Z., Catane, I.L.R., Amoroso, V.B., Ang, A.M.G., Porquis, H.C., Jacalan, D.R.Y. and Cruz, R.Y.D., 2025. Toxicity, anti-inflammatory, and phytochemical properties of *Christella parasitica* (L.) H. Lev. ex Y.H. Chang in Bukidnon, Philippines. Editorial Board, 59. https://doi.org/10.69721/TPS.J.2025.17.1.07

Eze, F.N. and Osamede, J.A., 2014. Quantitative phytochemical evaluation of stem bark extracts of Harungana madagascariensis. Br. J. Pharm. Res., 4(17): 2068. https://doi.org/10.9734/BJPR/2014/9988

Gaafar, A.A., Sami, I.A., Ahmed, M.F. and El-Hallouty, S.M., 2018. An insight into chemical content, biological effect and morphological features of *Pteris vittata* L., rarely growing in Egypt. Res. J. Chem. Environ., 22(10): 47–55.

Gacche, R.N., Shaikh, R.U. and Pund, M.M., 2011. *In vitro* evaluation of anticancer and antimicrobial activity of selected medicinal plants from Ayurveda. Asian J. Trad. Med., 6(3): 1–7.

Goswami, H.K., Sen, K. and Mukhopadhyay, R., 2016. Pteridophytes: evolutionary boon as medicinal plants. Plant Genet. Resour., 14(4): 328–355. https://doi.org/10.1017/S1479262116000290

Gracelin, D.H.S., Britto, A. and Kumar, B.J.R.P., 2013. Qualitative and quantitative analysis of phytochemicals in five Pteris species. Int. J. Pharm. Pharm. Sci., 5(1): 105–107.

Hao-bin, H., Cao, H., Jian, Y.F., Zheng, X.D. and Liu, J.X., 2008. Chemical constituents and antimicrobial activities of extracts from *Pteris multifida*. Chem. Nat. Compd., 44: 106–108. https://doi.org/10.1007/s10600-008-0031-y

Irshad, A., Noreen, S., Sajid, U., Jamal, M., Iqbal, M.A., Ullah, S., Sabtain, T., Ullah, S., Ibañez-Arancibia, E., De Los Ríos-Escalante, P.R.,

Belkahia, H., Ben Said, M. and Swelum, A.A., 2025. Molecular identification, risk factors' assessment and phylogenetic analysis of *Toxoplasma gondii* in goats from Malakand Division, Khyber Pakhtunkhwa, Pakistan. Trop. Anim. Health Prod., 49(21). https://doi.org/10.1007/s11259-025-10783-z

Juliasih, N.K.A. and Adnyana, I.M.D.M., 2023. Ethnopharmacology and species diversity of *Pteridophyta* in Cyathea Park, Bali: A field study and literature review. https://doi.org/10.21203/rs.3.rs-2497775/v2

Juliasih, N.K.A. and Adnyana, I.M.D.M., 2023. Utilization of *Pteridophyta* species in Cyathea Park, Bali, as traditional medicine agents: A field study and meta-synthesis review. Notulae Sci. Biol., 15(2): 11522. https://doi.org/10.55779/nsb15211522

Kumar, S., Mishra, A., and Pandey, A. K. (2013). Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models. BMC complementary and alternative medicine, 13(1): 120.

Khan, M., Haris, M., Riaz Khan, M., Ali, I., Nasreen, N., Sohail, M. and Ullah, S., 2024. Acaricidal efficacy of *Melia azedarach*, *Olea ferruginea*, and *Zanthoxylum armatum* against *Rhipicephalus microplus* from Khyber Pakhtunkhwa, Pakistan. Adv. J. Sci. Eng. Technol., 3(1): 99–114. https://doi.org/10.47264/idea.ajset/3.1.7

Khan, S., Jan, G., Bibi, H., Sher, J., Ullah, S. and Abidullah, S., 2018a. Phytochemical screening and antimicrobial activity of *Cichorium intybus* (Family: Asteraceae) and Medicago sativa (Family: Fabaceae), Peshawar, Pakistan. J. Pharmacogn. Phytochem., 7(3): 603–616.

Khan, S., Jan, G., Bibi, H., Ullah, K., Gul, F. and Ullah, S., 2018b. Plants traditional medication in the arid and semi-arid zone of Tehsil Domal, District Bannu, Khyber Pakhtunkhwa, Pakistan. J. Appl. Environ. Biol. Sci., 8(8): 14–21.

Khan, S., Jan, G., Bibi, H., Ullah, K. and Ullah, S., 2018c. Antimicrobial, phytochemical, and traditional studies of selected medicinal plants in Bajaur Agency, Pakistan. Int. J. Res. Pharm. Sci., 8(2): 4–21.

Latif, M., Umar, M.I., Rahim, A., Khan, S.A., Fatima, N., Hussain, I. and Basheer, M.K.A., 2020. Isolation and biological evaluation of some secondary metabolites from seeds of Silybum marianum. Pharmacogn. Mag., 16(68). https://doi.org/10.4103/pm.pm_315_19

Lyumugabe, L.F., Uyisenga, J.P., Bayingana, C. and Songa, E.B., 2017. Antimicrobial activity and phytochemicals analysis of Vernonia aemulans, Vernonia amygdalina, Lantana camara and Markhamia lutea leaves as natural beer preservatives. Am. J. Food Technol., 12(1). https://doi.org/10.3923/ajft.2017.35.42

Maguraushe, K.A., 2017. Phytochemical analysis, radical scavenging and anti-microbial activity of the leaves of Gomphocarpus fruticosus. Ph.D. Diss., Univ. of Namibia.

Mahmutović-Dizdarević, I. and Žujo, B., 2023. The review on the antimicrobial potential of maidenhair fern, *Adiantum capillus-veneris* L. Ann. Inst. Biol., 45: 41–58. https://doi.org/10.35666/2831-0705.2023.45.41

Mary, R.N.I., Meenashree, B. and Vasanthi, V.J., 2014. Screening of antibacterial activity and qualitative and quantitative analysis of phytochemicals in Vitex trifolia. Int. J. Curr. Microbiol. Appl. Sci., 3: 425–431.

Mir, S.A., Mishra, A.K., Reshi, Z.A. and Sharma, M.P., 2013. Preliminary phytochemical screening of some pteridophytes from District Shopian (J and K). Int. J. Pharm. Pharm. Sci., 5(4): 632–637.

Moussa, A.Y., Luo, J. and Xu, B., 2024. Insights into chemical diversity and potential health-promoting effects of ferns. Plants, 13(18): 2668. https://doi.org/10.3390/plants13182668

Mukhija, M., Singh, M.P., Dhar, K.L. and Kalia, A.N., 2015. Cytotoxic and antioxidant activity of Zanthoxylum alatum stem bark and its flavonoid constituents. J. Pharmacogn. Phytochem., 4(4): 86.

Nayak, N., Rath, S., Mishra, M.P., Ghosh, G. and Padhy, R.N., 2013. Antibacterial activity of the terrestrial fern *Lygodium flexuosum* (L.) Sw. against multidrug resistant enteric-and uro-pathogenic bacteria. J. Acute Dis., 2(4): 270–276. https://doi.org/10.1016/S2221-6189(13)60142-0

Nuwamanya, H., Lukubye, B., Kagoro-Rugunda, G., Ajayi, C.O., Wangalwa, R. and Olet, E.A., 2025. Phytochemical profile and antimicrobial activity of individual frond extracts of *Menisorus pauciflorus*, *Pteris catoptera*, *Conniogramme africana* and *Antrophyum mannianum*. https://doi.org/10.21203/rs.3.rs-5631852/v1

Olajuyigbe, O.O., Ijeyan, F.O. and Adeoye-Isijola, M.O., 2017. GC-MS analysis and antimicrobial effects of methanol stem bark extract of Trilepisium madagascriense DC. Int. J. Sci., 6(8): 34–45. https://doi.org/10.18483/ijSci.1366

Prabhakar, N.K., Gangwar, R., Adhikari, R.S., Nayaka, S., Arya, M. and Mehta, J.P., 2025. Phytochemical profiling and antibacterial efficiency of Hypotrachyna cirrhata and Parmotrema tinctorum lichen extracts from Madhyamaheshwar Valley, Garhwal Himalaya, Uttarakhand. Environ. Ecol., 43(1): 175–184. https://doi.org/10.60151/envec/ZDOF1463

Prasanna, G., Devi, R., and Ishwarya, G. (2019). In vitro evaluation of antidiabetic and cytotoxicity potentials of the rhizome extract of *Drynaria quercifolia* (L.) J. Smith. Asian J Pharm Clin Res, 12.

Priya, V., Krishnan, M. and Nair, M.G., 2024. Exploring the phytochemical properties and therapeutic potential of *Cheilanthes swartzii*: A novel fern from the Western Ghats. J. Med. Plants By-Prod., 13(4).

Rai, M., 2025. Fighting multidrug resistance with herbal extracts, essential oils and their components. Elsevier.

Rakkimuthu, R., Naveenraj, B., Benitta, L. and Dhanya, K., 2018. Phytochemical screening and its antibacterial activity of the fern *Hemionitis arifolia* (Burm.) Moore. World J. Pharm. Res., 7(13): 603.

Rautray, S., Panikar, S., Sofia, A. and Rajananthini, A.U., 2018. Antioxidant and antimicrobial study of *Adiantum capillus-veneris* and *Pteris quadriureta* L. J. Med. Plants Res., 12(23): 359–368. https://doi.org/10.5897/JMPR2018.6635

Razaghi, P. and Abdel-Azeem, A.M., 2024. Diversity of endophytic fungi hosted by medicinal ferns: Biotechnological potentials and possible applications. Microb. Biosyst., 9(1): 105–128. https://doi.org/10.21608/mb.2024.366970

Sajid, M., Rahim, F., Ullah, S., Ullah, R., Bilqees, R. and Shakir, L., 2023. Qualitative and quantitative ethnobotanical study of Arrang Valley of District Bajaur, Khyber Pakhtunkhwa, Pakistan. J. Agric. For. Res., 2(3).

Salisu, A., Iliyasu, M. Y., Sahal, M. R., Titus, I., Isma'il, S., Umar, R. D., & Agbo, E. B. (2022). Antimicrobial potential of *Syzygium aromaticium* (clove) extracts on multidrug-resistant (MDR) uropathogenic bacteria isolated from clinical specimens in Bauchi, Nigeria. Methodology.

Shakir, L., Asif, S., Haq, A., Haq, S., Zada, K., Said, M., Sajid, M., Ullah, S. and Ullah, R., 2023. Phytochemical detection and medicinal studies of selected plants from war-affected areas of Khyber Pakhtunkhwa, Pakistan. J. Agric. For. Res., 2(5).

Shakir, L., Ullah, S., Suhail, M., Ullah, R., 2023. Phytochemical analysis, antipyretic and antifungal activities of *Solanum nigrum* L. Nat. J. Pharm. Sci., 3(2): 6–12.

Singh, M., Govindarajan, R., Rawat, A.K.S. and Khare, P.B., 2008. Antimicrobial flavonoid rutin from *Pteris vittata* L. against pathogenic gastrointestinal microflora. Am. Fern J., 98(2): 98–103. https://doi.org/10.1640/0002-8444(2008)98[98:AFRFPV]2.0.CO;2

Singha, S., Nath, R., Das, S., Kityania, S., Nath, D. and Das Talukdar, A., 2023. Phytochemicals from the Pteridaceae family and their prospects as future drugs. In: Bioactive Compounds in Bryophytes and Pteridophytes. Springer Int. Publ., Cham, pp. 421–442. https://doi.org/10.1007/978-3-031-23243-5_16

Sintayehu, B., Asres, K., Mazumder, A. and Bucar, F., 2010. Isolation of an antibacterial flavonoid glycoside from the leaves of Cineraria abyssinica. Ethiop. Pharm. J., 28(2): 87–94. https://doi.org/10.4314/epj.v28i2.3

Subhan, G., Ullah, S., Ali, S., Zainab, R., Sohail, M., Shakir, L. and Ullah, I., 2024. Floristic composition and phenology of vegetation in Dir Lower, Khyber Pakhtunkhwa. Plant Prot., 8(3): 469–486. https://doi.org/10.33804/pp.008.03.5264

Şuțan, N.A., Fierăscu, I., Fierăscu, R., Ionica, D. and Soare, L.C., 2019. Phytochemical analysis and in vitro assessment of *Polystichum setiferum* extracts for cytotoxic and antimicrobial activities. Caryologia, 72(2): 53–61.

Ugbogu, E.A., Akubugwo, I.E., Ude, V.C., Gilbert, J. and Ekeanyanwu, B., 2019. Toxicological evaluation of phytochemical characterized aqueous extract of wild dried *Lentinus squarrosulus* (Mont.) mushroom in rats. Toxicol. Res., 35: 181–190. https://doi.org/10.5487/TR.2019.35.2.181

Ugwu, C. and Anosike, C.A., 2020. Phytochemical screening and antioxidant potentials of methanol extract of Duranta erecta leaves. Asian J. Res. Biochem., 7(3): 16–26. https://doi.

org/10.9734/ajrb/2020/v7i330140

Ullah, R., Rahim, F., Sajid, M., Ullah, S., Ali, S., Shakir, L. and Subhan, G., 2024a. Ethnobotanical study of Munda Khazana, Dir Lower, Khyber Pakhtunkhwa. Pak. J. Weed Sci. Res., 30(3): 105–120.

Ullah, S., Ahmad, S.M., Ul Haq, I., Ullah, I., Bilqees, R., Ali, A., Khan, Y. and Sajid, M., 2023. Ethnobotanical study of Village Darangal Kambat, Tehsil Samarbagh, District Dir Lower, Khyber Pakhtunkhwa, Pakistan. Int. J. For. Res., 2023: 1–18.

Ullah, S., Iman, S., Rafiullah, S., Mahmood, R., Khan, P., Anwar, S., Khan, Z. and Ullah, R., 2023. Checklist of ethnobotanical plants of Tehsil Colony, Samarbagh, District Dir Lower, Khyber Pakhtunkhwa, Pakistan. J. Agric. For. Res., 2(3). https://doi.org/10.33545/26646064.2021.v3.i1a.63

Ullah, S., Jan, G., Gul, F., Israr, M., Khan, S., Khattak, M., Bibi, H. and Sher, J., 2018e. Phytochemical analysis, antipyretic, analgesic, anti-inflammatory, and antifungal activities of Pteris quadriaurita Retz. World J. Pharm. Pharm. Sci., 7(1): 857–876.

Ullah, S., Jan, G., Gul, F., Khan, S. and Sheer, J., 2018f. Antifungal, nutritional, and phytochemical investigation of Asplenium dalhousiae of District Dir Lower, Pakistan. J. Pharmacogn. Phytochem., 7(2): 3281–3288.

Ullah, S., Jan, G., Gul, F., Khan, S., Husna, Sher, J. and Abidullah, S., 2018c. Phytochemistry and antibacterial activities of some selected plants of war-affected area Bajaur Agency, Pakistan. J. Pharmacogn. Phytochem., 7(3): 415–422.

Ullah, S., Jan, G., Gul, F., Khan, S., Khattak, M., Bibi, H. and Sher, J., 2018h. Phytochemistry, anti-inflammatory and antipyretic activities of Adiantum capillus-veneris in Swiss albino mice. Int. J. Fauna Biol. Stud., 5(3): 19–25.

Ullah, S., Jan, G., Gul, F., Khan, S., Khattak, M., Ihsan, M. and Bibi, H., 2018d. Phytochemical and nutritional analysis of selected plants of District Buner, Pakistan. Int. J. Fauna Biol. Stud., 5(3): 111–117.

Ullah, S., Jan, G., Gul, F., Khan, S., Khattak, M., Sher, J. and Bibi, H., 2018g. Antifungal and phytochemical screening of selected medicinal plants of Malamjaba, Swat, Pakistan. Pharma Innov. J., 7(5): 176–180.

Ullah, S., Jan, G., Jan, F.G., Khan, S., Khattak, M., Bibi, H. and Ihsan, M., 2018. Phytochemical analysis, antipyretic and antifungal activities of *Cyrtomium caryotideum*. Biosci. Biotechnol. Res. Asia, 15(3): 577–589. https://doi.org/10.13005/bbra/2664

Ullah, S., Jan, G., Jan, F.G., Khan, S., Khattak, M., Bibi, H. and Ihsan, M., 2018b. Phytochemical analysis, analgesic, anti-inflammatory, and anti-bacterial activities of *Berberis lycium*. Int. J. Adv. Res., 6(7): 1150–1166. https://doi.org/10.21474/IJAR01/7470

Ullah, S., Khattak, M., Abasi, F., Sohil, M., Ihsan, M. and Ullah, R., 2019b. Antifungal, nutritional and phytochemical investigation of Actiniopteris radiata of District Dir Lower, Pakistan. Int. J. Hortic. Food Sci., 1(1): 1–8. https://doi.org/10.33545/26631067.2019.v1.i2a.21

Ullah, S., Saeed, M., Sohail, M., Sajid, A., Aziz, L., Noreen, A. and Shakir, L., 2025b. Indigenous knowledge of medicinal plants used by tribal communities of Hasham Valley, Dir Lower, Khyber Pakhtunkhwa. Pak. J. Weed Sci. Res., 31(1): 37–55.

Ullah, S. and Shakir, L., 2023. The effects of plant age on phytochemical and geographical distribution of *Euphorbia helioscopia* from Arrang District Bajaur. Pak. J. Weed Sci. Res., 29(4): 206–212.

Ullah, S., Shakir, L., Ali, S., Subhan, G., Sohail, M., Khan, I. and Ali, S., 2025c. Nutritional analysis, phytochemical and antifungal study of *Equisetum arvense* L. from Village Kharkay, Pak–Afghan border, District Dir Lower. Kashmir J. Sci., 4(1). https://doi.org/10.63147/krjs.v4i01.85

Ullah, S., Shakir, L., Sohail, M., Noreen, A. and Aziz, L., 2025a. Controlling of cotton leaf worm in Solanum nigrum L. at District Swat under semi-field conditions. Pak. J. Weed Sci. Res., 31(1): 56–65.

Ullah, S., Shakir, L., Subhan, G., Sohail, M., 2023. Different soybean varieties are affected by irrigation in Mardan, Khyber-Pakhtunkhwa, Pakistan. Pak. J. Weed Sci. Res., 29(4): 229–237.

Ullah, S., Shakir, L., Subhan, G., Sohail, M., Bilqees, R., Khan, F., Gulzar, U., Ali, S., Ullah, Z., Ali, S., 2024. Phytodiversity and conservation assessment of ethnobotanically significant flora in Khall Hagram Dara, Lower Dir, Khyber-Pakhtunkhwa, Pakistan. Plant Prot., 8(1): 143–162. https://doi.org/10.33804/pp.008.01.5084

Ullah, S., Shakir, L. and Ullah, R., 2023. Morphological and phytochemical study of *Cirsium arvense* from District Mardan, Pakistan. J. Bioinform. Biotechnol. Res., 1(1): 1–7. https://doi.org/10.61440/JBBR.2023.v1.01

Ullah, S., Sohail, M., Niaz, K., Khan, S. and Khattak, M., 2018. Phytochemistry and antibacterial activity of *Convolvulus arvensis* Linn against *Escherichia coli*. J. Chem. Biol. Phys. Sci., 8(4): 2249–1929. https://doi.org/10.24214/jcbps.B.8.4.89204

Ullah, S., Sohil, M., Khattak, M., Ihsan, M. and Begum, L., 2019d. List of some selected pteridophytes from Maidan Valley of Dir Lower, Khyber Pakhtunkhwa, Pakistan. Int. J. Hortic. Food Sci., 1(2): 15–22. https://doi.org/10.33545/26631067.2019.v1.i2a.24

Ullah, S., Ullah, I., Khan, M., Zamir, M., Khan, B.T., Naz, R., Sohil, M., Ihsan, M. and Abasi, F., 2019c. Phytochemical analysis and antibacterial activity of *Ajuga bracteosa, Bergenia ciliata,* and *Amaranthus viridis* from District Lower Dir, Village Maidan Banda of Khyber Pakhtunkhwa, Pakistan. Int. J. Biosci., 14(5): 403–412.

Ullah, S., Ullah, I., Naz, R., Sohil, M., Ihsan, M. and Abasi, F., 2019a. Phytochemical's screening and chromatographic separation of bio-active compound from the roots of Berberis lyceum. J. Biotechnol. Bioinform. Res., 1(1). https://doi.org/10.33545/26180723.2019.v2.i1a.19

Ullah, S., Ullah, R., Ullah, F., Khan, G.Z., Shakir, L., Sardar, F. and Ullah, R., 2021. Traditional uses of plants and their role in the community development of Sheen Ghar Valley, District Lower, Khyber Pakhtunkhwa, Pakistan. Int. J. Agric. Nutr., 1(1). https://doi.org/10.33545/26646064.2021.v3.i1a.64

Uprety, Y., Lacasse, A. and Asselin, H., 2016. Traditional uses of medicinal plants from the Canadian Boreal Forest for the management of chronic pain syndromes. Pain Pract., 16(4): 459–466. https://doi.org/10.1111/papr.12284

Ushie, O.A., Longbap, B.D., Iyen, S.I., Ugwuja, D.I., Azuaga, T.I. and Fidelis, H., 2022. Phytochemical screening and antioxidants investigations of ethyl acetate and acetone leaf extracts of Thaumatococcus danielli. Res. J. Pharm. Chem. Biol. Sci., 3(1): 1–6.

Vanlalpeka, R., Ngamlai, E.V., Ralte, V., Vanlalhluna, P.C. and Mehta, S.K., 2024. Phytochemical profiling and pharmacognostic activity of *Lycopodiella cernua* (L.) Pic. Serm. from Mizoram, India. Res. J. Pharm. Technol., 17(5): 2385–2390. https://doi.org/10.52711/0974-360X.2024.00373

Zareef, H., Gul, M.T., Qureshi, R., Aati, H. and Munazir, M., 2023. Application of ethnobotanical indices to document the use of plants in traditional medicines in Rawalpindi District, Punjab, Pakistan. Ethnobot. Res. Appl., 25: 1–29. https://doi.org/10.32859/era.25.49.1-29