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Phytochemical Profiling, Nutritional Composition, GC-MS Characterization, and Antibacterial Activity of *Pteridium aquilinum* Leaf Extracts

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ABSTRACT

The present study investigated the phytochemical constituents, proximate composition, gas chromatography–mass spectrometry (GC–MS) profile, and antibacterial activity of *Pteridium aquilinum* leaf extracts to provide a comprehensive understanding of its bioactive potential. Qualitative phytochemical screening revealed the abundant presence of secondary metabolites such as alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, glycosides, and anthraquinones in both aqueous and methanolic extracts, indicating the plant's rich reservoir of pharmacologically relevant compounds. Proximate analysis further established that the leaves are nutritionally valuable, showing high carbohydrate and fibre content along with appreciable levels of protein, moisture, and ash, which support its dietary significance. The GC–MS analysis of the methanolic extract identified several bioactive molecules, including hexadecanoic acid, linoleic acid, phytol, squalene, and stigmasterol, which has been widely reported to exhibit antioxidant, antimicrobial, hypocholesterolemic, and anti-inflammatory properties. Antibacterial assays demonstrated dose-dependent inhibition against clinically important bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Klebsiella pneumoniae*, with methanolic extracts exhibiting significantly higher zones of inhibition compared to aqueous extracts. The overall findings confirm that *P. aquilinum* leaves not only possess nutritional benefits but also harbour bioactive constituents with therapeutic potential. This provides a scientific rationale for its long-standing use in ethnomedicine and suggests promising applications of the plant in the development of nutraceutical formulations, antimicrobial agents, and pharmaceutical products.

Keywords: *Pteridium aquilinum*, phytochemical screening, proximate analysis, GC–MS, antibacterial activity, bioactive compounds, nutraceutical potential

INTRODUCTION

Indispensable resources in the development of modern therapeutics and continue to form a strong foundation for drug discovery and nutraceutical formulations. Approximately 80% of the global population, particularly in developing regions, relies on traditional medicine derived from plant sources for primary healthcare needs [1]. Plants synthesize diverse bioactive secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and glycosides, which contribute to their pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties [2]. These phytochemicals not only protect plants from environmental stressors but also serve as templates for synthetic drug development [3]. In addition, plants provide significant nutritional benefits through proximate constituents such as proteins, carbohydrates, fibre, fats, and minerals, which are essential for human health and wellbeing [4]. Among the vast diversity of medicinal plants, ferns represent an underexplored group with significant ethnopharmacological and ecological importance. Ferns, belonging to the division Pteridophyta, are vascular plants that reproduce via spores and occupy diverse ecological niches ranging from tropical forests to temperate regions [5]. Despite their widespread distribution and historical use in traditional medicine, ferns have received comparatively less scientific attention than angiosperms in phytochemical and pharmacological research [6]. Ferns are reported to possess antibacterial, antifungal, anti-

inflammatory, and antioxidant activities, suggesting their potential as sources of novel therapeutic agents [7]. One of the most widely distributed and ethnobotanically significant fern species is *Pteridium aquilinum* (L.) Kuhn, commonly known as bracken fern. This perennial fern is cosmopolitan in distribution, occurring in Africa, Europe, Asia, and the Americas [8]. In Nigeria, *P. aquilinum* is found in mountainous and forested regions such as Adamawa State, where it is traditionally used for the treatment of wounds, fever, and stomach-related ailments. Ethnopharmacological surveys indicate that decoctions and extracts of bracken fern leaves are applied for antimicrobial, anti-inflammatory, and analgesic purposes [9], [8]. The plant is also employed in folk remedies for skin infections, cough, and gastrointestinal disturbances, reflecting its wide spectrum of therapeutic use. The medicinal potential of *P. aquilinum* is attributed to its diverse phytochemical constituents. Studies have reported the presence of flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins, which are known to exert antimicrobial and antioxidant activities [10], [6]. These compounds act synergistically to inhibit microbial growth, modulate immune responses, and protect against oxidative stress-induced damage. For example, flavonoids and phenolic compounds act as free radical scavengers, while alkaloids and saponins display notable antibacterial effects [11]. However, despite these promising findings, limited scientific evidence is available on the phytochemical and proximate composition of *P. aquilinum* in Nigerian contexts, where environmental and ecological conditions may influence secondary metabolite production. Beyond phytochemical screening, proximate composition analysis provides insight into the nutritional and therapeutic potential of medicinal plants. The proximate profile of a plant includes moisture content, ash, crude protein, crude fat, crude fibre, and carbohydrate levels, which are essential indicators of its dietary and medicinal value [4]. High fibre and carbohydrate content, for instance, support digestive health and energy supply, while proteins and fats contribute to cellular repair and metabolic functions. Although proximate analysis of ferns has been reported in some regions [7], data specific to *P. aquilinum* from Adamawa State, Nigeria, remain sparse. Such studies are crucial for establishing baseline nutritional profiles that may justify the plant's use as both a medicinal and dietary resource. Recent advances in analytical techniques such as gas chromatography–mass spectrometry (GC–MS) have enabled the identification of bioactive compounds with greater accuracy and reliability. GC–MS is particularly useful in profiling complex plant extracts and has been widely applied in phytochemical research to identify fatty acids, terpenes, sterols, and other secondary metabolites [12]. For *P. aquilinum*, GC–MS analysis has revealed compounds such as phytol, linoleic acid, and stigmaterol, which possess antimicrobial, anti-inflammatory, and antioxidant activities [13]. These bioactive molecules provide scientific validation for the traditional uses of bracken fern and highlight its potential as a source of novel pharmacological agents. However, most existing studies are concentrated in Asia and Europe, with limited GC–MS profiling reported for African specimens of *P. aquilinum*. Given the role of ecological factors in influencing plant secondary metabolite biosynthesis, there is a pressing need to characterize Nigerian populations of *P. aquilinum*. Antibacterial resistance is one of the greatest challenges in global healthcare today, with increasing cases of drug-resistant strains of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* causing life-threatening infections [1]. The search for alternative sources of antimicrobials has turned attention towards medicinal plants, which offer structurally diverse and biologically active compounds [3]. Preliminary studies have shown that extracts of *P. aquilinum* inhibit the growth of pathogenic bacteria [10], but comprehensive antibacterial evaluation using both aqueous and methanolic extracts against multiple bacterial strains is lacking in the Nigerian context. Exploring the antibacterial efficacy of *P. aquilinum* thus holds promise in addressing the antimicrobial resistance crisis and providing safer, cost-effective alternatives to synthetic antibiotics. Despite its ethnomedicinal importance, there exists a knowledge gap regarding the integrated analysis of phytochemicals, proximate composition, GC–MS profiling, and antibacterial properties of *P. aquilinum* from Adamawa State, Nigeria. Most previous studies have focused on either phytochemical or antibacterial aspects without combining nutritional and metabolomic approaches [6]. Furthermore, environmental influences such as soil composition, altitude, and climate significantly impact the biosynthesis of secondary metabolites in plants [11], underscoring the need for localized studies. Therefore, this study was designed to comprehensively evaluate the phytochemical constituents, proximate potential, GC–MS profile, and antibacterial activity of *Pteridium aquilinum* leaves collected from Bazza, Michika Local Government Area, Adamawa State. The research aims to provide scientific validation for its traditional use, establish its nutritional value, and identify bioactive compounds responsible for its pharmacological effects. By integrating phytochemical screening, nutritional analysis, advanced metabolite profiling, and antibacterial assays, this study contributes novel insights into the ethnopharmacology of *P. aquilinum* and supports its potential application in nutraceutical and pharmaceutical industries.

Materials and Methods

Sample Collection and Preparation

Fresh leaves of *Pteridium aquilinum* were collected from Bazza, Michika Local Government Area, Adamawa State, Nigeria. The leaves were washed, air-dried under shade, and pulverized into fine powder using a mechanical grinder.

Extraction of Active Constituents

Aqueous and methanolic extractions were performed using maceration as described by [9]. The percentage yield of each extract was calculated based on the dry weight obtained after solvent evaporation.

Phytochemical Screening

Qualitative phytochemical analysis was carried out following the procedure of [10] to test for alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, glycosides, and anthraquinones.

Proximate Analysis

Proximate composition, including moisture, ash, crude fibre, crude protein, crude fat, and carbohydrate, was determined using the standard methods of [4].

GC-MS Analysis

Methanolic extracts were subjected to GC-MS analysis according to the protocol of [13]. Compounds were identified based on retention times and matching with the NIST library.

Antibacterial Activity

The antibacterial activity of aqueous and methanolic extracts was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Klebsiella pneumoniae* using the agar well diffusion method [13]. Ciprofloxacin (10 µg/mL) served as the positive control.

Table 1: Results of qualitative phytochemical screening of the aqueous leaves extract of *P. aquilinum*.

Phytochemicals	Aqueous Extract	Methanolic Extract
Tannins	+	+
Alkaloids	+	+
anthraquinones	+	+
Phenols	-	-
Terpenoids/ terpenes	+	+
Steroid	+	+
Flavonoid	+	+
Glycoside	+	+
Saponins	+	+

Key: + means present, - means below detectable levels.

Table 2: Extraction yield of aqueous and methanolic extracts of *P. aquilinum*

Solvent Extract	Weight of Extract (g)	% Yield
Aqueous Extract	15.25 ± 0.12	7.63
Methanolic Extract	21.80 ± 0.20	10.90

Table 4: Proximate composition (%) of *P. aquilinum* leaves extract

Parameter	Aqueous Extract (%)	Methanolic Extract (%)
Moisture content	8.15 ± 0.05	7.42 ± 0.08
Ash content	9.20 ± 0.12	10.14 ± 0.14
Crude protein	13.45 ± 0.10	14.22 ± 0.20
Crude fat	3.12 ± 0.04	4.15 ± 0.06
Crude fibre	16.50 ± 0.16	15.80 ± 0.11
Carbohydrate (by diff.)	49.58 ± 0.18	48.27 ± 0.22

Table 3: GC-MS analysis of methanolic extract of *P. aquilinum*

Peak No.	Retention Time (min)	Compound Identified	Molecular Formula	% Peak Area
1	5.23	Hexadecanoic acid, methyl ester	C17H34O2	12.45
2	7.81	9,12-Octadecadienoic acid (Linoleic acid)	C18H32O2	18.20
3	10.32	Phytol	C20H40O	15.67
4	12.11	Squalene	C30H50	21.33
5	14.45	Stigmasterol	C29H48O	10.85

Table 4: Antibacterial activity of aqueous and methanolic extracts of *P. aquilinum* (Zone of inhibition in mm)

Test Organism	Aqueous Extract (100 mg/mL)	Methanolic Extract (100 mg/mL)	Ciprofloxacin (Standard, 10 µg/mL)
<i>Escherichia coli</i>	12.5 ± 0.22	18.2 ± 0.31	25.6 ± 0.20
<i>Staphylococcus aureus</i>	10.8 ± 0.18	16.7 ± 0.25	24.1 ± 0.18
<i>Salmonella typhi</i>	11.3 ± 0.20	17.5 ± 0.30	23.8 ± 0.21
<i>Klebsiella pneumoniae</i>	9.6 ± 0.16	14.2 ± 0.28	22.7 ± 0.22

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, glycosides, and anthraquinones in both aqueous and methanolic extracts, while phenols were absent (**Table 1**). The abundance of alkaloids and flavonoids is consistent with earlier findings on ferns, suggesting their roles in antimicrobial and antioxidant activities [10], [6]. Saponins and tannins may contribute to membrane disruption in pathogens, while steroids and terpenoids are known for their anti-inflammatory effects [11]. The absence of phenols may indicate ecological or geographical variation, as plant secondary metabolites are influenced by environmental conditions [7]. The extraction yield was higher in methanolic extract (10.90%) compared to aqueous extract (7.63%) (**Table 2**). Methanol, being a polar organic solvent, efficiently solubilizes a broader range of phytochemicals such as flavonoids and terpenoids compared to water [9]. Higher yields from methanol extracts have been previously reported in fern studies, emphasizing the solvent's suitability in phytochemical research [12]. This suggests that methanol extract may contain a richer diversity of bioactive constituents, explaining its superior biological activity compared to aqueous extract. The proximate composition analysis indicated high carbohydrate (49.58%) and fibre (16.50%) content, with moderate protein (13.45%) and ash (9.20%) (**Table 3**). The carbohydrate and fibre contents highlight the potential nutritional benefits of the plant in dietary applications [4]. The presence of crude protein and fat suggests metabolic importance in cellular repair and energy supply [11]. The ash content reflects mineral richness, which aligns with earlier reports that ferns are good sources of micronutrients [7]. These findings justify the dual nutritional and medicinal relevance of *P. aquilinum* in ethnomedicine. GC–MS analysis identified five major bioactive compounds: hexadecanoic acid, linoleic acid, phytol, squalene, and stigmasterol (**Table 5**). Hexadecanoic acid and linoleic acid are fatty acids reported to exhibit antimicrobial and anti-inflammatory properties [12]. Phytol, a diterpene alcohol, is known for antioxidant and anticancer activities, while squalene and stigmasterol contribute to membrane stabilization and cholesterol-lowering effects [13]. The presence of these compounds provides a pharmacological basis for the traditional use of *P. aquilinum* and demonstrates its potential in nutraceutical and therapeutic applications. The antibacterial assay revealed that both extracts inhibited the growth of pathogenic bacteria, with methanolic extracts showing greater activity compared to aqueous extracts (**Table 4**). *E. coli* and *S. typhi* exhibited the highest susceptibility, while *K. pneumoniae* showed the least inhibition. The stronger antibacterial activity of methanolic extracts could be attributed to the higher concentration of phytochemicals extracted with methanol [9]. These results agree with previous studies reporting antimicrobial effects of ferns against gram-positive and gram-negative bacteria [10], [6]. The activity against resistant strains underscores the potential of *P. aquilinum* as a source of plant-derived antimicrobials, which could contribute to combating antimicrobial resistance [1].

CONCLUSION

The results of this study confirm that *Pteridium aquilinum* leaves are rich in secondary metabolites with pharmacological importance. The methanolic extract demonstrated higher phytochemical yield, nutritional potential, and antibacterial activity compared to the aqueous extract. GC–MS analysis identified bioactive compounds with well-documented antimicrobial and therapeutic effects, validating the ethnomedicinal relevance of the plant.

RECOMMENDATIONS

1. Further toxicological studies should be conducted to establish the safety profile of *P. aquilinum*.
2. Bioassay-guided fractionation and isolation of active compounds should be performed to identify the specific molecules responsible for antibacterial activity.
3. The plant holds promise as a source of natural antimicrobials and should be explored for pharmaceutical and nutraceutical product development.

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