International Journal of Research and Review
[Indexed, Peer-Reviewed Journal]
Volume 12; Issue: 12; December 2025
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Chemical, Antioxidant and Antidiabetic Potential of the Fern *Pteridium aquilinum* (L.) *Kuhn ('Makungu')* Consumed in Brazzaville (Republic of Congo)

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DOI : https://doi.org/10.52403/ijrr.20251294

ABSTRACT

Pteridium aquilinum (L.) Kuhn (Dennstaedtiaceae) is a fern known as 'Makungu' in the Republic of Congo. This plant is consumed as food by the Congolese and used in traditional Congolese medicine for various ailments, including diabetes. However, its potential as a functional food remains poorly documented. This study evaluated the phytochemical, functional food, antioxidant and antidiabetic potential of *Pteridium aquilinum* consumed in Congo. Phytochemical screening by thin-layer chromatography revealed the presence of polyphenols, flavonoids, triterpenes, saponosides and amino acids. Analysis of the fronds revealed a high crude protein content (20.33 ± 0.58) and mineral content (total ash at 9.25 ± 0.49), suggesting high nutritional potential. The phenolic compound assays showed a total polyphenol content (248.10 ± 0.03 mg EAG/g ES) and total flavonoid content (216.61 ± 0.02 mg EQ/g ES) in the hydroethanolic extract. This richness correlates with very high antioxidant activity, measured by the DPPH test, with an inhibitory concentration of 51.83 µg/mL for the hydroethanolic extract. *In vivo* evaluation in Wistar rats made diabetic by streptozotocin demonstrated significant antidiabetic activity. The ethyl acetate fraction of the decoction induced the greatest reduction in blood glucose levels, reaching 42.3% after 3 hours of administration. These results confirm that *Pteridium aquilinum* has dual potential, both as a phytotherapeutic agent for diabetes management and as a source of essential nutrients, thus justifying its status as a functional food in Congolese pharmacopoeia and culinary tradition.

Keywords: *Pteridium aquilinum*, antidiabetic, antioxidant, polyphenols,

INTRODUCTION

Diabetes mellitus is now a major public health problem. According to the latest estimates from the International Diabetes Federation (IDF, 2024), diabetes currently affects around 25 million adults in the African region. This geographical area has the highest prevalence of undiagnosed cases in the world, an alarming situation that delays patient care. The projections for the coming decades are equally worrying: the IDF predicts that Africa will see the highest demographic growth of the disease, with the

number of people with diabetes expected to more than double to nearly 60 million by 2050 [1].

In Congo, the cost of modern treatment remains prohibitively high for a large part of the population, leading many patients to turn to traditional medicine in the hope of finding a cure [2].

Pteridium aquilinum (L.) Kuhn, a cosmopolitan fern known locally as "Makungu", is a plant with both nutritional and medicinal properties, attracting growing scientific interest. Its young fronds, eaten as a leaf vegetable in several regions of the Congo, are renowned in traditional medicine for treating fever, bronchial conditions and diabetes [3].

Despite its local importance, few scientific studies have included a comprehensive assessment of its pharmacological properties and nutritional profile to validate its uses. The present study aims to fill this gap by pursuing the following objectives: (i) to determine the proximate chemical composition of *P. aquilinum* fronds; (ii) to characterise its secondary metabolite profile using qualitative and quantitative methods; (iii) to evaluate its antioxidant potential in vitro; and (iv) to verify its antidiabetic activity in vivo in an animal model.

1. Plant material

Young fronds (terminal buds) of *P. aquilinum* (Figure 1) were collected in the marshy areas of the Kombo-Massengo district (9th arrondissement, Djiri), north of Brazzaville, Republic of Congo. Botanical identification was confirmed at the herbarium of the Institute for Research in Exact and Natural Sciences (IRSEN) in Brazzaville.

Figure 1: Leafy stems of *P. aquilinum*

2. Preparation of plant extracts

- **Decoction**
  200 g of finely chopped fresh fronds were boiled in 1 litre of distilled water for 25 minutes. The filtrate was freeze-dried to obtain the dry extract.
- **Hydroethanolic extract (PAE)**
  200 g of dried frond powder were macerated in 1 litre of a water/ethanol mixture (50/50, v/v) for one week with stirring. The filtrate was evaporated to dryness under reduced pressure.
- **Fractionation of the decoction**
  The dry decoction obtained was dissolved in water and subjected to successive liquid-liquid extraction with solvents of increasing polarity to obtain fractions with petroleum ether (F-EtP), ethyl acetate (F-AcOEt) and methyl ethyl ketone (F-MEC). The residual aqueous phase (F-Aq) was retained.
- **Petroleum ether extract (PAP)**
  200 g of dried frond powder was macerated in 1 litre of petroleum ether for three days with stirring. The filtrate was evaporated to dryness under reduced pressure.

3. Phytochemical analyses of the plant.

3.1. Screening of chemical metabolites

The search for major families of secondary metabolites was carried out by thin-layer chromatography (TLC) on silica gel (Merck 60 F254), using total extracts (decocted and hydro-ethanolic macerates) and fractions obtained by liquid-liquid extraction, according to Wagner's method [4].

The table below shows the specific eluent and developer systems used.

Table 1: Components of thin-layer chromatography screening

Chemical family	Eluent system	Developers
Phenolic compounds phenolic	Butanol/acetic acid/water (6:1:1) Ethyl acetate/formic acid/water (8:1:1)	Neu's reagent, $AlCl_3$
Amino acids	Butanol/acetic acid/water (6 :1 :1)	Ninhydrin
Glucoside	Butanol/acetic acid/water (6 :1 :1)	Thymol sulphuric acid
Terpenoids	Petroleum ether/ethyl acetate (7 :3)	Vanillin sulphate
Alkaloids	Butanol/acetic acid/water (6 :1 :1)	Dragendorf's reagent Bouchardat reagent

3.2. Determination of chemical compound content in plant organs

3.2.1. Total polyphenol content

The total polyphenol content was determined using the Folin-Ciocalteu reagent method. 100 µL of extract (decocted, hydroethanolic extract) was mixed with 500 µL of Folin Ciocalteu reagent and 400 µL of $Na_2 CO_3$ at 75% (m/v). The mixture obtained was stirred and left in the dark at room temperature for 10 minutes. The absorbance was measured at 760 nm using a ZUZI model 4211/50 UV spectrophotometer. The blank is prepared by replacing the extract with the water-ethanol mixture (50/50).

Polyphenol contents are expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE). [5]

3.2.2. Total flavonoid content

The flavonoid content was determined using the aluminium trichloride ($AlCl_3$) method. 500 µL of extract was mixed with 1500 µL of ethanol, 100 µL of 10% (m/v) $AlCl_3$, 100 µL of 1M sodium acetate and 2.8 mL of distilled water. The mixture was stirred and left to incubate away from light at room temperature for 30 minutes. The blank was obtained by replacing the extract with a 50/50 water-ethanol mixture. The absorbance was measured at 415 nm using a ZUZI model 4211/50 UV spectrophotometer.

Flavonoid contents are expressed in milligrams of quercetin equivalent per gram of dry extract (mg EQ/g DE). [6]

3.2.3. Determination of lipid content (MG) [7]

The lipids contained in 5 g of dried and crushed fronds were extracted using a Soxhlet extractor with 200 mL of hexane for 6 hours. The solvent was evaporated in a rotary evaporator (IKA HB 10 basic) to obtain a dry extract.

3.2.4. Determination of protein content [8]

0.1 g of dried and defatted fronds were used to determine the crude protein content of *P. aquilinum* using the Micro-Kjeldahl method. The protein content was obtained by multiplying the total nitrogen content by a conversion factor of 6.25.

3.2.5. Determination of total carbohydrate content (G) [9]

The carbohydrate content (G) was estimated using the difference method. According to this method, the carbohydrate content is calculated by subtracting from 100 per cent the sum of the percentages of moisture (H), fat (MG), protein (P) and ash (C) contained in the sample.

Carbohydrates (C) = 100 - [H + A + F + P]

*Where **H** = % moisture, **C** = % ash, **MG** = % lipids and **P** = % protein.*

The moisture content (H) and crude ash content (C) were determined in accordance with the protocol defined by Ossoko et al 2021 [7].

4. Evaluation of the antioxidant activity of the plant organ [10]

0.1 mL of plant extract was mixed with 1 mL of a methanolic solution of DPPH (100µM). The decrease in absorbance at 517 nm was

measured using a UV-Visible spectrophotometer after 20 minutes of incubation. Vitamin C was used as the reference compound.

The following formula was used to calculate the inhibition index (II):

$$\% II = \frac{At_0 - At_{20}}{At_0} x100$$

where At_0 is the absorbance at t = 0 minutes and At_{20} is the absorbance at t = 20 minutes

The activity was expressed as 50% inhibitory concentration (IC50), which corresponds to the concentration of extract required to trap 50% of DPPH radicals.

5. Evaluation of antidiabetic activity

Antidiabetic activity was assessed *in vivo* in male Wistar rats (200-350 g), in accordance with ethical rules for animal experimentation.

The rats, which were healthy at the start, were given single intravenous injections of streptozotocin (STZ) at 55 mg/kg to induce diabetes. After the operation, only rats with fasting blood glucose levels above 1.40 g/L were selected and retained for further analysis.

The diabetic rats, fasted for 16 hours, were divided into groups (n = 5) and received oral gavage with a mixture of water and Tween 40 (negative control), glibenclamide at a dose of 5 mg/kg (positive control), or the different fractions of the decoction (400 mg/kg). Blood glucose levels were measured using a glucometer from blood samples taken from the tail at T0, T1h, T2h and T3h.

6. Statistical analysis of data obtained

The results are expressed as mean ± standard deviation (SD). Comparisons between groups were performed using analysis of variance (ANOVA) followed by Student's t-test. The significance threshold was set at $p < 0.05$.

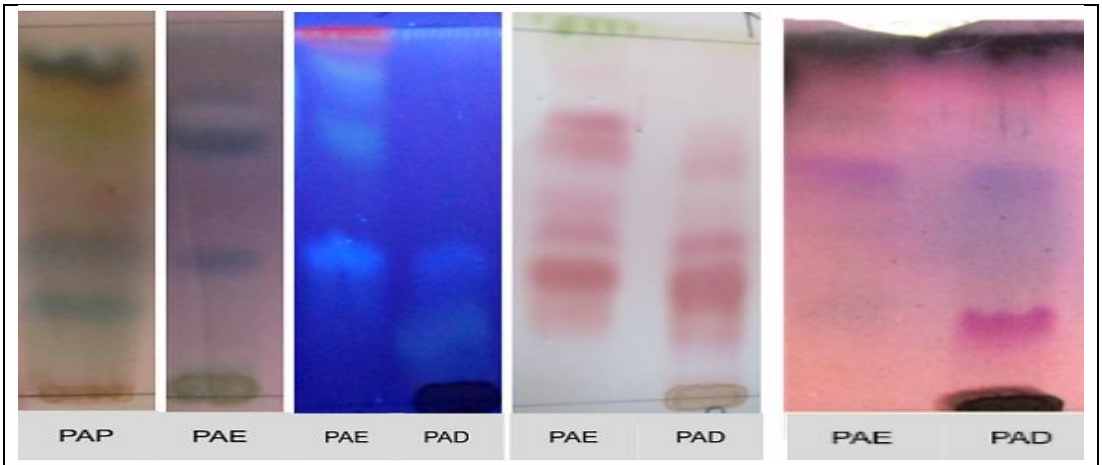
7. RESULTS

7.1. Phytochemical composition

7.1.1. Identification of metabolites in the organ of *P. aquilinum.*

Figure 2 and Table 2 illustrate the results of the search for certain chemical families in the different extracts of *P. aquilinum*.

Qualitative screening by CCM revealed the presence of several families in *P. aquilinum* extracts, including triterpenes, sterols, polyphenols, flavonoids, saponosides and amino acids.



Terpenoids And sterols	Phenolic Compounds	Amino acids	Saponosides / Glycosides

Figure 2: Chromatograms (CCM) of different extracts of *P. aquilinum*

Table 2: Characteristics of chemical families identified by CCM

Chemical family sought	Extract used	Frontal ratio (Rf)	Colour observed	Presumed chemical family
Terpenoids and Steroids	Petroleum ether	0.26	Red	Triterpenes
		0.44	Yellow	Sterols and steroids
		0.65	Light blue	Sterols and steroids
		0.82	Green	Sterols and steroids
	Hydroethanolic (PAE)	0.38	Blue	Triterpenes
		0.75	Green	Sterols and steroids
Phenolic compounds	Hydroethanolic (PAE)	0.04; 0.33	Bluish white	Phenolic acids
	Aqueous decoction (PAD)	0.38	Bluish white	Phenolic acids
		0.71; 0.92	Blue	Flavonoids
Saponins / Glycosides	Hydroethanolic (PAE)	0.57	Blue	Saponins
		0.8	Brown	Saponins
	Aqueous decoction (PAD)	0.18	Pink	Saponosides
		0.57	Blue	Saponosides
Nitrogen compounds (Amino acids)	Hydroethanolic (PAE)	0.17	Brown rose	Amino acids
		0.30; 0.42; 0.50; 0.64; 0.73	Pink	Amino acids
	Aqueous decoction (PAD)	0.12; 0.23; 0.38; 0.58; 0.64	Pink	Amino acids

7.1.2. Determination of chemical compound content in plant organs

7.1.2.1 Total polyphenol and flavonoid content of *P. aquilinum* extracts

Quantitative research (Table 3) confirms the plant's high phenolic compound content, particularly in the hydroethanolic extract.

Table 3: Total polyphenol and flavonoid content of *P. aquilinum* extracts

Extract	Total polyphenols (mg EAG/g ES)	Total flavonoids (mg EQ/g ES)
Decoction (PAD)	125.79±0.04	171.73±0.02
Hydroethanolic (PAE)	248.10±0.03	216.61±0.02

7.1.2.2 Nutritional compound content

Proximate analysis of *P. aquilinum* fronds revealed significant nutritional potential, with particularly high protein and ash content. The detailed composition is presented in Table 5.

Table 5: Proximal nutritional composition of *Pteridium aquilinum* fronds

Lipid content	7.07% ± 0.41
Protein content	20.33% ± 0.58
Carbohydrate content	49.48% ± 0.20
Water content	13.87% ± 0.17
Ash content	9.25% ± 0.49

7.1.3. Evaluation of the antioxidant activity of the plant organ

UV spectrophotometer analysis revealed high activity for both extracts. The IC50 values obtained for the hydroethanolic extract (PAE: 51.83 µg/mL) and the decoction (PAD: 52.03 µg/mL) are lower than that of the reference antioxidant, vitamin C (IC50 = 146.42 µg/mL). These results confirm the strong potential of *P. aquilinum* to neutralise free radicals.

7.1.4. Evaluation of antidiabetic activity

The evaluation of antidiabetic activity *in vivo* proved positive (Table 4). Administration of fractions of the *P. aquilinum* decoction induced a gradual and significant reduction in blood glucose levels in diabetic rats compared to the control group. The ethyl acetate (F-AcOEt) and methyl ethyl ketone (F-MEC) fractions were the most effective, causing reductions in blood glucose levels of 42.3% and 40.4% respectively after 3 hours.

Table 6: Effect of fractions of *P. aquilinum* decoction (400 mg/kg) on blood glucose levels in diabetic rats

Treatment	Initial blood glucose (g/L)	% reduction in blood glucose levels after 3 hours
Control (water-Tween)	2.78 ± 0.11	14.0
F-AcOEt	1.96 ± 0.09	42.3
F-MEC	1.98 ± 0.08	40.4
F-Aq	2.10 ± 0.10	20.9

8. DISCUSSION

This study highlights the medicinal and nutritional potential of the variety of *Pteridium aquilinum* harvested in the Congo. From a pharmacological and medicinal perspective, thin-layer chromatography (TLC) analysis of chemical families revealed a rich and diverse profile in itsextracts. The presence of several families was confirmed, including triterpenes, sterols, polyphenols, flavonoids, saponosides and amino acids. The phytochemical profile of the extracts analysed is dominated by phenolic compounds, with higher levels in the hydroethanolic extract (PAE: 248.10 mg EAG/g). These results are consistent with studies conducted in Côte d'Ivoire [11] and South Korea [12], which identify ethanol extracts as being richer in polyphenols than aqueous extracts. Some studies, notably those by San Francisco et al 1984 [13] and Imperato 1995[14], have specifically identified phenolic compounds such as caffeic acid, p-coumaric acid, and derivatives of quercetin and kaempferol in European specimens.

In nutritional terms, the crude protein content (20.33%) of the Congolese sample is remarkably high for a leaf vegetable. This result corroborates the work carried out in Nigeria by Awe & Amobi (2015) [15], who reported similar values, and places this fern well above most wild vegetables consumed. Studies in the Indian Himalayas [16] have also reported protein contents ranging from 19% to 21%, suggesting that this high protein value is a stable characteristic of the species, regardless of its geographical origin. In addition, the high ash content (9.25%) is a strong indicator of mineral richness. Literature reviews, such as that by Kathleen A. Lederle 1991 [17], confirm that *P. aquilinum* is a notable source of potassium, phosphorus, calcium and magnesium. Our results therefore suggest that consumption of the fronds, in addition to protein intake, could play a significant role in meeting essential micronutrient requirements.

Although the use of *P. aquilinum* as a functional food is well documented in Congo, the consumption of P nevertheless presents some toxicity risks. Indeed, the literature reports the presence of ptaquiloside (PT), a carcinogenic norsesquiterpene glycoside, in raw fronds [18-19].

Processes such as decoction (as for our PAD extract) and prolonged soaking with water changes are known to effectively break down ptaquiloside. It is therefore very likely that decoction, used both for consumption and traditional medicine in Congo, is an effective detoxification process. The antidiabetic activity observed in our decoction (PAD) reinforces the idea that the method of medicinal preparation is synergistic with safety. Targeted toxicological studies remain essential, however, to ensure safe use on a large scale.

This phytochemical richness translates into strong antioxidant activity (IC50 ≈ 52 µg/mL for aqueous and hydroethanolic extracts), well above that of vitamin C (IC50 = 146.42 µg/mL) under our experimental conditions. As oxidative stress is a key factor in the pathogenesis of diabetes and its complications [20], this strong antioxidant potential is in itself a relevant therapeutic mechanism.

With regard to *in vivo* antidiabetic activity, the significant reduction in blood glucose levels (up to 42.3%) by the ethyl acetate fraction (F-AcOEt) is a major finding. This moderately polar fraction is known to concentrate flavonoids, triterpenes and polyphenols. Our results corroborate the studies conducted on *P. aquilinum* in the Democratic Republic of Congo by Mangambu J.D et al 2014 [3]. This work, like

that of Chizoba Ojimelukwe et al 2024 [21], demonstrated that the ethyl acetate fraction exerted the strongest inhibition on the key enzymes of carbohydrate metabolism, α-amylase and α-glucosidase. Inhibition of these enzymes delays the digestion of polysaccharides, thereby smoothing postprandial blood glucose levels. Other studies on related fern species such as *Pteris sp.* have also linked α-glucosidase inhibitory activity to specific flavonoids [22]. Furthermore, the hypoglycaemic effect has been proven *in vivo* in diabetic rats by Unegbu et al 2021 [23], who observed partial restoration of the islets of Langerhans after administration of *P. aquilinum* extracts. Our *in vivo* data therefore provide further evidence for the traditional uses reported in Congo. [2]

9. CONCLUSION

The aim of this work was to evaluate the chemical, antioxidant and antidiabetic potential of *Pteridium aquilinum* harvested in Congo. The search for chemical families by thin-layer chromatography (TLC) revealed a rich and diverse profile in the extracts. The presence of several families was confirmed, including triterpenes, sterols, phenolic compounds, saponosides and amino acids. Quantitative analysis of total polyphenols and flavonoids confirmed this phytochemical richness, with, among other things, a polyphenol content of 248.10 mg EAG/g ES and a flavonoid content of 216.61 mg EQ/g ES for the hydroethanolic extract. These various results (phenolic profile) were confirmed by the measurement of antioxidant activity, as evidenced by very low IC50 values (PAE: 51.83 µg/mL; PAD: 52.03 µg/mL), indicating strong antioxidant activity, superior to that of vitamin C under our experimental conditions. In addition, the in vivo study validated the antidiabetic potential, with the decoction fractions inducing a significant reduction in blood sugar levels (up to 42.3%). All of these pharmacological results, coupled with the nutritional analysis that revealed a high protein content (20.33%), allow us to confirm the dual medicinal and nutritional potential of *P. aquilinum* and provide a solid scientific basis for its traditional use as a functional food plant.

P. aquilinum, already consumed by the population, nevertheless requires additional toxicological analyses to ensure its safety, in particular by measuring the residual ptaquiloside content after traditional preparations, such as decoction, in order to encourage or regulate its consumption.

This work opens up several avenues for further research: the convincing results obtained, particularly for antidiabetic activity, justify bioguided fractionation to isolate and purify the active molecules. It will also be necessary to elucidate their specific mechanisms of action, such as α-glucosidase inhibition, in order to fully exploit the therapeutic potential of this local resource.

Declaration by Authors

Acknowledgement: None
Source of Funding: None
Conflict of Interest: No conflicts of interest declared.

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How to cite this article: Snelle MIAKAYIZILA BAONDA, Longin Justin Clair BONAZABA MILANDOU, Célestine NKOUNKOU LOUMPANGOU, Sévère-Grébel BABOUONGOLO, Ghislain KENDE, Jean Francy Istaelle KOUBAKA. *Chemical, antioxidant and antidiabetic potential* of the fern *Pteridium aquilinum* (L.) Kuhn *('Makungu') consumed in Brazzaville (Republic of Congo)*. *International Journal of Research and Review*. 2025; 12(12): 942-950. DOI: *https://doi.org/10.52403/ijrr.20251294*

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