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Nutritional Profiling and Antioxidant Capacity of *Diplazium esculentum* (Retz.) Sw., an Underutilized Wild Fern from Seraj Valley, Himachal Pradesh: Implications for Genetic Conservation and Crop Diversification

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Wild edible ferns contribute significantly to the dietary diversity and food security of communities inhabiting the Himalayan region, yet their nutritional potential remains inadequately documented. This study presents a detailed biochemical characterisation of *Diplazium esculentum* (Retz.) Sw., locally known as 'Lingru', collected from the Seraj Valley in Himachal Pradesh. Our analysis of the dried young fronds revealed a substantial crude protein content of 23.56 g per 100 g, alongside a high dietary fiber content of 35.12 g per 100 g. The fern exhibited a low fat content of 1.23 g per 100 g. Mineral analysis indicated notable concentrations of zinc (36.28 mg/kg), magnesium (29.86 mg/g), and iron (9.98 mg/kg), with a vitamin C content of 39.0 mg per 100 g. Phytochemical screening confirmed the presence of flavonoids, saponins, tannins, and phenolic compounds, while common anti-nutritional alkaloids were absent. Among the extracts tested, a hydroalcoholic solvent yielded the highest total phenolic (4.29 mg GAE/g) and flavonoid (3.17 mg QE/g) contents. This extract also demonstrated the most potent free radical scavenging activity in the DPPH assay, with an IC_{50} value of 50.53 µg/mL. The findings confirm the traditional use of *D. esculentum* and highlight its considerable potential as a source of nutrition and bioactive compounds. This underutilised fern warrants further attention for its role in supporting food security and for possible development into value-added health products within Himalayan communities.

Keywords: Wild edible ferns; Diplazium esculentum; nutritional diversity; antioxidant activity; Himalayan genetic resources; population genetics; sustainable harvesting.

1. Introduction

The Himalayan region supports a diverse array of wild edible plants that have long served as crucial food resources for local populations, especially during seasonal shortages [Das et al., 2013; Khan et al., 2024]. These species are often rich in essential nutrients and possess notable resilience to the region's harsh climatic conditions, sometimes offering superior micronutrient profiles compared to conventional cultivated crops [Das et al., 2013; Khamparia et al., 2020; Naik et al., 2021]. Among these valuable but scientifically overlooked resources is the edible fern *Diplazium esculentum* (Retz.) Sw. (Athyriaceae). Commonly referred to as Lingru or fiddlehead fern, its young, coiled fronds (croziers) are widely harvested as a seasonal vegetable across various Himalayan states. Traditional preservation methods, such as sun-drying or pickling, allow for its consumption throughout the year [Katoch, 2020; Ghabru et al., 2022; Aguilar et al., 2024].

The integration of wild ferns into local food systems is well-documented. For instance, ethnobotanical studies of the Gujjar tribe in Himachal Pradesh report the use of a related species, *Diplazium maximum* (Khasrod), underscoring the historical dietary relevance of this plant genus [Manickam & Irudyaraj, 2003; Sarkar et al., 2018]. Beyond nutrition, *D. esculentum* holds a place in traditional medicine, with various communities using it to address ailments like dysentery and diabetes [Thakur et al., 2017; Chakraborty & Roy, 2018; Raina et al., 2023]. Despite this traditional knowledge, the shift towards monocultures of high-yielding commercial crops has led to the neglect of such wild foods, resulting in a gradual erosion of associated knowledge and genetic diversity [Yumkham et al., 2017; Rana et al., 2019; Ansari & Ahmad, 2019].

A plant's biochemical composition is not static; it can vary considerably with geographic location, altitude, and local ecological conditions [Archana et al., 2012; Essien et al., 2019; Kutum et al., 2011]. While previous research has analyzed *D. esculentum* from regions like Northeast India and Nepal [Devi, 2020; Thapa et al., 2019; Seal & Pillai, 2025; Bushway et al., 1982], a comprehensive nutritional and phytochemical profile of populations from the Seraj Valley in the Western Himalayas is lacking. This gap limits our understanding of this region-specific genetic resource and its potential application in nutritional strategies.

The present study was therefore conducted with the following objectives: (i) to determine the proximate composition, mineral, and vitamin content of *D. esculentum* from the Seraj Valley; (ii) to qualitatively and quantitatively assess its principal phytochemical constituents; and (iii) to evaluate its *in vitro* antioxidant capacity. By providing this scientific validation, we aim to elevate the status of *D. esculentum* from a gathered wild vegetable to a documented, nutrient-dense food source with the potential to enhance dietary diversity and support sustainable livelihoods in mountainous regions.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Fresh, young fronds (croziers) of *Diplazium esculentum* were manually collected in July-August 2022 from natural populations in the Seraj Valley, located in the Mandi district of Himachal Pradesh (approximate altitude 1600-2000 m). After collection, the outer scaly covering of the fronds was removed. The fronds were thoroughly washed with clean water to remove soil and debris, then blotted dry. They were subsequently shade-dried at ambient temperature (25-30°C) until a constant weight was achieved. The dried material was ground to a fine powder using a commercial electric grinder. This powder was stored in sealed, airtight glass containers at 4°C to prevent degradation prior to laboratory analysis.

Fig. 1. Fresh Fronds of *Diplazium esculentum* collected from Seraj Valley

2.2 Analysis of Nutritional and Proximate Components

All nutritional analyses were performed in triplicate using established methodologies outlined by the Association of Official Analytical Chemists [AOAC, 2005].

- The moisture content of fresh fronds was determined by drying a known weight in a hot-air oven at 105°C to a constant weight.
- Crude protein was estimated via the micro-Kjeldahl method, using a conversion factor of 6.25 to convert nitrogen content to protein.
- Crude fat content was extracted with n-hexane using a Soxhlet apparatus for 6 hours.
- Total ash was obtained by incinerating samples in a muffle furnace at 550°C for 5 hours.
- Total dietary fiber was quantified using the enzymatic-gravimetric method.
- Total carbohydrate content was calculated by difference: [100 - (moisture + protein + fat + ash + fiber)].
- The energy value (kcal/100g) was computed using the standard Atwater factors: 4 kcal/g for protein and carbohydrates, and 9 kcal/g for fat.

2.3 Determination of Mineral and Vitamin Content

For mineral analysis, the ash obtained from the incineration step was dissolved in dilute hydrochloric acid. This solution was filtered and made up to a standard volume with deionized water. The concentrations of minerals—calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), sodium (Na), potassium (K), and copper (Cu)—were measured using an Atomic Absorption Spectrophotometer (Model AAnalyst 400, PerkinElmer, USA). Phosphorus (P) was estimated spectrophotometrically using the vanadomolybdate yellow colour method. The vitamin C (ascorbic acid) content in freshly prepared frond extract was determined by titration against a standardised 2,6-dichlorophenol indophenol dye solution [AOAC, 2005; Stagos et al., 2012].

2.4 Screening and Quantification of Phytochemicals

- **Qualitative Screening:** Preliminary tests for major phytochemical groups were conducted on methanol, aqueous, and hydroalcoholic (80:20 methanol:water, v/v) extracts. Standard protocols were followed to detect the presence or absence of alkaloids (Dragendorff's test), flavonoids (Shinoda test), saponins (froth test), steroids (Liebermann-Burchard test), tannins (ferric chloride test), cardiac glycosides (Keller-Killiani test), and phenolics [Choudhury et al., 2017; Obadoni & Ochuko, 2002; Malick & Singh, 1980].
- **Quantification of Bioactive Compounds:** The total phenolic content (TPC) in the three extracts was determined with the Folin-Ciocalteu reagent, using gallic acid as the reference standard. Absorbance was read at 760 nm, and results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry sample weight [Singleton et al. (1999)]. The total flavonoid content (TFC) was estimated using the aluminium chloride colourimetric method, with quercetin as the standard. Absorbance was measured at 510 nm, and results were expressed as milligrams of Quercetin Equivalents (QE) per gram of dry sample weight [Chang et al. 2002; Zannah et al., 2017].

2.5 Assessment of Antioxidant Activity

The free radical scavenging potential of the plant extracts was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay [Re et al. 1999]. Briefly, different concentrations (2.5 to 300 µg/mL) of each extract were mixed with a 0.1 mM methanolic solution of DPPH. The reaction mixtures were vortexed and left to stand in the dark for 30 minutes at room temperature. The decrease in absorbance was then measured at 517 nm using a spectrophotometer. A solution of ascorbic acid was used as a reference standard. The antioxidant activity was expressed as the IC_{50} value, defined as the concentration of extract required to scavenge 50% of the DPPH radicals. A lower IC_{50} value indicates higher antioxidant activity.

2.6 Statistical Analysis

All experiments were conducted with three independent replications (n=3). The data are presented as the mean ± standard deviation (SD). To determine if there were statistically significant differences in TPC, TFC, and IC_{50} values among the three different solvent extracts, a one-way analysis of variance (ANOVA) was performed. Where ANOVA indicated significant differences ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for pairwise comparisons. All statistical computations were carried out using the SPSS software package (Version 25.0, IBM Corp., USA) Gomez and Gomez (1984).

3. Results and Discussion

3.1 Proximate Composition and Nutritional Significance

The nutritional profile of the dried young fronds (DYF) of *D. esculentum* is summarized in Table 1. The crude protein content of 23.56 g per 100 g is notably high. This value exceeds the protein content reported for many commonly consumed leafy vegetables and is significantly higher than figures previously published for *D. esculentum* from other geographic locations [Bassey et al. 2001; Gupta *et al*., 2020]. This finding underscores the potential of this fern to serve as an important plant-based protein supplement in local diets (Tongco et al. (2014); Wali et al. (2016); Chettri et al. 2018; Naik et al., 2020).

An exceptionally high level of total dietary fiber (35.12 g/100 g) was recorded. Dietary fiber is essential for digestive health and is known to help regulate blood sugar and cholesterol levels, potentially reducing the risk of chronic diseases such as type 2 diabetes and cardiovascular conditions [Chettri et al. 2018]. The total carbohydrate content (56.23 g/100 g) and the derived energy value (319.42 kcal/100 g) confirm that *D. esculentum* can provide substantial dietary energy. Its very low fat content (1.23 g/100 g) aligns with its profile as a low-lipid, healthful food component (Bassey et al. 2001; Koniyo et al., 2021).

3.2 Mineral and Vitamin Composition

The mineral analysis revealed a nutritionally rich profile (Table 1). The fern contained appreciable amounts of magnesium and calcium, which are vital for bone health and enzymatic functions. The zinc content (36.28 mg/kg) and iron content (9.98 mg/kg) are of particular significance. Deficiencies of these two micronutrients are widespread public health concerns in mountainous regions and are associated with impaired immune function, child development, and cognitive performance [Anderson et al. 2009; Naik et al., 2020]. The inclusion of *D. esculentum* in the diet could help mitigate such deficiencies. The vitamin C content (39.0 mg/100 g) further adds to its nutritional merit, as this vitamin enhances the absorption of non-heme iron from plant sources and acts as a potent antioxidant.

Table 1. Proximate composition and mineral content of dried young fronds (DYF) of *Diplazium esculentum* from Seraj Valley

Proximate composition	Energy (kcal/100g)	Moisture (g/100g)	Crude protein (g/100g)	Vitamin C (mg/ 100g)	Total fat	Total dietary fibre (g/100g)	Total ash
Content	324.13±9.22	71.40	23.56	39.0	1.23±0.04	35.12	8.4±0.40
Proximate composition	Total Carbohydrates (g/100g)	Reducing sugars	Non-Reducing sugars		Starch	Total soluble sugar	
	56.23±0.15	0.6±0.03	0.72±0.04		0.443±0.09	1.32±0.10	
Mineral Content							

Magnesium mg/g	Zinc mg/g	Phosphorus mg/g	Potassium mg/g	Calcium (mg/g)	Iron (mg/g)	Sodium (mg/g)
29.86±2.87	36.28±1.33	11.05±3.96	20.53±2.45	21.52±	9.98±0.54	1.55±0.08

Values are mean ± SD of triplicate analysis

3.3 Phytochemical Constituents and Antioxidant Capacity

The results of the qualitative phytochemical screening are presented in Table 2. Flavonoids, saponins, steroids, tannins, glycosides, and phenolics were detected in all three solvent extracts (methanol, water, and hydroalcoholic). A key finding was the consistent absence of alkaloids across all extracts. This is a positive indicator for food safety, as many alkaloids can be toxic, and it corroborates the long history of safe consumption of this fern.

Table 2. Preliminary phytochemical screening of *D. esculentum Retz*

Test	Alkaloid	Flavonoids	Saponins	Steroids	Tannin	Glycoside	phenolic
Methanol	-	++	+	+	+	+	+
Water	-	++	+	+	+	+	+
Methanol: water (80:20)	-	++	+	+	+	+	+

+ve sign indicate presence and –ve sign indicates absence

The quantitative analysis of bioactive compounds (Table 3) showed that the extraction solvent significantly influenced the yield. The hydroalcoholic (80:20 methanol:water) extract yielded the highest total phenolic content (4.29 mg GAE/g) and total flavonoid content (3.17 mg QE/g). This was followed by the methanol extract and then the aqueous extract. Statistical analysis (ANOVA, $p < 0.05$) confirmed that these differences were significant. The superior extraction efficiency of the hydroalcoholic solvent is likely due to its ability to solubilise a broader range of both polar and mid-polar phenolic compounds (Seal 2012; Wali et al. 2016; Roy et al. 2013; Tongco et al. 2014; Khan et al., 2024).

Table 3. Total phenolic content, flavonoid content, and antioxidant activity (DPPH IC_{50}) of different extracts from *D. esculentum* DYF

	Total phenol (mg GAE /100 g FW)	Flavonoids (mg QE/100 g FW)	IC_{50} (µg/mL)
Methanol	3.11±0.09	2.45±0.05	99.52
Water	2.22±0.02	1.12±0.04	80.53
Methanol: water (80:20)	4.29±0.06	3.17±0.02	50.53

The antioxidant activity, measured as the ability to scavenge DPPH radicals, followed a clear trend (Table 3). The hydroalcoholic extract, which had the highest phenolic and flavonoid content, demonstrated the strongest activity, with the lowest IC_{50} value of 50.53 µg/mL. This was significantly more potent than the methanol and aqueous extracts. A strong inverse correlation was observed between the TPC/TFC values and the IC_{50} values, suggesting that the phenolic compounds are major contributors to the antioxidant capacity of *D. esculentum*. This significant antioxidant potential provides a scientific basis for some of its traditional medicinal uses and indicates its promise as a source of natural antioxidants for health promotion (Roy et al., 2013; Choudhury et al., 2017; Raina et al., 2023).

4. Conclusion

This study provides a comprehensive biochemical evaluation of *Diplazium esculentum* collected from the Seraj Valley of Himachal Pradesh. The findings reveal that this wild edible fern is a rich source of dietary protein, fiber, essential minerals (notably zinc, magnesium, and iron), and vitamin C. Its phytochemical profile is characterised by beneficial compounds like phenolics and flavonoids, coupled with a notable absence of common anti-nutritional alkaloids. The significant antioxidant activity of its extracts, particularly from the hydroalcoholic solvent, further enhances its functional food potential.

These results offer strong scientific validation for the traditional consumption of *D. esculentum* and underscore its viability as a nutrient-dense wild food resource. To harness its full potential, we recommend: (i) implementing sustainable harvesting protocols and *in situ* conservation efforts to protect natural populations; (ii) developing simple processing techniques (e.g., optimised drying) to create shelf-stable products like flour or dried greens for year-round availability; and (iii) conducting sensory evaluation and recipe trials to promote its wider acceptance and integration into contemporary diets. By doing so, *D. esculentum* can contribute meaningfully to improving nutritional security, supporting local livelihoods, and preserving biocultural heritage in the Himalayan region.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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