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RESEARCH ARTICLE

Comparative nutritional and phytochemical screening of a wild edible fern: *Diplazium maximum* (D. Don) C. Chr. from north-western Himalayan regions of Himachal Pradesh

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Abstract

A comparative assessment of *Diplazium maximum* (D. Don) C. Chr. young edible fronds revealed significant variations ($p \leq 0.05$) in nutritional and bioactive properties across Shimla (SA), Mandi (SB) and Chamba districts (SC) of Himachal Pradesh. SC and SB fronds exhibited the highest content of moisture (92.78 %), total energy (1353.61 KJ/100 g), crude fat (2.86 %), total ash (12.64 %) and total carbohydrates (50.78 %), dietary fiber (40.54 %), total soluble sugar (1.60 %), respectively. The significantly highest ($p \leq 0.05$) protein (28.02 %), calcium (Ca 0.12 %), iron (Fe 0.0157 %), copper (Cu 0.0026 %) and manganese (Mn 0.0021 %) content was recorded at SA. Antioxidant profiling showed the significant activity in SB hydro-alcoholic extract, demonstrating the lowest IC_{50} values (53.31 and 50.69 µg/mL) for 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assays corresponding to the highest total phenolics (TP=90.71 mg GAE/g) and flavonoids (TF=75.65 mg QE/g) concentration. Phytochemicals screening also confirmed the presence of alkaloids, phenolics, flavonoids, terpenoids, glycosides, tannins and saponins. Total chlorophyll (50.53 µg/100 g) and total carotenoids (7.48 µg/100 g) content varied significantly ($p \leq 0.05$) across all sites, being highest in SB and SC fronds respectively. Fatty acids profiling through gas chromatography-mu ass spectrometry (GC-MS) revealed dominance of methyl palmitate (85.5 %) and methyl lignocarate (18.97 %). Overall, *D. maximum* fronds exhibit strong nutritional, bioactive and antioxidant potential, supporting their value as a sustainable wild edible resource for enhancing food security and future nutraceutical applications.

Keywords: antioxidant; nutritional analysis; phytochemicals; phytonutrients

Introduction

With the world's population anticipated to grow by 10 billion individuals by 2050, the demand for food and nutrition has become a serious global public health concern. According to WHO (2017), inadequate availability of protein and minerals rich foods has resulted in global malnutrition, which is responsible for approximately 45 % of mortality among the children under the age of 5 years and around 462 million underweight adult population. To counteract these challenges, the local, traditional, less explored and wild edible plant species that are rich in nutrients are required to be introduced as dietary supplements in our daily diet to improve mineral and protein deficiencies as well as to reduce pressure on cultivated food crops (1–3).

Indian Himalayan region (IHR) is home to diverse edible flora, among which wild edible fern species have a special place and many of these have been consumed till date by local and tribal communities residing in the foothills of Himalaya throughout the world. They can serve as an alternative food source in agriculturally difficult areas (4). The ancient Chinese texts also exemplified the remarkable health benefits of consuming these edible ferns for approximately 3000 years. The consumption of these traditional wild edibles with therapeutic qualities to treat several illness dates back thousands of years in India (5). Despite not commonly being cultivated in mainstream agriculture, edible ferns have long been utilised as supplementary food sources or as a substitute for staple crops during times of famine (6). Furthermore, a comprehensive ethnobotanical study reported the usage of nearly 144 fern species for both culinary and medicinal purposes across diverse regions of China (7). Indigenous fern species hold considerable importance in a particular locality in terms of social and economic spectrum. They continue to serve as an essential resource for food, traditional medicine and cosmetic applications, reflecting the enduring reliance of people on their native flora.

Even though pteridophytes provide substantial nutritional and medicinal advantages, the scientific community has paid relatively little attention to them (8). Among these, *Diplazium* constitutes the dominating fiddlehead fern genus, including a few well-known edible species viz. *Diplazium sammatii*, *D. proliferum*, *D. maximum* and *D. esculentum*. In Himalayan plains (at or below 1000 m mean sea level (MSL)), *D. esculentum* is a popular edible species, whereas in higher altitudinal ranges (1000–2750 m MSL), *D. maximum* becomes the predominant edible species. The latter

is known by several local names such as 'lingra', 'lingar' and 'kasror' in Shimla, Mandi and Chamba, Kangra districts of Himachal Pradesh, India (9–11). Their fresh fronds are harvested, cleaned, chopped and pickled, even preserved in dried form for year-round usage as they only appear during the monsoon (12). A special cuisine called '*MADHRA*' was made out of young fronds and served as delicacy in Himachali '*DHAMS*'. While another species, *D. esculentum* was also reported to have significant nutritional and therapeutic uses, like healing properties against fever, measles, dermatitis, dysentery, diarrhoea, gastrointestinal disorders, constipation, diabetes, analgesic and anthelmintic (13–18). Both the fern species have been eaten as delicacy and seasonal leafy vegetables by the tribal communities of Nepal, India, Tibet, China and Pakistan (6).

Exclusively, the young, succulent and tender fronds are cooked and eaten by human beings (4, 19). Fresh bundles of *D. maximum* young fronds have been sold in the local vegetable markets of Himachal Pradesh and their pickle has been sold at very high price, about 2-3 times expensive as fresh ones (10). *D. maximum* fronds are the tastiest among other locally consumed fern species and a reservoir of vital nutrients like iron (Fe), zinc (Zn), potassium (K), calcium (Ca), phosphorous (P), vitamin C, different proteins, glycosides and several antioxidant molecules (20).

Beyond the nutritional and health advantages, 'lingar' has a high degree of adaptability to marshy and damp environments and is extremely resistant to a range of biotic and abiotic stresses. Thus, this fern might have unique phytochemistry that has not been explored much till date scientifically. All these qualities make *D. maximum* a highly valued locally available leafy vegetable that can solve the hunger and nutritional security concerns of the world's expanding population. Despite growing interest in *D. maximum* as a nutritionally rich wild leafy vegetable, existing literature provides only fragmentary and site-specific information, with limited attention to ecological or spatial variability. Previous reports evaluated some selected nutritional traits of greenhouse-established population of *D. maximum* but did not discuss how environmental and edaphic conditions actually influence the nutrient composition, antioxidant properties, or phytochemical abundance (20). This represents a major research gap, particularly for Himalayan species, where habitat heterogeneity can drive significant biochemical variations.

These findings encouraged us to conduct a comprehensive, comparative and multi-site analysis of *D. maximum* nutrients, minerals, total phenolics, flavonoids, antioxidant potential and phytochemicals screening across three selected north-western Himalayan (NWH) regions of Himachal Pradesh. This investigation provides a scientific basis for validating this fern species' potential as a medicinally relevant functional food. Moreover, by discussing the effect of ecological determinants on studied parameters, this study advances the current knowledge on the under-utilised fern species and provides new insights into the nutritional quality of *D. maximum*.

Materials and Methods

Plant sample collection

A field survey was conducted to have insight into the distribution pattern of *D. maximum* in Shimla, Mandi and Chamba districts of Himachal Pradesh. Plant material was randomly collected in the month between April-July (2022–23), monsoon season, from the forest fringes of these selected NWH regions, including Tagnu, Gasoli Andhra, Munchara, Arhal lots in Shimla (SA), Junghi, Pandhar, Rohanda lots in Mandi (SB), Sillagharat, Khajiar, Dugli lots in Chamba (SC), based on the notable population density of the fern (Fig. 1 and Table 1). Only the tender young shoots were harvested, as the stipe becomes highly sclerenchymatous on maturity, rendering it unsuitable for consumption. From each site, 100 plants were collected and all plants from the respective site were pooled for further analysis. The herbarium specimens from all the 3 sites were deposited in Botanical Survey of India (BSI), Northern Regional Centre, Dehradun, India dated 14th September 2022, for identification and authentication purposes. The specimens were authenticated by S K Singh and assigned accession numbers 1165, 1166 and 1167. All the specimens were identified as a single species, *D. maximum.*

Sample preparation

The outer brown colored hairy layer of the fresh young edible fronds was peeled out following thorough cleaning to free them from dirt and dust, initially with tap water, followed by distilled water. Next, fronds were chopped to an average size of 4.0 cm × 4.0 cm and then shade-dried for 1–2 months until a constant weight was achieved and grounded into a coarse powder of an average particle size of 500 µm using a pestle mortar. The powdered form of *D. maximum* young fronds (Fig. 2) was represented as DMYF and stored at 4 ± 2 °C in air -tight glass jars for further experimentation.

Table 1. Geographical coordinates and details of sampling sites in Himachal Pradesh

Sl. No.	Sites	Locations	Latitude	Longitude	Altitude (m) MSL
1.	SHIMLA (SA)	Andhra	31°18'11.78496"	77°53'10.44276"	2145
2.		Arhal	31°12'55.20852"	77°41'38.75964"	2144
3.		Tagnu	31°18'27.21456"	77°59'47.59584"	2746
4.		Gasoli	31°17'32.1108"	77°52'31.77804"	2439
5.	MANDI (SB)	Munchara	31°11'18.78576"	77°46'5.51208"	1958
6.		Junghi	31°25'29.49024"	77°5'59.93772"	1920
7.		Pandhar	31°26'14.61624"	77°3'12.27132"	2301
8.		Raighad	31°28'19.04016"	77°12'0.22356"	2622
9.		Rohanda	31°28'9.7464"	77°1'51.32532"	2358
10.	CHAMBA (SC)	Janjehli	31°32'33.57528"	77°12'27.85896"	1955
11.		Sillagharat	32°39'53.02656"	76°11'44.13408"	1935
12.		Dugli	32°30'34.60356"	76°3'13.68504"	2215
13.		Khajiar	32°32'45.33"	76°3'35.93412"	1948

abbreviations used: MSL =mean sea level

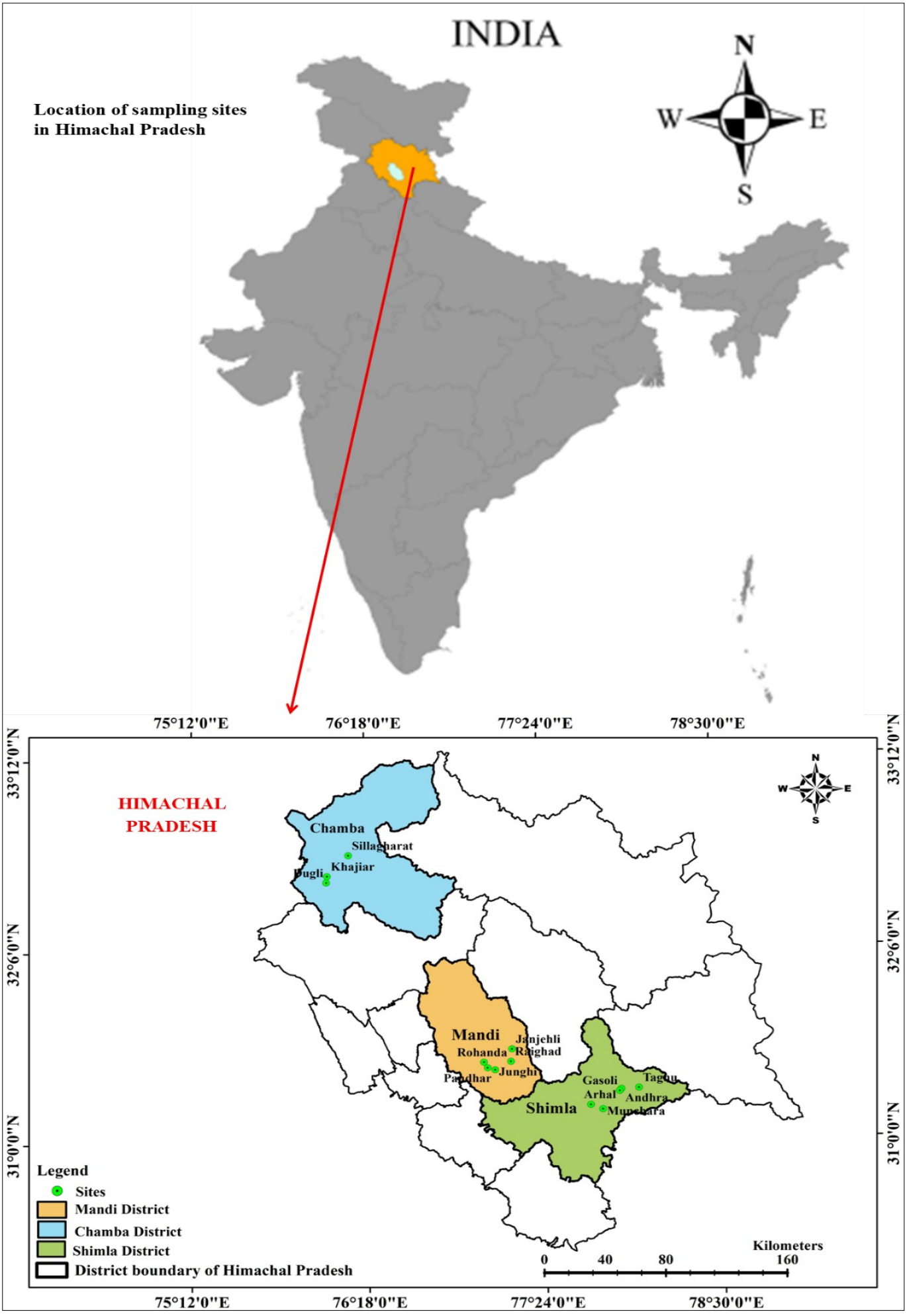


Fig. 1. Study area map of Himachal Pradesh marked with sampling sites (green tag).

Fig. 2. *D. maximum* (A, B) young fronds sold at the vegetable market; (C) cleaned and chopped young fronds; (D) powdered DMYF; (E) young fronds pickle; (F-G) *MADHRA* made from young edible shoots.

Proximate composition

A comparative nutritional analysis was performed of 3 selected region's DMYF following Association of Official Analytical Chemists (AOAC) standard procedures (21). To determine the moisture content, fresh edible fronds (10 g) were oven dried (i-therm AI-7781, 220-240 V) at 110 °C until a constant weight was achieved, followed by cooling in a desiccator and finally the dry weight was recorded. The crude protein (CP) content was pooled on the basis of total nitrogen content, determined using a Kjeldahl instrument (KEL PLUS-CLASSIC DX). A standard nitrogen-to-protein conversion factor of 6.25 (N × 6.25) was applied to estimate the total protein content. DMYF was extracted using n-hexane in a Soxhlet apparatus (MAC-SEU-6T-WGP) for fat and crude fibre estimation. The total carbohydrate content (TCC) was determined by the difference method (i.e. 100 - (% moisture + % ash + % crude fat + % protein) (20). While soluble carbohydrates, starch, total soluble sugars (TSS) and reducing sugars were also estimated (22–24). The glucose (0-100 µg/mL) was used as reference standard against total soluble carbohydrate and reducing sugar content. Non-reducing sugar content was determined by subtracting reducing sugar content from TSS. The total dietary fibre content was estimated according to serial enzymatic digestion method (25). The final residue obtained was dried (60 °C), grounded and utilized for insoluble dietary fibres estimation, whereas the soluble dietary fibre was measured from the supernatant obtained from the previous step. Ultimately, from the collective soluble and insoluble fibre content, the total dietary fibre value was calculated. The total ash (TA) content was estimated by calcinating the DMYF sample in a pre-weighed porcelain crucible at 550 °C in a muffle furnace until constant weight was attained. Following desiccator cooling, the ash was weighed which represented the TA content that was further used for estimation of mineral elements like copper (Cu), Zn, Fe, manganese (Mn), magnesium (Mg), K, Ca, P through atomic absorption spectrophotometry (AA-7000 AAS SHIMADZU) from CSKHPKV-Palampur (21). The total energy content of DMYF was calculated by multiplying the total fat, CP and TCC by conversion factors 37.7, 16.7 and 16.7 respectively (26).

Fatty acids profiling

The Soxhlet extracted crude fat from DMYF was subsequently utilised for fatty acids determination after converting it into fatty acid methyl esters (FAMEs) by refluxing with 5 % methanolic HCl (for 2 hr) (27). FAMEs were extracted using n-hexane and washed sequentially with 5 % NaCl solution and 2 % potassium bicarbonate ($KHCO_3$). Thereafter, FAMEs were dried over anhydrous Na_2SO_4, concentrated under vacuum and finally dissolved in HPLC grade n-hexane. From this, 2.0 µL of filtered FAME extract was sent to outsource (CIL, Bhatinda, Punjab, India) for GC-MS (GCMS-QP2010 Ultra TSQ 8050 NX) profiling of fatty acids. Finally, the identity of the FAMEs was confirmed by matching their retention time with the reference FAME fragmentation patterns and mixture with reliable and original standards (C-8-C-24, FAME mix, SUPELCO) (20).

Phytochemical analysis

Qualitative screening

DMYF was separately extracted with 3 unlike solvents, namely, methanol, hydro-alcohol (1:1) and aqueous using Soxhlet apparatus and filtered extracts were concentrated using a rota-evaporator. Thereafter, the individual extract (1 mg/mL) was re-dissolved in respective solvent to screen them for the presence or absence of different bioactive compound groups, viz. alkaloids, flavonoids, terpenoids, triterpenes, glycosides, steroids, reducing sugars, proteins, tannins and phenolic compounds etc.

Carbohydrates: To the plant extract (test sample), few drops of alcoholic α-naphthol in Molisch's test and Benedict's reagent in Benedict's test were added separately in 2 test tubes, following gradual and careful pouring of concentrated H_2SO_4 (0.2 mL) down the test tube inner walls in earlier test, while, heating of the mixture in a water bath in latter test. The formation of a purple to violet coloured ring at the interface of the 2 liquids in the former and reddish-brown precipitates in the latter test confirmed the presence of carbohydrates (28).

Fehling's test: Test sample was reacted with an equal amount of Fehling A and Fehling B solutions following heating in a water bath. The appearance of brick red cuprous oxide precipitates confirmed the presence of reducing sugar (28).

Glycosides:

Legal test: Few drops of NaOH (10 %) and freshly prepared sodium nitroprusside solution were added sequentially to the test sample, later turning the reaction mixture blue, which signified the glycosides presence (29).

Tannins and phenolics:

Ferric chloride test: Test sample was allowed to react with $FeCl_3$ solution (5 %), resulting into formation of a blue green colored solution, which indicated tannins presence (30).

Lead acetate test: The formation of black precipitates upon adding lead acetate to the test sample (2 mL) confirmed the presence of phenolic compounds (30).

Alkaloids:

Wagner's test: The formation of reddish-brown precipitate on the addition of Wagner's reagent to the test sample confirmed the alkaloids presence (28).

Hager's test: Yellow precipitates formation upon reacting the test sample with Hager's reagent indicated a positive response for the alkaloids (28).

***Mayer's test* (potassium mercuric iodide):** Mayer's reagent formed yellow-colored precipitates on reaction with the test sample, revealing alkaloids presence (28).

Sterols and Terpenoids:

Salkowski test: Addition of concentrated H_2SO_4 (2 mL) in a glass test tube containing the test sample and formation of a yellow ring at the junction of the 2 solutions, which turned red after 1 min, demonstrated the sterols and terpenoids presence (29).

Flavonoids:

Shinoda/Mg-HCl ribbon test: Few magnesium ribbon fragments were added to the test sample, followed by the drop-wise addition of concentrated HCl. The development of the pink-scarlet, crimson red, or occasionally green to blue coloration confirmed flavonoids presence (30).

Alkaline reagent test: Few drops of NaOH solution and diluted acetic acid were added sequentially to the extract, which later formed a bright yellow colour that finally turned colourless, confirming the flavonoids presence (30).

Proteins: Heating of the test sample with 1 mL of ninhydrin solution (0.5 %) resulted in purple coloured solution, confirming the amino acids and proteins (31).

Saponins test: To the test sample, distilled water (5 mL) was added, boiled and filtered, followed by the addition of again distilled water (3 mL) to the filtrate, which was shaken vigorously (5 min). Frothing (persisted even on heating) indicated saponins presence (28).

Total chlorophyll and carotenoids

The 100 mg of DMYF was extracted using acetone (3 times), followed by subsequent filtration and concentration under vacuum. Next, the extract was diluted properly before quantification of total carotenoids and chlorophyll (Chl) and absorbance was read at respective wavelength of 450 and 661.5 (Chl a), 645 (Chl b) as per Lichtenthaler's equation (20, 32).

$$\text{Chlorophyll (a) (µg/mL)} = 11.24 \times \text{O.D } 661.5 - 2.04 \times \text{O.D } 645$$

$$\text{Chlorophyll (b) (µg/mL)} = 20.13 \times \text{O.D } 645 - 4.19 \times \text{O.D } 661.5$$

$$\text{Total chlorophyll (µg/mL)} = [7.05 \times \text{O.D } 661.5 + 18.9 \times \text{O.D } 645]$$

$$\text{Total carotenoids (µg/mL)} = (1000 \times \text{OD } 450 - [1.9 \times \text{Chl a} + 63.14 \times \text{Chl b}])/214$$

Total phenolics and flavonoids estimation

A comparative spectrophotometric (Evolution 201, UV/Vis Spectrophotometer, Thermo-Scientific) estimation of total phenolics (TP) and total flavonoids (TF) content of both the methanolic and hydro-alcoholic (1:1 ratio v/v) DMYF extracts was done using Folin-Ciocalteu (FC) reagent method and aluminium chloride assay (33, 34). The concentrated extracts were dissolved in their respective solvents (1 mg/mL). Briefly, in the TPC assay, to 300 µL of test sample (made final 500 µL by diluting with distilled water), 250 µL of FC reagent and 1.25 mL of Na_2CO_3 solution (20 %) were added. Next, the reaction amalgam was mixed properly and incubated at room temperature (RT) for 30 min. The absorbance was measured at 765 nm wavelength and TP content was represented as Gallic acid equivalents (GAE mg/g). Gallic acid (50–350 µg/mL) was used as a reference standard ($r^2 = 0.98$).

In the aluminium chloride assay, to 350 µL of test sample, 5 % of $NaNO_2$ solution (150 µL) was added. Next, the reaction amalgam was incubated at RT (10 min) to which 10 % of $AlCl_3$ solution (150 µL) was added, following incubation again at RT for 5 min. After adding 1 mL of NaOH (1 M), the reaction amalgam was diluted to 5 mL with distilled water. Next, the absorbance was read at 510 nm. The TFC was calculated and values were represented as Quercetin equivalents (QE mg/g). Quercetin (50–350 µg/mL) was used as a reference standard ($r^2 = 0.99$).

Antioxidant activity determination of DMYF extracts

Again, a comparative determination of potential free radicals scavenging activity of DMYF methanolic, hydro-alcoholic (1:1) and aqueous extracts (20–200 µg/mL) was done *in-vitro* following slightly modified standard assays, namely DPPH and ABTS (50–300 µg/mL) (35). The former assay was rapid and sensitive, relying on the DPPH reduction by the antioxidant molecules, resulted in bleaching of the purple coloured freshly prepared methanolic DPPH solution (0.5 mM). This indicated the plant extract's free radical scavenging activity i.e., determined as a decrease in the absorbance at 517 nm. The values at 50 % inhibition of free radicals (i.e., IC_{50}) by the extracts were measured by employing ascorbic acid (20–140 µg/mL) as a reference standard ($r^2 = 0.99$).

Statistical analysis

All the experiments were performed in triplicates, repeated 3 times and findings were presented as the mean ± standard deviation (SD). All statistical analysis was done in IBM SPSS Statistics for Windows, version 31 (IBM Corp., Armonk, N.Y., USA). ANOVA was employed as a statistical test, followed by Tukey HSD post-hoc test at $p \leq 0.05$ significance level. Normality and homoscedasticity of all datasets were verified before conducting ANOVA using the Shapiro-Wilk and Levene's tests respectively. For pairwise comparisons, we applied a post-hoc Tukey HSD test with an alpha level of 0.05 (i.e. $p \leq 0.05$). For TP and TF content, a two-way ANOVA was applied.

Results and Discussion

The present comparative study on *D. maximum* nutritional and phytochemicals screening across 3 select NWH sites of Himachal Pradesh revealed that this wild edible fern species was flourishing well at an altitude range from 1000–2750 m MSL (Fig. 3, Table 1). This fern is rich in a number of nutritious and medicinally important bioactive components; thus, it has a high potential for inclusion in our daily food basket. This can boost our immunity by enriching our routine diet with a variety of nutritive biomolecules.

Proximate composition analysis

Freshly harvested young fronds of *D. maximum* from all 3 sites exhibited comparably high moisture content (91.15 to 92.79 %), characteristic of leafy vegetables, with no significant site-wise differences. Similarly, total energy, crude fat, dietary fibres, TA, soluble and overall carbohydrate content remained statistically uniform across 3 locations (Table 2). Notably, SA-fronds were recorded with significantly higher nitrogen (4.48 %) and CP (28.02 %) content (Table 2) in comparison to other 2 sites, indicating greater nutritional richness in this population. TSS was statistically highest in SB, while SA and SC showed non-significant differences. Reducing sugar content was significantly lower in SA, whereas, comparable in SB and SC. Non-reducing sugar content was significantly lower in SC, whereas, comparable in SA and SB. Starch content showed moderate site-wise variation, with SB exhibiting significantly lower levels compared to SA, while SC remained intermediate (Table 2).

Fatty acids profiling

The comparative GC-MS analysis of young frond's FAME extracts confirmed the presence of different types of fatty acids. The total fatty acids composition accounted dominance of palmitic acid (77.54–85.57 %) followed by methyl lignocarate or methyl tetracosanoate (11.23–18.97 %) and different polyunsaturated fatty acids (PUFAs) namely, linoleic acid (2.77 % in SA and 1.49 % in SB but not present in SC), oleic acid (1.21 % in SB only) and arachidonic acid (0.71 % in SA only). Among all SB and SA were demonstrated with the maximum amount of palmitic acid (85.57 %) and methyl lignocarate (18.97 %) respectively (Fig. 4, Table 3).

Mineral content

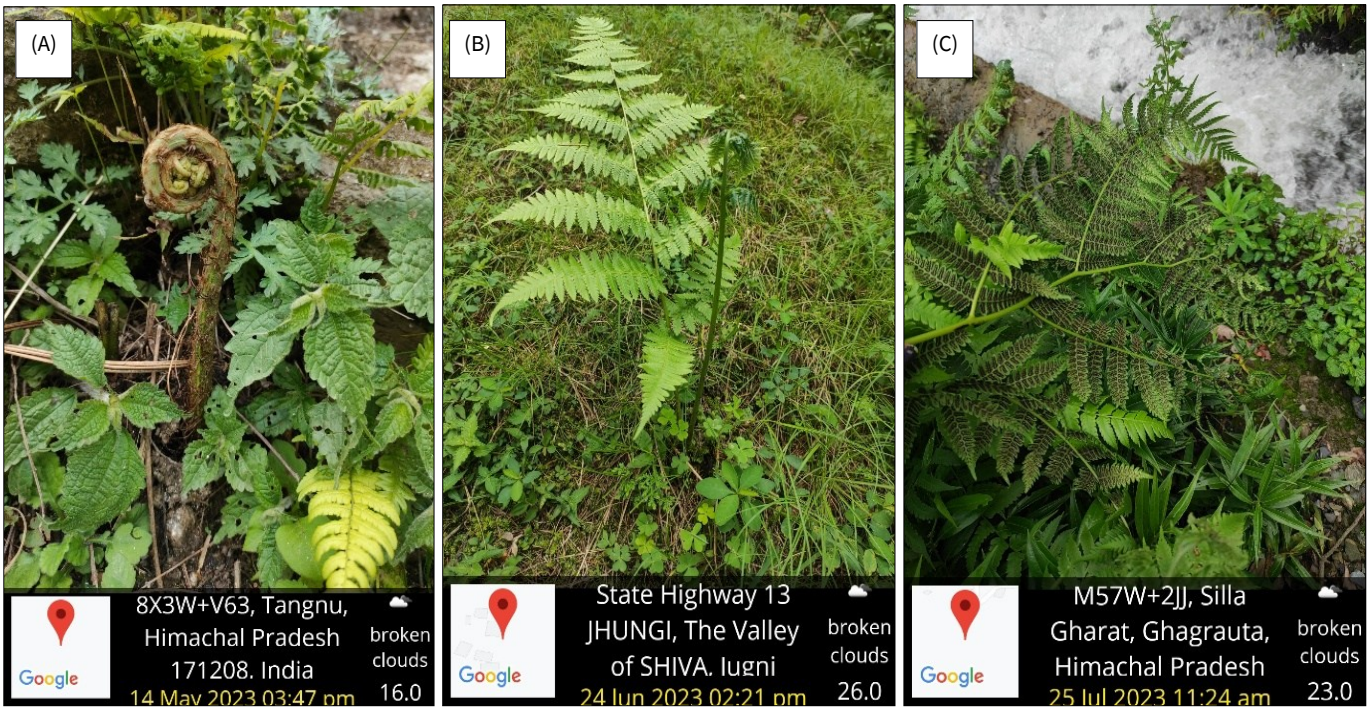


Fig. 3. *Diplazium maximum* in its natural habitat (A) young edible fronds (Tangnu, Shimla); (B) both mature and circinately coiled young fronds (Junghi, Mandi); (C) mature fronds bearing linear shaped sori on abaxial surface (Sillagharat, Chamba).

Table 2. Comparative proximate composition of *D. maximum* young edible fronds across 3 study sites

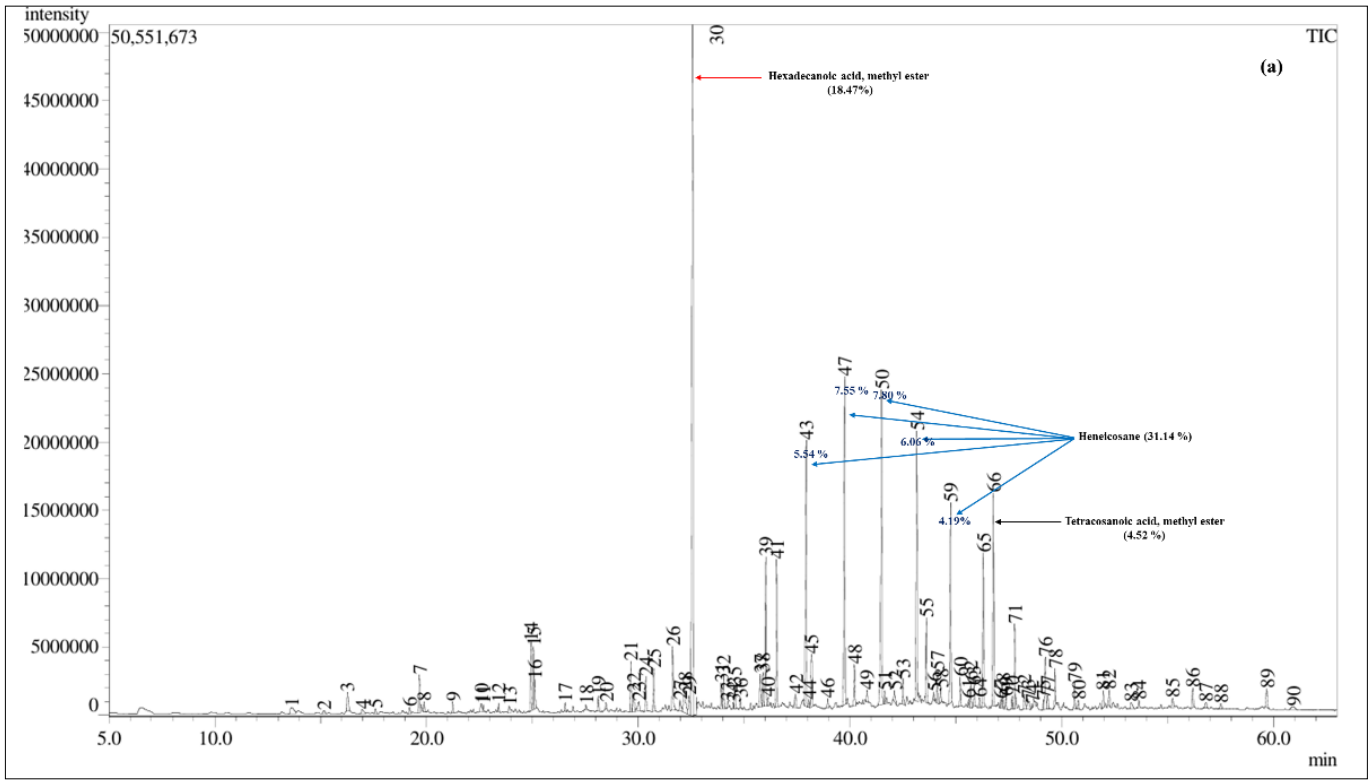
Nutritional composition	Content (g/100 g dry weight of DMYF)		
	SA	SB	SC
Moisture content	91.15±1.31[a]	92.29±0.61[a]	92.78±0.37[a]
Total energy (KJ/100g)	1350.60±17.01[a]	1312.29±35.07[a]	1353.61±10.44[a]
Total nitrogen	4.48 ± 0.03[a]	3.87 ± 0.02[b]	3.82 ± 0.03[b]
Crude protein	28.02 ± 0.16[a]	24.21 ± 0.13[b]	23.85 ± 0.19[b]
Crude fat	2.64 ± 1.0[a]	1.59 ± 1.89[a]	2.85 ± 1.52[a]
Soluble carbohydrate	24.03 ± 8.06[a]	22.48 ± 8.49[a]	29.05 ± 6.60[a]
Total soluble sugar	1.24 ± 0.78[b]	1.60 ± 0.10[a]	1.10 ± 0.26[b]
Reducing sugar	0.13 ± 0.04[b]	0.57 ± 0.08[a]	0.65 ± 0.21[a]
Non-reducing sugar	1.11± 0.04[a]	1.03 ± 0.02[a]	0.45 ± 0.21[b]
Starch	0.88 ± 0.02[a]	0.61± 0.07[b]	0.71 ± 0.16[ab]
Dietary fibre	39.88±0.49[a]	40.54±0.80[a]	39.70±0.78[a]
Total ash	12.35 ± 0.49[a]	11.76 ± 0.69[a]	12.64 ± 0.83[a]
Total carbohydrate	46.89±1.23[a]	50.77±2.37[a]	50.75±3.00[a]

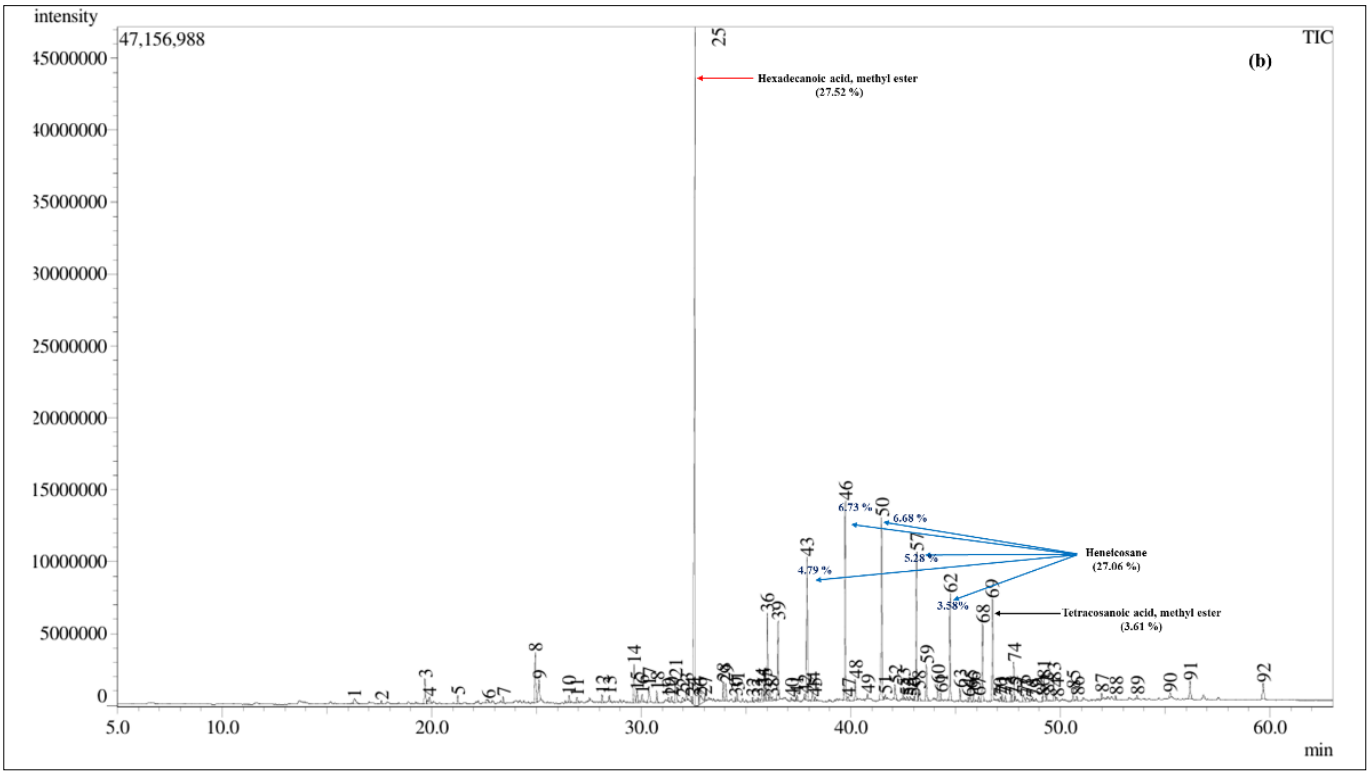
All values are mean ± SD (n=3); Abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF. Mean values within a row having different lowercase superscript letters denote significant difference as per Tukey HSD at $P \leq 0.05$. Letter a is significant to b and c.

Table 3. Comparative fatty acids profiling of *D. maximum* young fronds across three study sites (SA, SB, SC)

Fatty acids composition				Content (g/100 g)			% Composition		
IUPAC	**Common name**	**Nomenclature**	**Type**	**SA**	**SB**	**SC**	**SA**	**SB**	**SC**
Hexadecanoic acid, methyl ester	Methyl palmitate	C16:0	Saturated	**18.47**	**27.52**	**21.0**	77.54	85.57	82.19
Octadecanoic acid, 9,10-dihydroxy-, methyl ester	Methyl 9,10-dihydroxystearate	C18:0	Saturated	-	0.16	0.18	-	0.50	0.70
9,12-Octadecadienoic acid (Z, Z)-, methyl ester	Methyl linoleate	C18:2	PUFA (omega-6)	0.66	0.48	-	2.77	1.49	-
Arachidonic acid	5,8,11,14-Eicosatetraenate	C20:4 n6	PUFA (omega-6)	0.17	-	-	0.71	-	-
Tetracosanoic acid, methyl ester	Methyl lignocarate	C24:0	Saturated	4.52	3.61	4.37	18.97	11.23	17.10
Cis-9-Octadecenoic acid, methyl ester	Methyl oleate	C18:1	Monounsaturated fatty acid (MUFA) omega-9	-	0.39	-	-	1.21	-

abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF





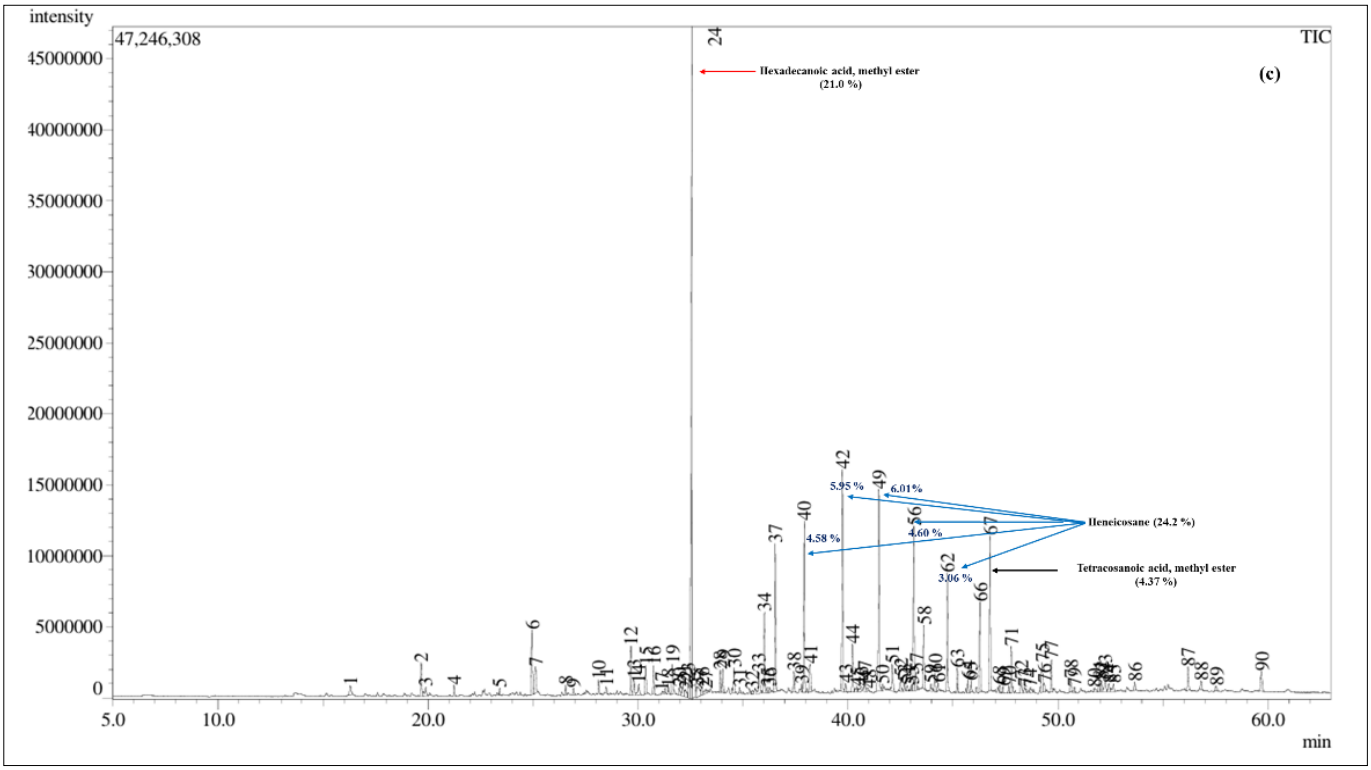


Fig. 4. GC-MS chromatograms of *D. maximum* young fronds showing fatty acids composition (A) SA; (B) SB; and (C) SC (major peaks were labelled and their corresponding area percentage was mentioned).

DMYF was found to contain substantial amounts of 8 different nutritionally important elements, out of which 4 were macro-nutrients (i.e., Mg, K, P, Ca) and rest 4 were micro-nutrients (i.e., Fe, Zn, Cu, Mn). Among these, the amount of potassium (5.34 %) and phosphorous (0.31 %) was significantly higher in SB and SC respectively. While SA fronds were recorded to possess significantly higher amounts of Ca (0.12 %), Fe (0.0157 %), Cu (0.0026 %) and Mn (0.0021 %). SC fronds were estimated with a significant proportion of Mg (0.12 %) and Zn (0.0060 %) (Table 4).

Phytochemical analysis

Qualitative screening

Preliminary screening of any plant for the presence of different bioactive molecules is crucial, as it produces a baseline data for further quantification and biological activity determination of these molecules. The results showed that all the DMYF samples and extracts were potentially a rich source of several biomolecules groups like alkaloids, phenols, flavonoids, glycosides, tannins, terpenoids, saponins (Table 5).

Table 4. Comparative nutritional element content in *D. maximum* young fronds across 3 study sites

Nutritional elements	Content (mg/Kg)		
	SA	SB	SC
Magnesium (Mg)	1198 ± 1.00 ^{b}	1193.10 ± 2.95 ^{b}	1206.96 ± 2.67 ^{a}
Potassium (K)	51400 ± 98.02 ^{c}	53400 ± 97.03 ^{a}	52600 ± 39.31 ^{b}
Phosphorous (P)	2609 ± 25.51 ^{c}	2699.67 ± 20.01 ^{b}	3100 ± 39.13 ^{a}
Calcium (Ca)	1200 ± 16.09 ^{a}	1100 ± 21.07 ^{b}	1100.33 ± 30.37 ^{b}
Iron (Fe)	157.09 ± 6.13 ^{a}	114.62 ± 1.15 ^{b}	87.97 ± 2.20 ^{c}
Zinc (Zn)	49.46 ± 0.71 ^{b}	46.82 ± 1.01 ^{c}	60.2 ± 0.78 ^{a}
Copper (Cu)	26.25 ± 1.19 ^{a}	23.31 ± 1.15 ^{b}	24.61 ± 0.53 ^{ab}
Manganese (Mn)	21.48 ± 0.62 ^{a}	20.40 ± 0.56 ^{ab}	20.03 ± 0.18 ^{b}

All values are mean ± SD (n=3); Abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF. Mean values within row having different lowercase superscript letters denote significant difference as per Tukey HSD at $P \leq 0.05$. Letter a is significant to b and c.

Table 5. Preliminary phytochemical analysis of different solvent extract of *D. maximum* young fronds

Plant compounds	Methanolic			Hydro-alcoholic			Aqueous		
Alkaloids	**SA**	**SB**	**SC**	**SA**	**SB**	**SC**	**SA**	**SB**	**SC**
Phenols	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+

(+) indicates presence of compound group and absence is indicated by a - sign. Abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF.

Total chlorophyll and carotenoids

The total carotenoid and total chlorophyll content (µg/100 mg of DMYF) was found to be 6.75 ± 0.20 (SA), 7.32 ± 0.18 (SB), 7.48 ± 0.43 (SC) and 45.28 ± 0.60 (SA), 50.53 ± 0.20 (SB), 48.69 ± 0.72 (SC) respectively. Further the chl a and b content (µg/100 mg of DMYF) were estimated to be 25.82 ± 0.07 (SA), 24.08 ± 0.27 (SB), 25.11 ± 0.25 (SC) and 16.05 ± 0.29 (SA), 20.19 ± 0.27 (SB), 22.31 ± 0.12 (SC) (Table 5). Significant variations ($p \leq 0.05$) were found in chlorophyll a, b and total chlorophyll content across all sites, while

Table 6. Total chlorophyll and carotenoid content (µg/100 mg of DMYF)

Sample	Chl a	Chl b	Total Chl	Total Carotenoid
SA	25.82 ± 0.07^{a}	16.05 ± 0.29^{c}	45.28 ± 0.60^{c}	6.75 ± 0.20^{b}
SB	24.08 ± 0.27^{c}	20.19 ±0.27^{b}	50.53 ± 0.20^{a}	7.32 ± 0.18^{ab}
SC	25.11 ± 0.25^{b}	22.31±0.12^{a}	48.69 ± 0.72^{b}	7.48 ± 0.43^{a}

All values are mean ± SD (n=3); Abbreviations used: Chl= Chlorophyll; SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF. Mean values within column having different lowercase superscript letters denote significant difference as per Tukey HSD at $P \leq 0.05$. Letter a is significant to b and c.

total carotenoid content was relatively similar between site A and B, site B and C (Table 6).

DMYF total phenolics and flavonoid content

Among 3 different plant extracts, both the TPC (90.71 ± 5.63 mg GAE/g) and TFC (75.65 ± 3.79 mg QE/g) were significantly highest ($p \leq 0.05$) in the hydro-alcoholic extract of SB-DMYF (Table 7). A two - way ANOVA revealed that both TP and TF content of DMYF varied significantly ($p \leq 0.05$) across all sampling sites and the extraction solvent used.

Anti-oxidant assays

The lowest IC_{50} value reflects the higher antioxidant activity of the plant extract. Among all the DMYF extracts across 3 study sites, sample B hydro-alcoholic extract showed the significantly highest ($p \leq 0.05$) free-radical scavenging activity as demonstrated by its lowest IC_{50} value in ABTS assay (50.69 ± 0.45 µg/mL) and DPPH assay (53.31 ± 0.95 µg/mL) (Fig. 5, 6, Table 8).

D. maximum (giant fern), a widely consumed wild edible species in the Himalayan region, holds significant potential for advancing human nutrition and strengthening local food security. Plants native to the Himalayan regions produces a diverse array of metabolites and their quality and quantity fluctuate with changing climatic conditions like altitude and temperature in different elevations (36). These environmental factors play a major role in shaping the nutritional and phytochemical profiles of wild edible plants in the NWH regions. These conditions also influence the soil key physicochemical characteristics such as organic carbon levels, nitrogen content and pH, which may enhance or limit nutrient levels across plant tissues (37). Therefore, the observed variations in *D. maximum* nutrient concentrations were driven by soil characteristics such as altitude, aspect degree, field capacity, available water and phosphorus and potassium levels.

In contrast to the earlier work on the same species, where young fronds were obtained from greenhouse-grown plants, our study utilised the plant material collected directly from 3 natural sites (20). This allows us to capture the true ecological site-specific variability across 3 distinct Himalayan regions (SA, SB, SC), offering novel insights not addressed in the previous studies. This provides the first comparative and more comprehensive evidence of how local ecological and edaphic factors influence the nutritional composition and antioxidant potential in *D. maximum*.

Table 7. Comparative TPC and TFC of *D. maximum* young fronds (DMYF) across 3 study sites (SA, SB, SC)

Plant compounds	Methanol			Hydro-alcoholic			Aqueous		
	SA	SB	SC	SA	SB	SC	SA	SB	SC
Total phenolics (mg GAE/g)	19.89±0.38^{c}	48.84±4.88^{a}	23.43±0.01^{bc}	89.74±1.83^{a}	90.71±5.63^{a}	88.44±0.34^{a}	76.24±1.53^{ab}	70.79±0.01^{b}	80.53±2.23^{a}
Total flavonoids (mg QE/g)	27.00±0.71^{b}	34.54±1.28^{a}	19.65±4.24^{c}	52.08±4.38^{b}	75.65±3.79^{a}	46.60±1.69^{b}	21.32±5.67^{b}	29.64±0.79^{a}	24.86±0.71^{ba}

All values are mean ± SD (n=3), Abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF. Mean values within row having different lowercase superscript letters denote significant difference as per Tukey HSD at $P \leq 0.05$. Letter a is significant to b and c.

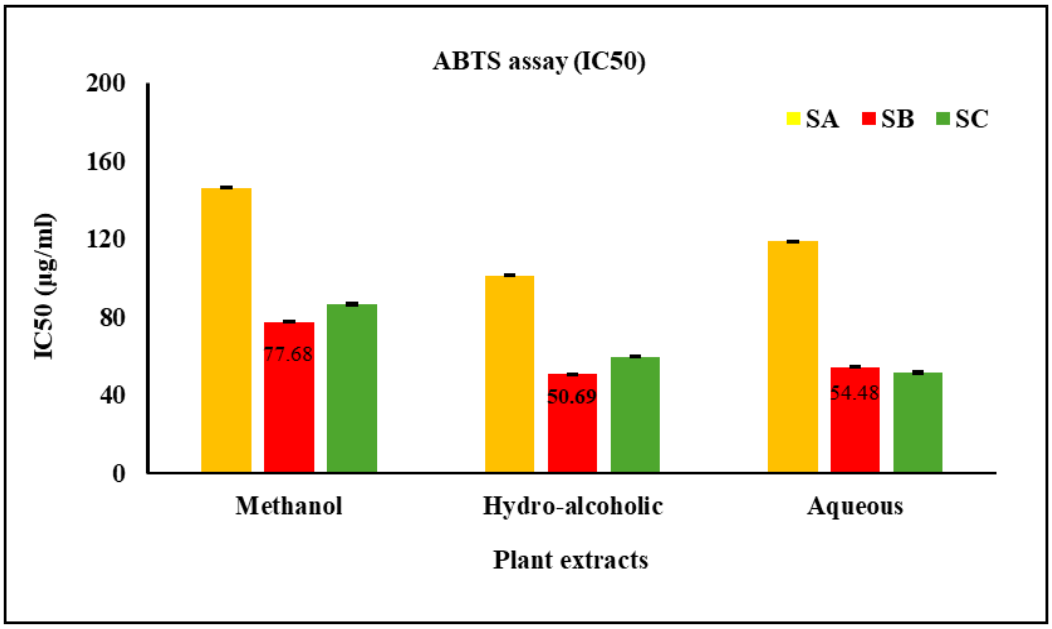


Fig. 5. Comparative IC_{50} value of ABTS assay of methanol, hydro-alcoholic and aqueous extracts of *D. maximum* young fronds across 3 study sites (SA, SB and SC) (n=9).

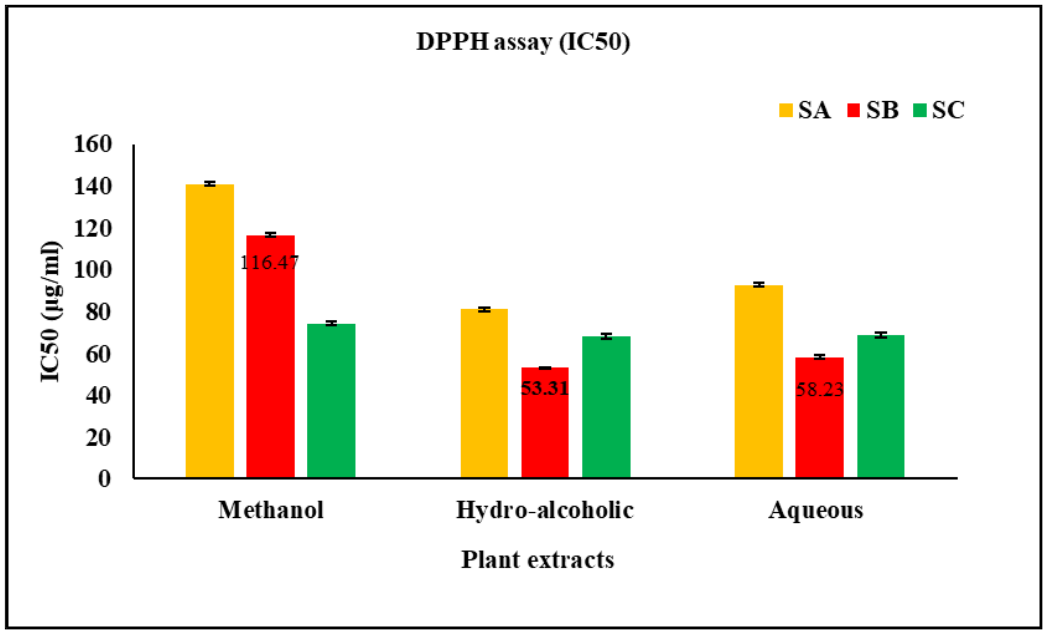


Fig. 6. Comparative IC-50 value of DPPH assay of methanolic, hydro-alcoholic and aqueous extracts of *D. maximum* young fronds across 3 study sites (SA, SB and SC) (n=9).

Table 8. Comparative antioxidant activity assessment of DMYF across 3 study sites following DPPH and ABTS assays showing IC-50 values (µg/mL)

Antioxidant assays IC-50 (µg/mL)	Methanol			Hydro-alcoholic			Aqueous		
	SA	SB	SC	SA	SB	SC	SA	SB	SC
ABTS	146.29±0.28^{a}	77.68±0.30^{c}	86.69±0.25^{b}	101.21±0.20^{a}	50.69±0.35^{c}	59.63±0.39^{b}	118.73±0.50^{a}	54.48±0.35^{b}	51.60±0.40^{c}
DPPH	140.82±0.90^{a}	116.47±0.82^{b}	74.20± 0.89^{c}	81.00±0.85^{a}	53.31±0.10^{c}	68.03±0.96^{b}	92.66±0.90^{a}	58.23±0.95^{c}	68.67±0.99^{b}

All values are mean ± SD (n=3), Abbreviations used: SA= Shimla DMYF; SB= Mandi DMYF; SC= Chamba DMYF. Mean values within row having different lowercase superscript letters denote significant difference as per Tukey HSD at $P \leq 0.05$. Letter a is significant to b and c.

Edible fern species have not yet undergone thorough characterisation; therefore, limited research is available on their nutritional information. For this reason, the current information was compared to that of popular green leafy vegetables like celery, spinach and moringa. The observed moisture content was consistent with earlier reports on some wild edible fern species like *D. esculentum, Tectaria coadunata, Matteuccia struthiopteris, Dryopteris cochleata* and *D. maximum* that varied from 80.0 to 92.4 % (20, 38–41). This might be because forest fringes have a more consistent humid environment, which is responsible for higher moisture retention in the ferns growing there. The moisture content of celery, spinach and moringa likewise varied from 78–95 % (42).

DMYF starch, TSS, nitrogen and CP content was nearly similar to the earlier study on the same species i.e., 0.64 % (starch) and 1.51 % (TSS). 4.06 % (nitrogen) and 25.39 % (protein) (20). Similarly, CP content (23.85 - 28.02 %) was approximate to that of other leafy vegetables, namely, *D. esculentum* (31.2 %) (40), *Amaranthus viridis* (22.73 %) (43), *A. spinosus* (27.71 %) (44), *U. dioica* (26.06 %) (45). This protein content was approximately equal to that found in some conventional vegetables like broccoli (*Brassica oleracea* var. *italica*), having 22–31.94 % (46–48) and spinach powder (*Spinacia oleracea*), having 27.8 % (49). Remarkably, the DMYF had a 2.5-fold higher protein content compared to young edible shoots of *D. esculentum* and *D. sammatii,* having 10–10.5 % on a dry weight basis (38, 50), while, on a fresh weight basis, it was 5.5–6.0 % (41, 51). According to this information, *D. maximum* could be exploited as a potential protein source for wider consumption. Moreover, this edible fern seems to be a potential amino acid-rich food for frequent consumption (20).

Total nitrogen and CP content were significantly higher in SA fronds across all 3 study locations. This pattern aligns with the earlier reports on the same species and *Bergenia stracheyi* collected from different elevations in the Rudraprayag district of Uttarakhand (20, 52). The observed findings regarding site SA were possibly driven by interacting edaphic, high altitude (maximum 2750 m above sea level (ASL)) and light-canopy factors. Soil nitrogen (N) supply and related edaphic properties (organic C, moisture, pH, texture) are primary determinants of plant N status, because greater soil N availability increases root uptake and hence plant N concentration (53), which in turn supported the higher CP content in SA-DMYF. Altitudinal gradients can further modulate this pattern via climatic effects (lower temperature, altered growing season length and soil mineralisation rates) that influence plant N demand and allocation. Several studies reported higher protein or leaf N at particular elevations, where soil N supply and photosynthetic demand are relatively well matched (54). In addition, a more open canopy or higher incident photosynthetically active radiation (PAR) at SA could explain elevated N and CP (55).

Protein, carbohydrates and fats constitute the 3 major food components responsible for supplying energy. The total energy content of DMYF was comparatively lower than that found in *D. sammatti,* having 1743.05 KJ/100 g (50), but slightly higher than *D. maximum* (1336.45 KJ/100 g) grown in green house conditions (20) and *U. dioica* (1332.53 KJ/100 g) (45). The fern

species growing in the natural conditions have exposure to varied and fluctuating light intensities with a wide spectrum, therefore, undergo more efficient photosynthetic reactions and hence, higher energy storage compared to lower light and a more uniform microclimate in artificial conditions (56). However, among the 3 samples, SC edible fronds were recorded with higher total energy content. Overall, *D. maximum* energy content was also significantly higher than other frequently consumed green leafy vegetables including moringa, lettuce, other fiddlehead ferns and spinach (20).

Total carbohydrate content (50.77 %) was lower than earlier study on the similar species (61.36 %) and *Berberis chitria* (55.26 %) but comparable to *A. spinosus* (50.68 %) (20, 44, 57). TCC was slightly higher than that of *D. esculentum* (44.3 %) (37), *U. dioica* (33.08 %) (45) and 3 *Ceropegia* species (34.4–46.16 %) (58). This might be due to more variable natural environmental conditions like fluctuating light intensities, competition for nutrients and several stresses which can divert the resources towards the production of protective metabolites (tannins) and thereby limit the carbohydrate production.

There is numerous health benefits associated with dietary fibres, such as enhancing gut flora and preventing type 2 diabetes, other non-communicable diseases and dyslipidemia (59). The amount of dietary fibre and crude fat was slightly greater and similar to the previous studies and crude fat was lower than other *Diplazium* and *Tectaria* sp. (20, 41). Fat indirectly controls the in and out movement of substances from the cell. Additionally, dietary lipids act as carriers for vitamins A, D, E and K, as well as for various hormones. The crude fat content varied in the range of 1.59–2.85 %, which was comparable to *Elaeocarpus sikkimensis* (1.55 %), *Chenopodium quinoa* (2.8–4.50 %), *Zanthoxylum armatum* (2.63 %) and *Cordia obliqua* (2.65 %) (60, 61, 44). Total ash content was approximately similar to another leafy vegetables namely, *A. viridis* (13.31 %) and *A. spinosus* (13.35 %) (62, 44). Overall, only a subset of parameters, particularly nitrogen, CP and selective carbohydrate fractions, demonstrated meaningful site-associated variations, whereas most proximate components remained consistent across the 3 regions.

SB showed dominance of methyl palmitate, which was comparatively higher as reported earlier in the same species and equal to *Prinsepia utilis* (18.90 %) (20, 63). A high proportion of palmitic acid is associated with adverse cardiometabolic effects relative to unsaturated fats. Thus, current public-health guidance recommends limiting saturated fatty acids (SFA) to ≤ 10 % of total energy and replacing SFA with unsaturated fats wherever possible (64). The fatty acid composition of DMYF was comparable with ostrich fern, *M. struthiopteris* (38).

Another dominant FAME, methyl lignocarate has been used as an antioxidant, biofuel, emollient or thickening agent, in high-performance lubricants synthesis, food and therapeutic supplements in pharmaceutical industries (65, 66). While methyl oleate is a major biodiesel component, used in biodegradable lubricants and eco-friendly solvents, possessing anti-inflammatory and antioxidant properties and also employed in plant protection formulations (67–69). Arachidonic acid (ARA) and linoleic acid (LA) are omega-6 PUFAs; the latter amount was comparable to that found in seed kernel oil of *Neolitsea pallens* (2.75 %) (63). Higher LA content improves the essential fatty acid value of any oil and can favourably substitute SFA in the diet (70). The gamma linolenic acid (GLA), a metabolised form of LA, serve as a substrate during prostaglandin synthesis, which is crucial for maintenance of nerve blood flow (71). ARA has been recorded to play a significant physiological and pharmacological role during embryogenesis, ultimately impacting the health of newborns (72–74). Oleic acid (MUFA) is generally associated with beneficial effects on blood lipids and metabolic health when it replaces SFA and its presence helps attenuate the negative signalling associated with palmitate-rich fats so managing and preventing obesity (75). These results concluded that DMYF is a rich reservoir of various essential PUFAs, MUFAs and saturated fatty acids, so it can have several therapeutic health benefits. But the relatively high palmitate fraction warrants caution for direct dietary recommendation; therefore, suggested some processing or blending strategies (e.g., mixing with high-MUFA/PUFA-rich foods) to improve the SFA profile. Moreover, the unique phytochemicals associated with *D. maximum* can be beneficial in identifying and validating a fern species, as they can also act as chemotaxonomic markers (20).

Phytonutrients like Ca, K, Fe, Zn play a crucial role in the maintenance of good health. Among the macronutrients quantified in various wild edible plant species, after N, K occurred in the greatest amounts, followed by Ca, Mg and P. A somewhat similar trend was also observed in our study, with K as the highest amounted element, followed by P and Ca = Mg. K was slightly higher than *C. obliqua* (4.35 %) and *A. spinosus* (4.18 %), whereas P content was comparable to *N. pallens* seed's kernel (3160 mg/Kg), *C. obliqua* (3567 mg/Kg), *U. dioica* (2721 mg/Kg) (44, 63). Mg and Ca content was comparable to *B. aristata* (1160 mg/Kg) and *Eriolobus indica* (1240 mg/Kg) fruit (60, 76). Ca was comparatively lower (1927–2005 mg/Kg), while Fe lied in the range (112.0–202 mg/Kg) of *D. esculentum* (40, 41, 77) and near to *Oxalis corniculata* (161.71 mg/Kg) (78). Fe concentration was reported to be highest in leaves and young shoots (60). Zn content was comparable to that of *D. maximum* (46.82–60.2 mg/Kg) (20), *A. spinosus* and *Z. armatum* (49.7 and 47.6 mg/Kg) (44). Hence, the young shoots of *D. maximum* are a rich reservoir of essential macro and micro-elements; therefore, this fern of full potential can be exploited in resolving nutrient deficiency problems.

Phytochemical screening of DMYF showed similar phytocompounds as reported earlier in the same species and in *D. esculentum* (14, 20, 39, 79, 80). Nearly similar (total carotenoid and chl a) and higher (total chl and chl b) pigments content was reported earlier (20). The latter might be due to *D. maximum* preference for shady and humid-moist locations for its optimum growth and survival, as shading promotes these photosynthetic pigments accumulation in tea leaves, which is useful in improving its quality, quantity and antioxidant properties (81).

Significant differences were also found in TF and TP content of DMYF across 3 regions, which might be due to the number of microclimatic factors prevailing at the concerned region of sample collection, like average temperature, air pressure, rainfall and light intensity, in addition to pedological characteristics (82–84). Additionally, different solvents had different extraction yields, which also influenced the TP and TF content. The higher phenolic content obtained than flavonoids correspond to high antioxidant activity, as phenols have a higher redox potential and can absorb and neutralise free radicals (85). Among different underutilised Himalayan wild edible plants, *Asystasia ganjetica* hydro-alcoholic extract showed comparable TPC (91.80 mg GAE/g) to our study. Similarly, a comparable TFC was estimated in Chamomile i.e.,

Matricaria chamomilla (74.48 mg QE/g) and ethanol extract of *Viola canescens* roots (75.46 mg/QE/g) (62, 86, 87).

The significantly highest free radical scavenging activity possessed by SB-DMYF hydro-alcoholic extract was comparable to an earlier study on the same species and lied in the range (i.e., 37–282 µg/mL) of *V. canescens* and was slightly higher than *Momordica dioica* (43.05 µg/mL) (20, 44, 87). This stronger antioxidant activity was contributed by the high amount of both TFC and TPC, together with the other phytocompounds like carotenoids. The elevated TPC and TFC in SB might be likely driven by microclimatic stresses (higher light/UV, temperature variation, or mild water deficit) and soil constraints (lower N/P or reduced moisture), both of which are known to activate the phenylpropanoid pathway. Such stress-induced up-regulation of key enzymes leads to greater accumulation of phenolics and flavonoids, explaining the enhanced antioxidant potential at this site (88).

Limitations and future work

Although most edible ferns are considered safe, some species (e.g., *Pteridium aquilinum*) contain ptaquiloside and other anti-nutritional factors such as thiaminase or oxalates (89). Traditional culinary practices (boiling, blanching) are believed to mitigate many heat-labile anti-nutrients from ferns. The consumption of *D. maximum* after drying and boiling in water added with some ghee was demonstrated (90). No published evidence currently reports these toxic glycosides in *D. maximum*, but systematic safety screening is lacking. Therefore, dedicated toxicological profiling is still required in the future, which targets the fern-related hazards.

Conclusion

The present study demonstrates that *D. maximum* is a nutritionally rich wild edible fern with high moisture content, substantial mineral concentrations (especially K, P, Ca, Mg, Fe) and significant levels of bioactive molecules (phenolics, glycosides, flavonoids, alkaloids, steroids, fatty acids). The clear site-based variation observed across the 3 Himalayan locations further underscores the influence of local environmental and edaphic factors on its biochemical quality. The abundance of essential nutrients and natural antioxidants highlights the species' potential for dietary integration, especially in nutritionally vulnerable Himalayan communities. Its consistent nutrient richness also indicates strong prospects for commercialisation, including incorporation into herbal formulations, dehydrated leafy vegetable powders, or functional food products. The findings indicate that wild edible plants serve as valuable nutrient sources for rural communities and are comparable to many commercial fruits. The study recommends the cultivation of select wild edible species within traditional agroforestry systems to ease harvesting pressure on natural forests while providing economic benefits to small-scale farmers. Future research should prioritise the isolation and structural characterisation of key bioactive compounds, in-depth evaluation of their biological activities and the exploration of processing and preservation methods to retain nutrient stability. Additionally, developing value-added functional foods and conducting multi-season ecological studies will help establish *D. maximum* as a reliable and sustainable nutritional resource. Overall, this study provides foundational evidence to promote the species from a traditional forest food to a potential nutraceutical ingredient.

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Authors' contributions

PS wrote the original draft, performed the whole experiments and statistical analysis, data curation, visualization. PK conceptualized, supervised and provided the essential resources to carried out the research work. LT, MD, NG and PG edited and performed certain part of formal analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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