

## Nutraceutical and antioxidant potential of selected wild edible plants from the cold-arid desert of Ladakh, India

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### Abstract

Ladakh's wild edible plant species (WEPs) provide a vital source of food, nutrition, and medicine to the region's indigenous populations. However, WEPs' nutritional and antioxidant properties are missing in the region. The current study examined the nutritional values, total phenolic, total flavonoid, and antioxidant capabilities of 11 WEPs from the cold-arid region of Ladakh, India. *Lactucata tatarica* had the greatest carbohydrate content ( $36.48 \pm 0.15 \text{ mg g}^{-1}\text{Dw}$ ) among 11 wild edible plant species. *Malva verticillata* had a greater glucose concentration ( $292.74 \pm 0.01 \text{ ng g}^{-1}\text{Dw}$ ), and *Urtica hyperborea* had higher protein and vitamin C levels ( $38.07 \pm 2.44$  and  $49.95 \pm 1.18 \text{ mg g}^{-1}\text{Dw}$ , respectively). The plant samples collected were also shown to have high total phenolic, flavonoid, and antioxidant capabilities. Our findings revealed that these species should be promoted as a natural source of nutraceuticals, with the potential to supplement the diets of locals. To boost food availability in a location noted for its intense weather conditions and short vegetative period, an agroforestry system that promotes sustainable use and increases the output of these species is critical.

**Keywords:** antioxidants; carbohydrate; Ladakh; protein; vitamin C; wild edible plants

### Introduction

Wild edible plants (WEPs) have been the source of sustenance across the planet for a very long time. These plants provide vital nutritional components such as carbohydrates, protein, and antioxidants (Jacinto-Azevedo *et al.*, 2021). The utilization of wild edible plants among the ethnic people of Ladakh, India, has had a very long tradition from time immemorial (Nirmala *et al.*, 2022). According to a recent claim, the number of vascular plant species in the region may range between 1250 and 1500, but only some are consumed as phytofood (Gairola *et al.*, 2014; Batool *et al.*, 2023). Previous studies reported around 164 species of wild edible plants used by the indigenous communities of Ladakh (Rana *et al.*, 2012).

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*Prunus armeniaca* L. and *Bunium persicum* are the most widely cultivated edible plants. However, most edible plants produced in the wild are favored because of their nutritional and therapeutic characteristics (Nirmala *et al.*, 2022). The cold desert climatic conditions, minimal yearly precipitation, and low oxygen content not only enhance the endurance of these wild plants to harsh biotic and abiotic stress when compared to farmed crops but also load the plants with antioxidants (Kaur *et al.*, 2013). These edible plants are a valuable source of nutrition for the residents, especially during the difficult and prolonged winters that exist in the region (Ballabh *et al.*, 2007). During the winter, the leafy portion of these plants is properly dried, packed, and stored in distinctive structures locally known as “Sadong”, “Tsothbang” and “Charches” to prevent frosting, decay, and mechanical injuries (Ali *et al.*, 2012). However, owing to a lack of understanding of the nutritional benefits and prevention of diet-related disorders, the majority of these species are either underutilized or disregarded (Nirmala *et al.*, 2022).

There is a multitude of scientific evidence that supports the nutritional value of WEPs being comparable to or even higher than that of many cultivated varieties (Rana *et al.*, 2012). Various authors (Ballabh *et al.*, 2007; Murugan *et al.*, 2010; Dorjey *et al.*, 2012; Rana *et al.*, 2012; Boesi, 2014; Dorjey, 2015; Haq *et al.*, 2021) have reported the WEPs of Ladakh, but very few have evaluated the nutritional values or antioxidant properties of these wild edible plants. Nutritional values were evaluated only for *Lepidium latifolium* Linn. (Kaur *et al.*, 2013; Ali *et al.*, 2021). However, antioxidants values were analyzed for phyto-cocktail of (*Hippophae rhamnoides* L., *P. armeniaca*, and *Rhodiola imbricata* Edgew) (Dhar *et al.*, 2013), *R. imbricata* (Tayade *et al.*, 2013), *P. armeniaca* (Ali *et al.*, 2022), *Urtica hyperborea* Jacq. ex Wedd. (Raj *et al.*, 2012), *Nepeta longibracteata* Benth, *Allardia tomentosa* Regel, *R. imbricata*, *Delphinium brunonianum* Royle, *L. latifolium*, *Echinacea purpurea* (L.) Moench, and *Perovskia abrotanoides* Karel (Avasthi *et al.*, 2016), *Capparis spinosa* L. (Bhojar *et al.*, 2018) and *H. rhamnoides* (Sharma *et al.*, 2014).

Even though these plants constitute an essential component of the indigenous’ traditional diet, and the edible sections may be preserved for longer usage throughout winter months, there isn’t much research on most of these plants’ nutritional and antioxidant capabilities. In light of this, the current study intends to assess the nutritional makeup and phytochemical analysis of selected 11 wild edible plants from Ladakh, India, including *Capparis spinosa*, *Chenopodium album* L., *Lepidium latifolium*, *Plantago major* L., *Dysphania botrys* (L.) Mosyakin & Clemants, *Urtica hyperborea*, *Carum carvi* L., *Lactuca tatarica* C.A. Mey., *Rumex patientia* L., *Taraxacum officinale* F.H. Wigg, and *Malva verticillata* L.

## Materials and Methods

### *Raw material: Collection, identification, and storage*

The plant components were collected from native wild habitats in various sites around Ladakh, India, as shown in Table 1. Plant materials were collected on a sunny day during the early stages of flowering for easier identification. After removing soil and plant residues, each wild-collected sample was carefully removed from the substrate and rinsed with deionized water. The plant leaves were dried and pounded into a fine powder with a pestle and mortar before being stored in an aseptic, controlled environment at  $-20^{\circ}\text{C}$  until further investigation. Flora of Ladakh (Stewart, 1916; Kachroo *et al.*, 1977; Polunin and Stainton, 1985; Kaul, 1997), and the herbaria of Regional Research Laboratory (Jammu), Jammu University, and Kashmir University, were used as taxonomic references for plant identification. All specimens were identified by the first author (Z.B) of this paper. The voucher specimens were deposited and preserved at the Janaki Ammal Herbarium of Indian Institute of Integrative Medicines Jammu for future reference. The botanical names of the plants were verified according to [www.worldfloraonline.org](http://www.worldfloraonline.org).

**Table 1.** General information and accession details about the investigated Ladakh wild edible plants

Plant name / Family/ [Voucher no.]	Local name	Part use	Location	Altitude (masl)
<i>Capparis spinosa</i> L./Capparaceae [RRLH-25429]	Kabra	Leaves	Garkone	3513
<i>Chenopodium album</i> L. /Amaranthaceae [RRLH-25401]	Snue	Leaves	Lankerchey	2938
<i>Lepidium latifolium</i> L. /Brassicaceae [RRLH-25419]	Shangsho	Leaves	Wakha	3473
<i>Plantago major</i> L. /Plantaginaceae [RRLH-24295]	Kararatse	Leaves	Panikhar	3239
<i>Dysphania botrys</i> (L.) Mosyakin & Clemants /Amaranthaceae [RRLH-25621]	Sanik, Hama	Leaves	Nubra	3114
<i>Urtica hyperborea</i> Jacquem. Ex Wedd. / Urticaceae [RRLH-25399]	Rdoastot, Zastot	Leaves	Nyoma	4227
<i>Carum carvi</i> L. /Apiaceae [RRLH-24051]	Kumbulik	Leaves	Sapi	4340
<i>Lactuca tatarica</i> C.A. Mey. /Asteraceae [RRLH-24078]	Skyabs	Leaves	Shargole	3177
<i>Rumex patientia</i> L. /Polygonaceae [RRLH-26958]	Shoma	Leaves	Sankoo	2975
<i>Taraxacum officinale</i> F.H. Wigg / Asteraceae [RRLH-25549]	Khorma	Leaves	Tai-Suru	3232
<i>Malva verticillata</i> L. /Malvaceae [RRLH-24738]	Sochilik	Leaves	Khachan	2890

*Preparation of methanol extracts*

1 g of powdered samples was used for extract preparations. The extracts were obtained by continuously stirring fine powder with 10 mL of 80% methanol at 40 °C for 8 hrs. This procedure was carried out three times. Using a rotatory vacuum concentrator and lyophilizer, the extracts were initially combined, filtered, and evaporated to dryness. Furthermost, the lyophilized extracts were re-dissolved in 2 ml of methanol and diluted to perform different nutritional and antioxidant assays.

*Nutritional values*

The nutritional values of wild edible plants were calculated as per the guidance given under Association of Official Analytical Chemists(AOAC, 2019).

*Estimation of total glucose*

Total glucose was determined using a glucose assay kit (GAGO-20) from Ms/- Sigma-Aldrich Co., St. Louis as described previously by Kaur *et al.* (2013) with certain adjustments. The colors' intensity was measured at 540 nm after it was stabilized using H<sub>2</sub>SO<sub>4</sub>. Total glucose was reported as ng/d dry weight and compared using the calibration curve generated with BSA standard curve (0.1–1 ng; R<sup>2</sup> = 0.9901).

*Estimation of total carbohydrate*

The total carbohydrate content from the dried WEPs leaves powder was evaluated by the anthrone method as described previously by Yemm and Wills (1954) with some modifications. 1 g of dried samples was heated with a reagent containing 5 ml of 2.5 N HCl, and then cooled at room temperature. By adding distilled

water and centrifuge, the volume was increased to 100 ml. It is necessary to increase the volume to 1 ml by adding distilled water and anthrone reagent and heating the solution for 8 minutes in boiling water. The absorbance of the dark green color was measured at 630 nm.

#### *Estimation of total protein*

The total crude protein was calculated using the Bradford reagent as previously described by Kaur *et al.* (2013). Total protein was precipitated with 20% TCA solution and then dissolved in 1N NaOH following centrifugation. Total protein was reported as mg/g dry weight and compared using the calibration curve generated with BSA standard curve (0.5-5 µg;  $R^2 = 0.969$ ).

#### *Estimation of Vitamin C*

A spectrophotometric technique reported by Chanwitheesuk *et al.* (2005) was used to estimate vitamin C. At room temperature, 0.5 g of extract was produced from dried plant leaves using 10 ml of oxalic acid-EDTA solution. 2.5 ml of oxalic acid-EDTA solution, 0.5 ml of the metaphosphoric acid-acetic acid solution, 0.1 ml of sulfuric acid solution, and 2 ml of ammonium molybdate reagent were added to the extract. The extract volume was raised to 25 ml adding distilled water. After 15 minutes, the absorbance of the molybdenum blue complex was measured at 760 nm. The content of Vitamin C was determined by using ascorbic acid solution as a standard solution.

#### *Estimation of total phenolic and flavonoid contents*

##### Total phenolic content

Total phenolic content was calculated by the method described previously by Kaur *et al.* (2013) with a few modifications. 10 µL of extract in 80 µL of methanol was combined with 100 mL of 1N FC reagent and incubated in the dark for 5 minutes. 200 mL of 20% Na<sub>2</sub>CO<sub>3</sub> was added to the solution after further incubating for 10 minutes. The absorbance was measured at 730 nm, and the phenolic content was estimated using the gallic acid standard curve (500-5000 ng;  $R^2 = 0.967$ ). The results were calculated as mg GAE/g dry weight of plant material.

##### Total flavonoids content

With a few modifications, the total flavonoid content was analysed via., the colorimetric method described by Abu Bakar *et al.* (2009). In a test tube, 10 µL of the extract was combined with distilled water, followed by 15 µL of 5% NaNO<sub>2</sub> solution. After 6 minutes, 300 µL of a 10% AlCl<sub>3</sub> H<sub>2</sub>O solution was added and allowed to stand for another 5 minutes before adding 100 µL of 1 M NaOH. Vortexing was used to thoroughly mix the solution. A spectrophotometer was used to quickly measure the absorbance at 510 nm using quercetin as standard curve (10-100 µg;  $R^2 = 0.999$ ). The results were reported as mg QE/g dry weight.

#### *Antioxidant capacity assay*

##### DPPH radical assay

The bleaching of purple-colored methanol solution of the DPPH radical was used to measure the free radical scavenging activities of WEPs as described by Guleria *et al.* (2011). A double-beam UV-VIS spectrophotometer was used to detect the absorbance at 517 nm. RSA was determined as a percentage of DPPH radical discoloration using the equation.

$$\% \text{ RSA} = [(A_0 - A_s) / A_0] \times 100$$

where A<sub>0</sub> is the absorbance of the control, and A<sub>s</sub> is the absorbance of a test compound.

### ABTS assay

The ABTS was estimated using a modified version of the approach described by Jiménez-González *et al.* (2018). 200  $\mu\text{L}$  of ABTS solution was added to 10  $\mu\text{L}$  of extract and stirred with a vortex before being allowed to react in the dark environment at room temperature for 2-6 minutes. The absorbance was measured after six minutes of incubation at 765 nm. The results were expressed as millimole Trolox per gram of dry weight plant extract (mmol TE/g DW) for ABTS assay.

### FRAP assay

The FRAP assay was evaluated as described by Benzie and Strain (1996) with a few modifications for plate reader analysis. 180  $\mu\text{L}$  of FRAP reagent was combined with 5  $\mu\text{L}$  of extract and incubated at 37 °C for 3-5 minutes before measuring the absorbance at 593 nm with a microplate reader. The findings were expressed as millimoles  $\text{Fe}^{2+}$  per gram of dry weight (mmol  $\text{Fe}^{2+}$ /g DW) for FRAP assay.

### *Statistical analysis*

In the current study, each experiment was tested three times in independent extractions, using three measurements for each extraction. The values for each replicate were presented as mean  $\pm$  standard deviation (SD). The data was analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test. The difference was considered statically significant when  $p < 0.05$ . Statistical analysis of the data was performed in SPSS v. 26.0 (IBM SPSS Statistics for Window Version 26.0. Armonk, NY: IBM Corp.).

## **Results**

### *Nutritional profile*

The highest carbohydrate content was recorded in *Lactuca tatarica* (36.48 mg/g DW), while the lowest was recorded in *Plantago major* (14.35 mg/g DW). Good levels of carbohydrates were also recorded in *Dysphania botrys* (36.14 mg/g DW), *Taraxacum officinale* (34.11 mg/g DW), *Malva verticillata* (31.51 mg/g DW), and *Carum carvi* (29.54 mg/g DW). The carbohydrate content for *L. tatarica* showed significant differences with all the other species except for *D. botrys* at  $p > 0.05$ . The carbohydrate content between *C. carvi*, *Capparis spinosa*, *M. verticillata*, and *Lepidium latifolium*, and between *L. tatarica* and *D. botrys* showed no significant differences based on Tukey test as illustrated in Table 2.

Glucose content in the selected wild edible plants from Ladakh ranged between 145.86 to 292.74 ng/g DW. The highest glucose content was recorded in *M. verticillata* (292.74 ng/g DW) and the least in *P. major* (145.86 ng/g DW). The other species with higher glucose content were *Capparis spinosa* ( $275.48 \pm 0.08$  ng/g DW), *Taraxacum officinale* ( $258.48 \pm 0.25$  ng/g DW), *U. hyperborea* ( $245.19 \pm 0.11$  ng/g DW), *D. botrys* ( $232.83 \pm 0.14$  ng/g DW) and *C. carvi* ( $231.23 \pm 0.11$  ng/g DW). The glucose content of *M. verticillata* differed considerably from the other 10 species at  $p < 0.05$ .

According to Table 2, the protein content in the selected 11 WEPs ranged from  $11.39 \pm 0.34$  mg/g DW to  $38.07 \pm 2.44$  mg/g DW. *U. hyperborea* from Nyoma ( $38.07 \pm 2.44$  mg/g DW) has the highest protein content while *P. major* collected from Panikhar ( $11.39 \pm 0.34$  mg/g DW) harbored the lowest among all. The difference in protein content of *U. hyperborea* was significant at  $p < 0.05$  as compared to the other selected WEPs.

The content of vitamin C in the wild edible plants used by indigenous communities of Ladakh is given in Table 2. The highest level of vitamin C was reported for *U. hyperborea* ( $49.95 \pm 1.18$  mg/g DW), followed by *Dysphania botrys* ( $49.75 \pm 4.68$  mg/g DW), and the least for *L. tatarica* ( $27.60 \pm 0.11$  mg/g DW). The

results for vitamin C content for *U. hyperborea* and *D. botrys* showed no significant difference. However, the vitamin C contents of *U. hyperborea* and *D. botrys* were significant from the rest of the other species at  $p > 0.05$ .

**Table 2.** Nutritional values of wild edible plants used by the indigenous communities of Ladakh

Plants	Carbohydrate (mg g <sup>-1</sup> DW)	Glucose (ng g <sup>-1</sup> DW)	Protein (mg g <sup>-1</sup> DW)	Vitamin C (mg g <sup>-1</sup> DW)
<i>Capparis spinosa</i>	29.29 ± 4.09c	275.48 ± 0.08b	18.52 ± 0.80c	41.41 ± 2.95b
<i>Chenopodium album</i>	16.67 ± 2.11e	215.31 ± 0.13f	21.35 ± 2.46b	38.09 ± 3.87b
<i>Lepidium latifolium</i>	28.38 ± 1.23c	215.16 ± 0.09f	25.35 ± 2.04b	38.58 ± 4.06b
<i>Plantago major</i>	14.35 ± 4.12e	145.86 ± 1.47h	11.39 ± 0.34d	31.66 ± 1.58c
<i>Dysphania botrys</i>	36.14 ± 3.37a	232.83 ± 0.14e	23.65 ± 0.38b	49.75 ± 4.68a
<i>Urtica hyperborea</i>	24.92 ± 2.18d	245.19 ± 0.11d	38.07 ± 2.44a	49.95 ± 1.18a
<i>Carum carvi</i>	29.54 ± 3.52c	231.23 ± 0.11e	20.69 ± 1.07b	34.06 ± 2.05b
<i>Lactuca tatarica</i>	36.48 ± 0.15a	245.29 ± 1.53d	20.22 ± 0.37b	27.60 ± 0.11c
<i>Rumex patientia</i>	24.55 ± 4.33d	219.95 ± 0.16g	22.43 ± 1.65b	30.10 ± 5.39c
<i>Taraxacum officinale</i>	34.11 ± 0.15b	258.48 ± 0.25c	24.21 ± 1.14b	36.30 ± 2.36b
<i>Malva verticillata</i>	31.51 ± 3.98c	292.74 ± 0.01a	27.14 ± 1.62b	37.12 ± 0.51b

The mean values across the column with different superscript letters denote significantly different as per the Tukey's post hoc test ( $p < 0.05$ ). Data is reported as mean SE. and shown as an average value of the three replicates i.e. n=3

#### *Total phenolic and flavonoid contents of the selected wild edible plants*

The total phenolic content (TPC) and total flavonoid content (TFC) of the investigated wild plants of Ladakh are presented in Table 3. TPC in the selected samples ranged from  $11.47 \pm 0.33$  to  $53.25 \pm 0.87$  mg GAE/g and TFC content ranged from  $19.83 \pm 5.16$  to  $64.87 \pm 3.51$  mg QE/g. The highest TPC was reported in *Urtica hyperborea* ( $53.25 \pm 0.87$  mg GAE/g) while *Lactucata tatarica* contained the lowest ( $11.47 \pm 0.33$  mg GAE/g) total phenolic content. The total phenolic content value for *U. hyperborea* showed a significant difference from the rest of the other species at  $p < 0.05$ . As far the total flavonoid content (TFC) is concerned, *Plantago major* and *Malva verticillata* had the highest ( $56.64 \pm 4.44$  mg QE/g) and lowest ( $19.83 \pm 5.16$  mg QE/g) TFC, respectively. The result of TFC for *P. major* is statistically different at  $p < 0.05$  from the rest of the other species. However, the TFC of *Carum carvi* and *U. hyperborea*, and between *L. tatarica* and *Dysphania botrys* did not show any statistical differences.

#### *Antioxidant capacity of selected wild edible plants*

##### DPPH radical scavenging activity

DPPH radical scavenging abilities of the wild edible plants are presented in Table 3. The highest DPPH radical scavenging activity was recorded in *Urtica hyperborea* ( $E_{50} = 11 \pm 2.86$  mg/ml), while the lowest antioxidant capacity was recorded in *Taraxacum officinale* ( $E_{50} = 39.86 \pm 2.37$  mg/ml). The DPPH radical scavenging activity of *Capparis spinosa*, *Chenopodium album*, *Rumex patientia*, and *Taraxacum officinale* exhibited no significant differences. On the other hand, DPPH activities of *U. hyperborea* showed a significant difference from the rest of the other 10 species at  $p < 0.05$ .

##### ABTS assay

For the ABTS assay, the antioxidant ability varied from  $111.11 \pm 19.60$  to  $237.04 \pm 26.71$  mmol Trolox g<sup>-1</sup>DW as illustrated in Table 3. *Lactuca tatarica* showed the highest ABTS antioxidant capacity ( $237.04 \pm 26.71$  mmol Trolox/g DW), followed by *Carum carvi* ( $232.10 \pm 34.21$  mmol Trolox/g DW) while the lowest ABTS activity was observed for *Urtica hyperborea* ( $111.11 \pm 19.60$  mmol Trolox/g DW). The result of ABTS

for *L. tatarica* showed significant differences ( $p < 0.05$ ) from those of *T. officinale*, *Malva verticillata*, *Lepidium latifolium*, and *U. hyperborea*.

#### FRAP assay

The antioxidant capacity based on FRAP assay for the selected wild edible plants used by the indigenous people of Ladakh varied from  $80.44 \pm 2.49$  to  $542.08 \pm 3.16$  mmol Fe<sup>2+</sup> 100 g<sup>-1</sup>DW. The highest FRAP value was recorded for *Plantago major* ( $542.08 \pm 3.16$  mmol Fe<sup>2+</sup> 100 g<sup>-1</sup>DW), followed by *Urtica hyperborea* ( $534.02 \pm 15.67$  mmol Fe<sup>2+</sup> 100 g<sup>-1</sup>DW) and the least for *Malva verticillata* ( $80.44 \pm 2.49$  mmol Fe<sup>2+</sup> 100 g<sup>-1</sup>DW). According to the Tukey test, the FRAP value for *Plantago major* was different significantly from the rest of the other species except for *U. hyperborea* (Table 3).

**Table 3.** The selected WEPs' total phenolic content, total flavonoid content, DPPH, FRAP, and ABTS values used by the indigenous communities of Ladakh

Plants	Total phenolic content (TPC) (mg GAE g <sup>-1</sup> )	Total flavonoid content (TFC) (mg QE g <sup>-1</sup> )	DPPH (E <sub>50</sub> mg ml <sup>-1</sup> )	ABTS (mmol Trolox g <sup>-1</sup> DW)	FRAP (mmol Fe <sup>2+</sup> 100 g <sup>-1</sup> DW)
<i>Capparis spinosa</i>	28.59 ± 0.21b	43.84 ± 4.72b	36.07 ± 5.91a	217.28 ± 8.55a	233.33 ± 20.42c
<i>Chenopodium album</i>	23.34 ± 0.55cd	36.22 ± 5.83b	34.66 ± 4.17a	204.94 ± 22.63a	103.37 ± 14.63e
<i>Lepidium latifolium</i>	27.34 ± 0.83b	39.48 ± 4.68b	27.50 ± 1.93b	160.49 ± 22.63b	344.49 ± 16.94b
<i>Plantago major</i>	24.14 ± 0.51c	56.64 ± 4.44a	31.29 ± 4.89b	229.63 ± 25.66a	542.08 ± 3.16a
<i>Dysphania botrys</i>	22.74 ± 0.43d	34.05 ± 4.22b	28.04 ± 5.00b	222.22 ± 25.66a	128.24 ± 8.05e
<i>Urtica hyperborea</i>	53.25 ± 0.87a	47.80 ± 5.16b	11.11 ± 2.86c	111.11 ± 19.60c	534.02 ± 15.67a
<i>Carum carvi</i>	17.80 ± 0.25f	45.75 ± 3.09b	31.14 ± 5.63b	232.10 ± 34.21a	309.37 ± 20.35b
<i>Lactuca tatarica</i>	11.47 ± 0.33h	36.78 ± 4.28b	30.50 ± 6.97b	237.04 ± 26.71a	122.93 ± 5.93e
<i>Rumex patientia</i>	19.46 ± 0.31e	64.87 ± 3.51a	39.72 ± 5.71a	217.28 ± 22.63a	179.55 ± 14.78d
<i>Taraxacum officinale</i>	21.72 ± 0.54d	46.28 ± 3.22b	39.86 ± 2.37a	197.53 ± 21.38b	176.03 ± 10.95d
<i>Malva verticillata</i>	15.24 ± 0.87g	19.83 ± 5.16c	25.06 ± 1.20b	185.19 ± 19.60b	80.44 ± 2.49f

The mean values across the column with different superscript letters denote significantly different as per the Tukey's post hoc test ( $p < 0.05$ ). Data is reported as mean SE, and shown as an average value of the three replicates i.e. n=3

## Discussion

Among the selected 11 WEPs, the carbohydrate value of only *Carum carvi*, *Capparis spinosa*, and *Taraxacum officinale* has been evaluated so far. Previous studies on seeds of *C. carvi* reported higher carbohydrate content than those reported in the leaves of the present study, e.g., *C. carvi* with a value of 78.7 mg/g (Nirmala *et al.*, 2022). The difference in the value could be contributed to the different plant parts used for analysis (Alam *et al.*, 2020). The carbohydrate content of other WEPs from other part of the globe as reported by Kobeasy *et al.* (2011) and Rezzan *et al.* (2013) were higher than those reported here. Thus, there exists a difference in terms of carbohydrate content between a wild edible plant grown in Ladakh and other global regions. This variation could be contributed to differences in geographical, climatic, vegetative, and/or other factors (Alam *et al.*, 2020; Rana *et al.*, 2019). To the best of our knowledge, there is no report on the carbohydrate content of *Lactuca tatarica*, *Malva verticillata*, *Urtica hyperborea*, and *Dysphania botrys* in literature.

The glucose content of the selected WEPs except for *Lepidium latifolium* had not been assessed in any of the previous studies and there is no reference data available to compare for the rest. The glucose content observed for *Lepidium latifolium* in the current study was within the similar range as reported by Kaur *et al.* (2013) from Ladakh. From Table 2, it is evident that the selected wild plants contained a high amount of simple

sugar like glucose, and thus could provide a considerable amount of glucose in our diet which in turn makes these plants a good source of food.

The protein content of *Carum carvi* seeds and *Capparis spinosa* was 31.12 g/100 g, and 2 g/100 g respectively as reported by Nirmala *et al.* (2022). These values were different from the ones reported in the present study. Kaur *et al.* (2013) also reported variations in protein content between the same plant parts as well as similar plants collected from different places of Ladakh. These differences could be secondary to the diversion of primary metabolites to produce secondary metabolites (Kaur *et al.*, 2013). Similar or even higher protein content was also reported for some wild edible plants found in Ladakh (Nirmala *et al.*, 2022), the Eastern Himalayan region (Tag *et al.*, 2014), and other parts of India (Gupta *et al.*, 2005; Kumar and Shiddamallayya, 2021; Vishwakarma and Dubey, 2011). On the other hand, protein content was found to be lower in this study than in some previously reported wild edible plant species from Western Himalayan regions (Maikhuri *et al.*, 2021).

The highest level of vitamin C was reported for *U. hyperborea* ( $49.95 \pm 1.18$  mg/g DW), followed by *D. botrys* ( $49.75 \pm 4.68$  mg/g DW), and the least for *L. tatarica* ( $27.60 \pm 0.11$  mg/g DW). The values of vitamin C for the recorded plants were found to have considerably higher as compared to some of the wild edible plants, underutilized green leafy vegetables, and locally available cultivated vegetables such as spinach and cauliflowers (Alam *et al.*, 2020; Gupta *et al.*, 2005; Nirmala *et al.*, 2022). Since humans are unable to synthesize vitamin C endogenously, it's essential an essential dietary component due to its antioxidant properties which enhance the immune system and help in preventing or treating numerous health-related issues (Samancioglu *et al.*, 2016). Ladakh, with extreme climatic conditions and short vegetative periods usually results in a shortage of cultivable leafy vegetables. Given these reasons, the leaves of these wild edible plants play an important role in the fulfillment of the nutritional needs of the local inhabitant and can be promoted as a source of natural source of Vitamin C in Ladakh.

The values for TPC and TFC of the selected WEPs are in accordance with the values reported by Kaur *et al.* (2013), Bhoyar *et al.* (2018), and Nirmala *et al.* (2022), who studied wild edible plants collected from Ladakh and elsewhere. Contrary to the current result, a higher value of TPC and a lower value of TFC for *Urtica hyperborea* collected from Ladakh were reported by Raj *et al.* (2012). Avasthi *et al.* (2016) also reported a similar or higher range of TPC and TFC for other high-altitude wild edible plants of Ladakh. These results indicate that geographical location, climatic conditions, time, and methods of cultivation do influence the content of TPC and TFC in edible plants (Rana *et al.*, 2019). Similar observations were also reported from other regions of India, and elsewhere in the world (Alam *et al.*, 2020; Jacinto-Azevedo *et al.*, 2021; Kumar and Shiddamallayya, 2021).

The antioxidant abilities of plants are believed to be mediated through phenolics and the different kinds of solvents as well as extractive procedures can markedly affect the antioxidant potential of the plant extract (Alam *et al.*, 2020). In the present study, the methanolic extract of the selected wild plants was assessed for antioxidant activities through DPPH, ABTS, and FRAP. The DPPH value observed for *U. hyperborea* was higher than the one reported by Xavier *et al.* (2011) and Raj *et al.* (2012) in Ladakh. This variation could be secondary to the collection of plants from different places and a similar pattern was also observed by Kaur *et al.* (2013), where different DPPH values were obtained for the same species collected from three different places. Similar or higher values were reported by Avasthi *et al.* (2016) and Razak *et al.* (2020) for some of the wild edible plants of Ladakh and neighboring Kashmir valley respectively. The presence of phenolic compounds present in the selected WEPs allows them to act as hydrogen donors, singlet oxygen quenchers, and reducing agents (Ali *et al.*, 2021; Xavier *et al.*, 2011). In previous studies, a positive correlation was observed between the content of phenolic compounds and their antioxidant DPPH potential (Ali *et al.*, 2021; Šamec *et al.*, 2018), which also hold in our case as well, since the total phenolic content for *U. hyperborea* is highest among the selected wild edible plants (Table 3).

Among the selected 11 WEPs, ABTS of only 2 species collected from Ladakh viz., *C. carvi*, and *U. hyperborea* has been evaluated so far. However, the computed units in prior studies from Ladakh by Bhoyar *et al.* (2018), Xavier *et al.* (2011) differed from those employed in this work, making comparison problematic. According to Bhoyar *et al.* (2018), the dried fruit of *C. spinosa* had the highest IC<sub>50</sub> for ABTS ( $0.086 \pm 0.002$  mg/ml), whereas the IC<sub>50</sub> in the leaves was between the range of ( $31.0 \mu\text{g/ml}$  to  $39.0 \mu\text{g/ml}$ ). The current investigation found that dried leaves of *C. spinosa* had an ABTS value of ( $217.28 \pm 8.55$  mmol Trolox g<sup>-1</sup>DW). On the other hand, the ABTS value recorded by Xavier *et al.* (2011) for *C. carvi*, and *U. hyperborea* were  $29.5 \pm 0.3$  and  $11.1 \pm 2.0$  mg Ascorbic acid eq/g DW respectively which was  $232.10 \pm 34.21$  mmol Trolox g<sup>-1</sup>DW for *C. carvi* and  $111.11 \pm 19.60$  mmol Trolox g<sup>-1</sup>DW for *U. hyperborea* in the present study.

The antioxidant potential via FRAP for plants collected from the Ladakh region was evaluated for only *U. hyperborea*, and no reference data was available to compare the rest of the species. The total antioxidant potential of the methanolic extract of *U. hyperborea* as determined by FRAP was  $560.59 \pm 18.72$  mmol Fe<sup>2+</sup>g<sup>-1</sup>DW in one of the previous studies conducted in Ladakh (Raj *et al.*, 2012). The recorded value ( $560.59 \pm 18.72$  mmol Fe<sup>2+</sup>g<sup>-1</sup>DW) by Raj *et al.*(2012) for *U. hyperborea* is comparable to that of the current study ( $534.02 \pm 15.67$  mmol Fe<sup>2+</sup> g<sup>-1</sup>DW). Avasthi *et al.* (2016) found that the antioxidant potential of seven distinct wild edible plants from Ladakh ranged from  $15.67 \pm 0.08$  to  $139.23 \pm 0.24 \mu\text{g/mL}$ . In the FRAP test, the antioxidant of *C. Carvi* collected from the northeast of Tunisia was recorded as ( $EC_{50}=18.00 \pm 1.00$  mg/mL). Given the different units used in the previous studies, it would be difficult to interpret the result. From the data obtained through different antioxidant assay methods, it was found that the selected WEPs showed good antioxidant capacities. Apart from their nutritional values, different parts of all the selected species are well documented in Ladakh to cure a wide range of diseases (Angmo *et al.*, 2012; Ballabh and Chaurasia, 2007; Gairola *et al.*, 2014; Haq *et al.*, 2021; Uniyal, 1981). There has been a plethora of evidence to suggest that antioxidants can neutralize free radicals which are responsible for the causation of various types of diseases (Kaur *et al.*, 2013). Thus, as a rich source of antioxidants, further isolation of antioxidant molecules of these WEPs would be beneficial, and these plants could be utilized for the preparation of crude extract.

Ladakh's rich ethno-botanic diversity contributes significantly to supporting the nutritional need of the local inhabitants (Batool *et al.*, 2023). The traditional community-based approach system, in which households assist one another in the cultivation and production of vegetables, not only reduce the labour cost but also increases post-production costs for their products. Farmers, notably Ladakhi women actively participate in the selling of locally produced vegetables in local markets (Stobdan *et al.*, 2017). The price of vegetables range between Rs 50 to 200 per kg for most of the cultivable plants such as coriander, beet leaf, mustard leaves. However, inhabitants continue to lack knowledge about the nutritional, economic, and medicinal merits of the wild edible plants which are well known to grown in harsh abiotic conditions and are cold tolerant (Murugan *et al.*, 2010). Due to a lack of understanding on this rich resource, there is high risk of overexploitation of these species for non-nutritional uses which could disrupt the natural ecosystem and lead to the extinction of these critical species. Hence, it is important to provide proper training for safe harvesting and cultivation of these species to enhance socio-economic status of the local communities.

## Conclusions

The purpose of this article is to provide information about the nutritional value, supplementary role, and economic potential of wild edibles of Ladakh. The 11 selected wild edible plant species from the cold-arid desert of Ladakh contain considerable amount of carbohydrate, glucose, protein, vitamin-C, total phenolic, flavonoid contents, and antioxidant capacities. The findings suggest that the wild plants investigated in this study are promising sources of key nutrients and could be employed in the production of functional foods. As

a result, it is acceptable to conclude that wild edible plants are nutrient-dense and might be used as an alternative source of nutrients to help alleviate poverty and ensure food and nutritional security. Domestication and promotion of commercial production of these wild edible plants would give major economic benefits to the farmers living in rural areas of the region. Furthermore, immediate action at the national level to gather their unique germplasm for ex-situ conservation is as important.

### Authors' Contributions

Conceptualization: ZB; Writing - original draft: ZB; Methodology: ZB; Investigation: ZB; Data curation: ZB, KS, JFL; Formal analysis: ZB, JFL, KS; Project administration: SG; Resources: SG; Software: ZB and KS; Supervision: SG; Validation: SG; Visualization: SG; Writing - review and editing: ZB, KS, JFL. All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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