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Research Article

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Nutritional composition and antioxidant capacity of *Urtica hyperborea*: A phytofood of Trans-Himalayan region of Ladakh, India

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ABSTRACT

Urtica hyperborea Jacquem. ex Wedd., a perennial plant of Urticaceae family is considered as a wild vegetable in the mountainous region of Ladakh. Due to its application in many forms of traditional culinary in every household during the harsh winter season, the plant ensures the food security. However, the nutritional composition and phytochemical analysis of *U. hyperborea* responsible for these beneficial features have not been explored widely. The present study aims to determine the nutritional composition (e.g., macromolecules, pigments, minerals, phenolics and flavonoid contents), antioxidant activity and the phytochemical analysis of this plant species present in Ladakh, India. The radical scavenging and antioxidant potential of the plant were evaluated by assays like 2,2–diphenyl–1–picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), hydroxyl (^oOH), and ferric ion reducing antioxidant power (FRAP) for different extracts prepared in water, methanol, ethyl acetate, and petroleum ether. Macromolecules such as protein, carbohydrate, total phenolic and flavonoid contents in *U. hyperborea* were found to be 62.28±6.67, 170.80±3.98, 24.47±0.39 and 5.43±0.97 mg g^{-1}, respectively. Similarly, dried powder of *U. hyperborea* was found to be rich in different mineral contents such as potassium, magnesium, sodium, manganese, zinc and iron. Among the various solvents used for exploring scavenging and antioxidant potential, aqueous extracts showed highest activity with 79.2% in DPPH assay as compared to other extracts. Similar trend was observed for other assays where aqueous extracts exhibited higher activity followed by methanolic, ethyl acetate and petroleum ether extracts. Significant positive linear correlations were observed between the radical scavenging/antioxidant activity of aqueous extracts and their content of phenolic/flavonoid compounds. The identification of phenolic compounds such as coumarin, quercetin, and ferulic acid confirm the antioxidative nature of the plant. Overall, rich macromolecule and mineral contents, as well as higher radical scavenging/antioxidant activities in aqueous extracts of *U. hyperborea* revealed that the plant has significant potential to be utilized as a phytofood source in harsh environmental conditions.

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INTRODUCTION

The type of foods consumed by the indigenous people represent the identity of the area or ethnic group or community. The foods sourced from the wild plants become an integral part of their custom and traditions, and it closely reflects the legacy of the traditional fore parents to consume the untapped natural plant resources that serve their daily nutrition needs (Nagar & Jain, 2016; Ballabh & Pullaiah, 2017; Singh & Bedi, 2018). The plant-based food habit of the people largely depends on the availability of the natural resources. For example, in India, about 1403 plant species are being valued as edible (Ray *et al.*, 2020). Various groups of communities in the central and western Himalayas are found to treasure about 647 wild plants as their food in many forms (Ballabh & Pullaiah, 2017). For instance, Sheena tribe of Kashmir, J&K consume 42 edible plants in their daily meals (Singh & Bedi, 2018), 57 species of plants are being used as food by tribes of eastern Arunachal Pradesh (Ngomle *et al.*, 2020). Likewise, 69 plant species in Ladakh are considered edible (Nagar & Jain, 2016). Moreover, Lamo *et al.* (2012) have

also reported the use of 28 plant species from cold desert of Ladakh for various culinary purposes since time immemorial. Apart from being consumed for their nutritional needs, many plants are considered for various medicinal properties such as, the edible fruit plant, *Eleagnus latifolia* L. is consumed in North-eastern Sikkim due to its good antioxidant, antibacterial properties, and contains various bioactive compounds and minerals (Dasila & Singh, 2022). Similarly, *Lepidium latifolium* L. in Ladakh possesses nutritional benefits as well as antioxidant properties (Kaur *et al.*, 2013).

One such wildly sourced vegetable is *Urtica* sp., a well imbibed vegetable in traditional food culture known by the local name as "Bichubutti" in Himachal Pradesh (Thakur *et al.*, 2020a), "Zwa or Zatsot" in Ladakh (Angchok *et al.*, 2009; Nagar & Jain, 2016) and "Stinging nettle" in the Himalayan region (Rawat *et al.*, 2020). Various *Urtica* sp. are being foraged as vegetable in the Himalayan belts including Sikkim (Sundriyal & Sundriya, 2004), Himachal Pradesh (Rana *et al.*, 2019; Thakur *et al.*, 2020b), Ladakh (Lamo *et al.*, 2012; Rana *et al.*, 2012; Nagar & Jain, 2016), Tibet, Nepal, and China (Boesi, 2014). Ethnobotanical explorations of *U. dioica* revealed the wound healing properties of its leaf extracts (Zouari-Bouassida *et al.*, 2017), and is also utilized for curing the anaemic patients (Rana *et al.*, 2019). The pharmacological properties of *U. dioica* highlight its use in hypertensive conditions, where it provides a vasodilating effect on the cardiovascular system (Testai *et al.*, 2002; Qayyum *et al.*, 2016; Vajic *et al.*, 2018). In addition, it is also known to possess anti-proliferative effect (Hodroj *et al.*, 2020), antimicrobial and antifungal properties (Saklani & Chandra, 2012). The *Urtica* sp. exhibit a variety of other biological activities such as anti-inflammatory, analgesic, anti-prostatic hyperplasia (Su, 2018), and hyper-uricemic (Han *et al.*, 2020).

Urtica hyperborea belonging to the family Urticaceae is a perennial plant found in high altitude (3800-5100 m) mountains in Ladakh (UT) region of Indian Himalaya. It is traditionally cooked as a vegetable in Ladakh (Angchok *et al.*, 2009; Nagar & Jain, 2016), and used for the treatment of cold, cough and rheumatism in the traditional health care system (Angmo *et al.*, 2012). Apart from being used as an indigenous dish, the information on dietary phytochemistry and biological activities of *U. hyperborea* is limited. Therefore, the study was designed to explore the functional food composition associated with dietary benefits, estimation of chlorophyll and carotenoid contents in addition to its antioxidant and radical scavenging activities. The objectives of the study were: (i) to determine the macromolecules, flavonoid, phenolic and pigment contents of the plant, (ii) to estimate the mineral and ash contents of the plant, (iii) to observe radical scavenging/antioxidant potential of the plant extracts prepared in four different solvents, and (iv) to find out the key factors responsible for the observed antioxidant potential. The findings of the study will provide detailed information on the nutritional composition and antioxidant potential of the plant, which may help in accelerating its cultivation to ensure food security during harsh conditions in the mountainous landscapes.

MATERIALS AND METHODS

Collection of Plant Material

The fresh plant samples of *Urtica hyperborea* (Figure 1) were collected during the peak of its growing season (June-July) from Leh district, Ladakh (UT) at 5193 m amsl (33°49'19.12" N and 077°56'33.07" E). A voucher specimen (accession number - 455) of the plant has been submitted to the Botanical Survey of India, Dehradun, India.

Estimation of Protein, Carbohydrate, Total Phenolic and Flavonoid, Chlorophyll and Carotenoid Contents

To determine the protein content, the extract was prepared as 10 mg mL^{-1} distilled water and kept overnight in order to dissolve the soluble proteins. The extract was then centrifuged at 12000 rpm (at 4 °C) for 20 minutes. The supernatant from the centrifuged samples were used for the analysis using the method described by Bradford (1976), where absorbance was taken at 595 nm. Bovine serum was used as a standard. The carbohydrate content was measured from the plant extract using the anthrone reagent as described by Loewus (1952), where the absorbance at 620 nm was measured spectrophotometrically. Glucose was used as a positive control.

The total phenolic content of the sample was estimated spectrophotometrically by following the Folin-Ciocalteu reagent method as given by Swain and Hillis (1959) using ferulic acid as a standard (absorbance at 700 nm), and expressed in terms of mg ferulic acid equivalents (FAE) g^{-1} of the plant. The total flavonoid content was determined using the methanolic aluminium chloride ($AlCl_3$) as per the method of Meda *et al.* (2005), and the absorbance was measured at 415 nm. The amount of flavonoid was expressed as mg QE g^{-1} of the plant, using quercetin as a standard.

For determining the chlorophyll and carotenoid contents of the plant, 25 mg of leaves were mixed with 4 mL of dimethyl sulphoxide, and the mixture was placed in an oven (at 60 °C) for 60 minutes as per the method described by Hiscox and Israelstam (1979). The absorbance was measured at 663, 645 and 470 nm for estimating the contents of chlorophyll (a, b, and total), and carotenoids. Further, the calculation and expression on a dry weight basis were done as per Batish *et al.* (2006).

Figure 1: *Urtica hyperborea* present in its natural habitat, Ladakh, India

Elemental Composition and Ash Content Analyses

ICP-MS elemental analysis

Elements like sodium (Na), magnesium (Mg), potassium (K), manganese (Mn), iron (Fe) and zinc (Zn) were estimated by the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) technique using Thermo Scientific ICP-MS iCAP instrument with model number iCAP RQ ICP-MS. For the sample preparation, pretreatment like digestion procedure was performed, where 200 mg of the sample was treated with 5 mL HNO_3 and 2 mL of HCl. It was then microwaved at 200 ºC for 1 hour and further diluted to 100 times using distilled water. From the diluted fraction, 10-20 mL of sample was filtered using the 0.4 mm filter. A calibration curve with multi-element standard was prepared in the range of 10 ppb to 500 ppb. When the linear curve was obtained, the sample was allowed to run to evaluate the presence of desired elements. The amount of mineral content is presented as mg 100 g^{-1} dry weight.

CHN-O analysis

The CHN-O elemental analyzer by Thermo Scientific was employed in order to determine the percentages of carbon (C), hydrogen (H), nitrogen (N), and oxygen (O) present in the dried plant material. For the sample preparation, pretreatment like digestion procedure was performed as mentioned in the element analysis section.

Estimation of ash content

The method described by Harris and Marshall (2017) was followed to estimate the ash content. The dry plant samples were placed in porcelain crucibles in a muffle furnace at 550-600 ºC for about 4-5 h, and obtained mass of ash content was presented in percentage (%) value.

Preparation of Plant Extracts

Different solvents ranging from polar to non-polar were used to prepare extracts of *U. hyperborea* following the protocol given by Abubakar and Haque (2020) with some minor modifications. Dried plant samples were immersed in various solvents such as water, methanol, ethyl acetate, and petroleum ether in a 1:20 (*w/v*) ratio. It was then homogenized for a few hours using a SPINOT digital hot plate magnetic stirrer by Tarsons at room temperature. The mixture was filtered the next day using Whatman filter paper #1 and allowed to evaporate the solvents. The final weight of the extracts was noted and the required concentrations were prepared accordingly. Further, the extracts were stored at 4 ºC prior to conducting the experiments.

Estimation of Radical Scavenging and Antioxidant Activity

2,2–Diphenyl–1–picrylhydrazyl (DPPH) activity

Scavenging activity of various extracts of *U. hyperborea* was determined by the procedure explained by Blois (1958) using DPPH (0.01 mM prepared in methanol) solution. The absorbance was measured at 517 nm, and ascorbic acid was used as a standard.

Hydrogen peroxide (H_2O_2) assay

The scavenging capacity of *U. hyperborea* was estimated according to the method given by Ruch *et al.* (1989) using 40 mM H_2O_2 as reaction mixture and the absorbance was measured at 230 nm. *Rosmarinus officinalis* L. (Rosemary) extract was taken as standard.

Hydroxyl radical (ºOH) scavenging activity

The hydroxyl radical (ºOH) scavenging activity of *U. hyperborea* was determined by following the method described by Wenli *et al.* (2004) with a few modifications. In brief, the reaction mixture was prepared by taking 0.02 mL of ferrous chloride (0.02 M), 0.50 mL of 1,10-phenanthroline (0.04 M), 1 mL of phosphate buffer (0.2 M, pH 7.2) and mixed with 1 mL of plant extract, followed by the addition of 0.05 mL of hydrogen peroxide (7 mM). The absorbance of the mixture was measured at 560 nm. Ascorbic acid was used as a standard for the assay. The hydroxyl radical scavenging activity of the extract was calculated using the following equation and is reported as percent (%) inhibition.

$$\% \text{ Scavenging activity} = [(A_{sample} - A_{control})/(A_{blank} - A_{control})] \times 100$$

Ferric ion reducing antioxidant power (FRAP)

The ferric ion reducing capability of the plant extract was estimated following the method described by Oyaizu (1986) with slight modifications. In brief, in a reaction mixture of 0.2 mL of extract, 0.6 mL of 0.2 M phosphate buffer (pH 6.6), 0.6 mL of potassium ferricyanide solution (10 gL^{-1}), 0.6 mL of trichloroacetic acid were added and centrifuged for 10 minutes at 3000 rpm. Then, 0.6 mL aliquot of the supernatant was mixed with 0.6 mL of distilled water and 0.125 mL of ferric chloride (1 gL^{-1}) solution in the test tube. After 10 mins, the absorbance was measured at 700 nm using a Shimadzu UV-1800 double beam spectrophotometer. Curcumin was taken as a standard for the assay. The reducing power was calculated using the equation as follows:

$$\% \text{ Inhibition} = [(A_{sample} - A_{control})/A_{sample}] \times 100$$

Chemical Characterization of *U. hyperborea* through Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography–Mass Spectrometry (LC–MS) was used to identify the phytochemicals present in *U. hyperborea* plant. Waters Micromass Q-Tof, micro-frame spectrometer fixed by separation unit 2795, Unisol C18 (4.6 mm × 250 mm) column fitted with VWR® with 5 µm thickness film was used for the experiment. The chemical constituents of the plant sample were identified based on their molecular mass. Overall data analysis was done using Mass Bank (https://massbank.eu/

MassBank/) and further verified from the literature available for the genus *Urtica*. The specific activities of various compounds identified in the LC-MS were described based on the previous studies.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was applied for the antioxidant assays performed to observe the variations with different concentrations. Significant observations based on ANOVA were further subjected to *post hoc* Tukey's test for differentiating the mean values at $p \leq 0.05$. The data are presented as mean±SE (n = 3) for different studied parameters. Linear regression analysis was performed for predicting the relationship between total phenolic/flavonoid contents and the radical scavenging/antioxidant activities of extracts. SPSS software package (ver. 16.0) was used for performing all the statistical analyses. Sigma Plot (ver. 11) was used for graphical presentation.

RESULTS

Macromolecule, Total Phenolic, Flavonoid and Pigment Contents of *U. hyperborea*

The protein content of *U. hyperborea* was found to be 62.28 mg g^{-1} which was 6.23% of the total plant biomass (Table 1). Likewise, the carbohydrate content was estimated to be 170.80 mg g^{-1} which represented 17.10% of the total plant biomass (Table 1). The phenolic content was found to be 24.47 mg FAE g^{-1} (Table 1). The flavonoid content was estimated in terms of quercetin equivalent, and was estimated to be 5.43 mg QE g^{-1} (Table 1). The chlorophyll *a* content of the plant was found to be 0.67 mg g^{-1} while its chlorophyll *b* content was found to be 0.24 mg g^{-1}. The total chlorophyll and carotenoid contents were found to be 0.98 mg g^{-1} and 0.13 mg g^{-1}, respectively (Table 1).

Mineral analyses and ash content of *U. hyperborea*

The elemental composition of *U. hyperborea* plant indicated the presence of several essential elements such as Na, Mg, K, Zn, Mn, Fe, as well as C, H, N, and O. The results revealed that the plant has a higher amount of K (278.86 mg 100 g^{-1}) followed by Mg (45.22 mg 100 g^{-1}). The concentration of essential elements was found to be in decreasing order of K > Mg > Na > Fe > Mn > Zn (Table 2). The C, H, N, and O contents presented in percentage were found to be 32.82%, 4.39%, 10.80%, and 32.22%, respectively (Table 2). The ash content constituted 23.3% of *U. hyperborea* plant biomass (Table 2).

Radical Scavenging and Antioxidant Analysis

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH scavenging activity of *U. hyperborea* was found to be highest (79.2%) in aqueous extracts as compared to methanolic (57.3%), ethyl acetate (26.4%) and petroleum ether (24.3%) extracts at 400 µg mL^{-1} concentration (Figure 2a). It can be noted that the aqueous extracts of the *U. hyperborea* exhibited relatively good antioxidant activity when compared with the standard i.e., ascorbic acid with 80% of scavenging activity.

Table 1: Protein, carbohydrate, total phenolic, total flavonoid, and pigment contents of *Urtica hyperborea*

Macromolecules	Quantity (mg g^{-1})
Protein	62.28±6.67
Carbohydrate	170.80±3.98
Total phenolic content	24.47±0.39
Total flavonoid content	5.43±0.97
Chlorophyll *a*	0.67±0.41
Chlorophyll *b*	0.24±0.15
Total chlorophyll content	0.98±0.20
Carotenoid	0.13±0.01

Values are represented as mean±SE.

Table 2: Elemental composition and ash content of *Urtica hyperborea*

Element	Quantity (mg 100 g^{-1})	Element	Percentage (%)
Sodium (Na)	6.75	Carbon (C)	32.88
Magnesium (Mg)	45.22	Oxygen (O)	32.23
Potassium (K)	278.86	Nitrogen (N)	10.81
Manganese (Mn)	2.22	Hydrogen (H)	4.40
Iron (Fe)	4.44	Ash content	23.33±3.34
Zinc (Zn)	0.14		

Hydrogen peroxide (H_2O_2) scavenging activity

Highest H_2O_2 radical scavenging activity was observed in the 400 µg mL^{-1} aqueous extracts (55.6%), followed by the methanolic (40%), ethyl acetate (29.9%) and petroleum ether (15.9%) extracts at the same concentration (Figure 2b). The scavenging activity of the aqueous extracts was found to be comparable to the standard used.

Hydroxyl radical (^{o}OH) scavenging activity

The hydroxyl radical scavenging activity of *U. hyperborea* was observed to be maximum (56.7%) in aqueous extracts at 400 µg mL^{-1} concentration as compared to methanol, ethyl acetate and petroleum ether extracts which exhibited the scavenging activity less than 30% (Figure 2d). When compared to its standard (ascorbic acid), it was found to be relatively low in methanol, ethyl acetate and petroleum ether extracts.

Ferric ion reducing antioxidant power (FRAP)

The FRAP assay of *U. hyperborea* revealed that the aqueous extracts showed maximum activity (55.6%) followed by methanolic extracts (34.6%), whereas low activity was observed in case of ethyl acetate (23.7%) and petroleum ether (19.7%) extracts at 400 µg mL^{-1} concentration (Figure 2c). The activity was found to be dose-dependent.

LC-MS Analysis

The chemical characterization via LC-MS revealed the presence of 28 compounds in the extracts of *U. hyperborea*

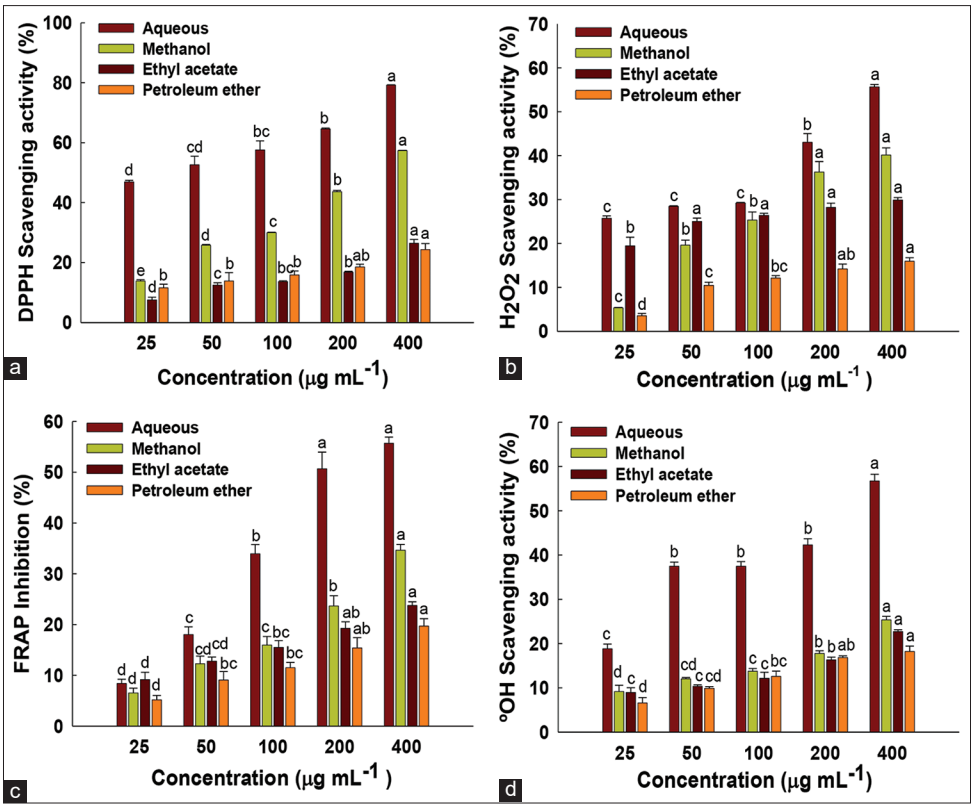

Figure 2: Antioxidant activity of *Urtica hyperborea* in different extracts: a) 2,2–diphenyl–1–picrylhydrazyl (DPPH) free radical scavenging activity, b) hydrogen peroxide (H_2O_2) scavenging activity, c) Ferric ion reducing antioxidant power (FRAP) activity, and d) hydroxyl (^{o}OH) scavenging activity. Different alphabets along bars represent significant differences at $p \leq 0.05$ for different concentrations, applying *post hoc* Tukey's test

(Table 3). Among different compounds identified, polyphenols (20) are found as the major class of compounds, followed by N-containing compounds (4), flavonoids (3) and terpenoids (1) present in *U. hyperborea* plant. Some of the important polyphenolic compounds identified were chicoric acid, coumaric acid, ferulic acid, peonidin 3-rutinoside, sinapic acid, and quinic acid. The N-containing compounds included glycine betaxanthin, leucine betaxanthin, serine betaxanthin and valine betaxanthin. Flavonoids observed in the *U. hyperborea* included 6-hydroxyluteolin, crysoeriol and quercetin. The terpenoid (viz., geranyl acetone) was also observed in the extracts of *U. hyperborea* (Table 3). The compounds found in *U. hyperborea* have various ethnobotanical uses and may help in providing antioxidative nature to the plant.

Relationship between Phenolic/Flavonoid Contents and Radical Scavenging/Antioxidant Activities

A strong positive correlation (r^2= >0.81 for phenolics and >0.90 for flavonoids) between antioxidant activities of different aqueous extracts with total phenolic and flavonoid contents was observed in this study (Figure 3). The trend observed for correlation between total phenolics with different aqueous extracts was in the order of FRAP > H_2O_2 > DPPH > ^{o}OH activity (Figure 3a). The trend observed for correlation between total flavonoids with different aqueous extracts was in the order of H_2O_2 > FRAP > DPPH > ^{o}OH activity (Figure 3b).

DISCUSSION

Urtica sp., a nutritious plant, is widely used as a vegetable which has high vitamin and mineral contents. It is used by Westerners as tea, juice and dried products. Based on the results of this study observed for different macromolecules, pigments, ash and elemental composition, it is safe to conclude that *U. hyperborea* is an important food crop with immense nutritional and economic potential. Important elements such as K and Mg were found to be present in higher concentrations. The protein and carbohydrate contents reported in the present study were found to be comparable with other *Urtica* species such as *U. dioica* (Said *et al.*, 2015; Radha *et al.*, 2021). A study on another *Urtica* species, *U. urens* revealed higher contents of the protein and carbohydrates compared to those observed in the present study on *U. hyperborea* (Afolayan & Jimoh, 2009). Chlorophyll and carotenoids which are present naturally in plants are frequent organic food components which impart colouration (Schoefs, 2002). Carotenoids are associated with various health benefits such as providing vitamin A to the body and acting as an antioxidative agent (Aadil *et al.*, 2019). The plant pigments such as chlorophyll a, b, and carotenoid in *U. hyperborea* were found to be 0.67, 0.24, and 0.14 mg g^{-1}, respectively, in the present study which were higher than other species of *Urtica*, such as *U. dioica* (Paulauskienė *et al.*, 2021). Joshi *et al.* (2014) reported the presence of essential amino acids and minerals like Ca, Fe, P, Mg and K in *U. dioica*. Available

Table 3: Chemical compounds present in *Urtica hyperborea* analysed by LC-MS

S. No.	Name of the compound	Formula	MW[a]	Class of compound	Biological activity of the compounds with reference(s)
1.	Quinic acid	$C_7H_{12}O_6$	192.2	Polyphenols	Anti-inflammatory agent (Zeng *et al.,* 2009)
2.	Coumaric acid ethylester (p-)	$C_{11}H_{12}O_3$	192.2	Polyphenols	Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, analgesic (Pei *et al.,* 2015)
3.	Scopoletin	$C_{10}H_8O_4$	192.2	Polyphenols	Anti-inflammatory activities and protective effects in multiple sclerosis (Zhang *et al.,* 2019)
4.	Ferulic acid	$C_{10}H_{10}O_4$	194.2	Polyphenols	Antioxidant, antimicrobial, anti-cancerous, antithrombotic, antidiabetic (Zduńska *et al.,* 2018)
5.	Geranyl acetone	$C_{13}H_{22}O$	194.3	Terpenoids	An important constituent of tomato and its flavour (Simkin *et al.,* 2004)
6.	Sinapic acid	$C_{11}H_{12}O_5$	224.2	Polyphenols	Antioxidant, anti-inflammatory, anticancer, cardioprotective, hepatoprotective, antidiabetic (Pandi & Kalappan, 2021)
7.	Chrysoeriol	$C_{16}H_{12}O_6$	300.3	Flavonoids	Antioxidant activity (Mishra *et al.,* 2003)
8.	Isorhamnetin 3-O-rutinoside	$C_{22}H_{22}O_{11}$	462.6	Polyphenols	Induce apoptosis in human myelogenous erythroleukaemia cells (Boubaker *et al.,* 2011)
9.	Chicoric acid	$C_{22}H_{18}O_{12}$	474.4	Polyphenols	Antioxidant, anti-inflammatory, obesity regulation and neuroprotective effects (Peng *et al.,* 2019)
10.	(+)-Catechin 3-O-glucose	$C_{21}H_{24}O_{11}$	452.4	Polyphenols	Antioxidant (Iacopini *et al.,* 2008).
11.	Coumarin	$C_9H_6O_2$	146.1	Polyphenols	Anti-inflammatory activities antioxidant, antimicrobial, anticoagulant, antitumoral (Borges *et al.,* 2005)
12.	Cymene-*p*	$C_{10}H_{14}$	134.2	Polyphenols	Antimicrobial, antioxidant, anti-inflammatory, antinociceptive, anticancer (Marchese *et al.,* 2017)
13.	6-Hydroxyluteolin	$C_{15}H_{10}O_7$	302.2	Flavanoid	Antioxidant, anti-inflammatory, antimicrobial and anticancer activities (López-Lázaro, 2009)
14.	Ellagic acid	$C_{14}H_6O_8$	302.2	Polyphenols	Antioxidant activity (Vattem & Shetty, 2005)
15.	Quercetin	$C_{15}H_{10}O_7$	302.2	Flavonoid	Antioxidant, anti-inflammatory agent (Lesjak *et al.,* 2018)
16.	Glycine- betaxanthin	$C_{11}H_{12}N_2O_6$	268.2	N-containing compounds	Antioxidant, anticancer, anti-inflammatory, and chemopreventive effects (Coy-Barrera, 2020)
17.	Leucine- betaxanthin	$C_{15}H_{20}N_2O_6$	324.3	N-containing compounds	Antioxidant, anticancer, anti-inflammatory, and chemopreventive effects (Coy-Barrera, 2020)
18.	Valine- betaxanthin	$C_{14}H_{18}N_2O_6$	310.3	N-containing compounds	Antioxidant, anticancer, anti-inflammatory, and chemopreventive effects (Coy-Barrera, 2020)
19.	Myricetin	$C_{15}H_{10}O_8$	318.2	Polyphenols	Antioxidant, anticancer, antidiabetic and anti-inflammatory activities (Semwal *et al.,* 2016)
20.	Naringenin	$C_{15}H_{12}O_5$	272.3	Polyphenols	Antidiabetic, antidepressant, immunomodulatory, antitumor, anti-inflammatory, DNA protective, antioxidant (Rao *et al.,* (2017)
21.	Pelargonidin	$C_{15}H_{11}ClO_5$	306.7	Polyphenols	Antidiabetic, antioxidant (Roy *et al.,* 2008)
22.	Protocatechuic acid 3-O-glucuronide	$C_{13}H_{14}O_{10}$	330.2	Polyphenols	Pro-apoptotic and anti-proliferative effects (Masella *et al.,* 2012)
23.	Serine-betaxanthin	$C_{12}H_{14}N_2O_7$	298.3	N-containing compounds	Antioxidant, cardiovascular, gastrointestinal, metabolic disorders, anticancer, anti-inflammatory, and chemopreventive effects (Coy-Barrera, 2020)
24.	Isorhamnetin	$C_{16}H_{12}O_7$	316.3	Polyphenols	Cardiovascular and cerebrovascular protection, anti-tumor, anti-inflammatory, antioxidation, organ protection, prevention of obesity (Gong *et al.,* 2020)
25.	Kaempferol 3-O-rutinoside	$C_{27}H_{30}O_{15}$	594.5	Polyphenols	Hepatoprotective effect (Wang *et al.,* 2015) anti-inflammatory effect (Hua *et al.,* 2021)
26.	Epicatechin	$C_{15}H_{14}O_6$	290.3	Polyphenols	Antioxidant (Iacopini *et al.,* 2008)
27.	(+)- Catechin	$C_{15}H_{14}O_6$	290.3	Polyphenols	Antioxidant, anti-inflammatory (El-Aziz *et al.,* 2012), anti-tumour (Singh *et al.,* 2011)
28.	Peonidin 3-rutinoside	$C_{28}H_{33}O_{15}$	609.6	Polyphenols	Antioxidant activity (Abdel-Aal *et al.,* 2018)

MW[a]- Molecular weight

reports on the genus *Urtica* revealed that the total phenolic and flavonoid contents of *U. hyperborea* observed in this study were found to be comparatively higher than other species like *U. dioica* (Sidaoui *et al.*, 2015). Similarly, ash content observed for *U. hyperborea* (23.33%) was found higher as compared to *U. dioica*, where it was found to be 4.73% (Radha *et al.*, 2021), 18.9% (Said *et al.*, 2015) and 20.9% (Mahlangeni *et al.*, 2015). Higher ash content observed in *U. hyperborea* further resulted in a decent amount of minerals as proven from the result of the present study. Potassium (K) was found to be higher than other minerals and estimated as 278.86 mg 100 g^{-1} in *U. hyperborea*, while a similar trend was observed in *U. dioica* as well (Paulauskienė *et al.*, 2021). *U. dioica* was found to be a rich source of minerals such as Zn, Fe and Mn (4.5, 19.64, and 4.65 mg 100 g^{-1}) as reported by Radha *et al.* (2021), however, these minerals were found to be comparatively lower in *U. hyperborea*.

From the LC-MS analysis, various compounds associated with proteins such as betaxanthin with glycine, leucine and valine were found to be present in *U. hyperborea*. It was reported that betaxanthin is dominantly found in the Chenopodiaceae family,

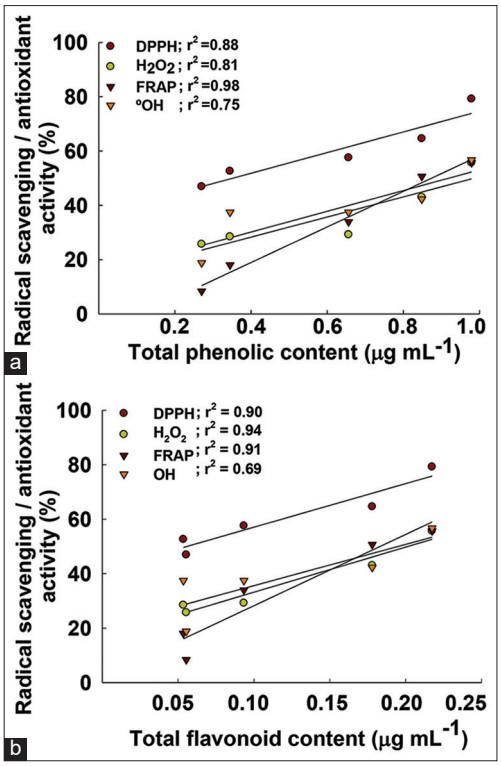

Figure 3: Relationship between a) total phenolic and b) flavonoid content of *Urtica hyperborea* with its radical scavenging/antioxidant activity

several other vegetables and fruits, including beetroots (Kugler *et al.*, 2014). They are responsible for antiradical or scavenging activities in the juices of many fruits (Sadowska-Bartosz & Bartosz, 2021). Another compound, geranyl acetone found in various fruits imparts aroma and flavour in addition to its antioxidant properties (Stobiecka, 2015). Functional food ingredients like chicoric acid which is a phenolic compound reported to be present in herbs like basil, lettuce, and chicory are associated with various health benefits such as antioxidant, anti-inflammatory, obesity regulation, and neuroprotective effects (Lee & Scagel, 2009; Peng *et al.*, 2019). Anthocyanin like peonidin-3-rutinoside is present in *U. hyperborea* which has antiradical properties and used as natural food coloration agent (Abdel-Aal *et al.*, 2018). Thus, the consumption of *U. hyperborea* enriched in these compounds may provide several health benefits.

Antioxidants provide resistance to oxidative stress by inhibiting lipid peroxidation and scavenging free radicals. The use of dietary plants rich in therapeutic antioxidants have a tendency to replace synthetic phenolic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) which are proven as harmful and responsible for deadly diseases (Sindhi *et al.*, 2013). The aqueous extracts were found to possess higher antioxidant activity as compared to the extracts prepared in methanol, ethyl acetate and petroleum ether. This revealed that most of the compounds present in *U. hyperborea* are readily soluble in water and can be easily utilized for dietary consumption. A positive correlation between the antioxidant activities of aqueous extracts with total phenolic and flavonoid contents was observed, making it safe to assume that these compounds contribute greatly to the antioxidant activity of this plant. The findings of the study were in consensus with the earlier studies reporting a positive relationship between the antioxidant activity and polyphenolic compounds (Wong *et al.*, 2006; Lizcano *et al.*, 2010). The presence of health boosting properties like carotenoids, fatty acids, polyphenolic compounds, essential amino acids, chlorophyll, carbohydrates, vitamins, and minerals are also reported in *U. dioica* (Kregiel *et al.*, 2018; Rawat *et al.*, 2020). Overall, the plant *U. hyperborea* has an immense potential to be utilized as a phytofood due to its high nutritive values and presence of several antioxidative agents.

CONCLUSION

Based on the findings of this study, it can be concluded that *U. hyperborea* has high nutritive value, and thus, can serve as a better alternative source of nutrients. Since this plant grows wild without any application of synthetic chemicals, it is an environmentally safe leafy vegetable providing health benefits to humans such as protection against oxidative stress. Owing to its immense nutritive value and health benefits, there is a need to promote its cultivation for eradicating the problems of malnutrition and food scarcity, especially under the adverse environmental conditions.

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