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Nutritional and anti-nutritional profiling of stinging nettle (*Urtica dioica* L.)

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Abstract

Stinging nettle grows profusely in the temperate regions. Such wildly growing plants were collected from different altitudes in Uttarakhand, India. They were profiled for various nutritional and anti-nutritional parameters. It is found to contain very high levels of various nutrients including vitamins, macro and micronutrients apart from protein and crude fibre. The amount of phytate, considered as an anti-nutritional factor, is found to be very low while none of them were found to contain saponins. However, another anti-nutritional parameter – oxalates, were found to be significantly higher than most of the foods. Apart, it was found to possess very high amount of anti-oxidant activity due to presence of higher amounts of polyphenols. The study also determined relationships between various parameters.

Keywords: stinging nettle, *Urtica dioica*, nutrition, anti-nutrition, profiling

Introduction

Malnutrition is lack of access to the recommended dietary allowance and could lead to stunting (inadequate height for age), wasting (inadequate weight for height). In India, stunting and wasting in children of below five years is at about 38% and 21% respectively. In children malnutrition is result of poor feeding as well as inadequate and unhealthy diet during pregnancy leading to lower IQ in later stages of development. Nutrient requirements - including those for energy, protein, minerals, vitamins and others - increase in adolescence to support adequate growth and development. With poor diet, anaemia and micronutrient deficiencies are higher. Proper functioning of endocrine system for promoting normal adolescent growth is essential and is sensitive to under nutrition [1]. In adults, its repercussions are reduced immunity, anaemia, reduction of muscle mass and function, reduced gastrointestinal function apart from psychological impact [2]. Adequate nutrition at every stage of life is not only very important for good human health but for the development of nation. The second goal of 17 sustainable development goals of the United Nation aims to "End hunger, achieve food security and improved nutrition, and promote sustainable agriculture" by 2030. The 2018 Global Nutrition Report reveals that the global burden of malnutrition is unacceptably high and now affects every country in the world. It is worrisome that despite increasing GDP and being fourth largest economy of the world, according to the global nutrition report (2020) [3], India is among 88 countries that are likely to miss global nutrition targets by 2025. Undernourishment is generally taken as malnutrition, yet the poor nutrition is also prevalent among affluent. Thus, along with financial inequality, changing food habits are also responsible for poor nutrition. In the recent past, food debate has shifted from food security to nutritional security as it is realized that food security that takes only calorie requirement into consideration does not addresses the nutritional deficiency that is the root cause of several health issues. Several research institutes have also

shifted their focus to develop varieties that are nutritionally rich. Increasing the dietary diversity, food fortification and supplementation are few of the measures to address malnutrition. Amongst these, diversifying food intake by including local wild plants that are reservoir of nutrition, is the easiest and most effective. One such plant species which is a power pack of nutrition and medicinal properties is stinging nettle, Urtica dioica. Nettle is a common plant of rich biodiversity in Uttarakhand, India. Though a native of Europe and Asia, it is now found throughout the temperate regions of the world. Urtica dioica is commonly known as stinging nettle or simply nettle. Vernacular name of the plant is Bichu Butti in Hindi and Vrishchhiyaa shaaka in Sanskrit [4, 5]. In local language of Kumaun region, nettle is also known as 'shishun', apart from having many other names in different parts of Uttarakhand. It is an herbaceous perennial flowering plant belonging to the family Urticaceae [6] of the order Rosales. It normally grows at an altitude ranging from 1200-3000 m [7] and can be found growing in common land, waste land, gardens, farmers field (as weed), hedges of the terraced fields and so on. Traditionally, as an edible item, young nettle leaves are cooked like any other green leafy vegetable after blanching. However, this rich green vegetable is disappearing fast from the traditional platter due to changing food habits despite being a rich source of protein and many essential minerals such as iron, calcium, magnesium etc. It also provides vitamins A, B1, B2, E and K. Apart, it is a rich source of many other trace elements such as Copper, Zinc, Manganese, Cobalt as required by human body [8, 12]. Also, when compared with commonly consumed spinach and parsley, nettle leaves had twice as much protein [13]. It has higher concentrations of essential amino acids than Brussels sprouts [14]. In Himalayan nettle, nutrient content was higher than the most popular and cultivated green leafy vegetables spinach and rayi [15]. Stinging nettle is an excellent plant species for addressing the food and nutritional security due to its rich nutritional content and ease of cultivation. It will be

excellent source of addressing Protein Energy Malnutrition due to rich protein content and being high in amount of energy. Despite being nutritionally rich and having host of medicinal values [16, 17], it has been considered as a weed species [18] by certain researchers. This study has been initiated to determine nutritional as well as anti-nutritional properties of stinging nettle growing widely at different altitudes in Uttarakhand.

Materials and Methods

Stinging nettle leaves were collected from plants growing at altitudes ranging from about 590 to 2600 metres in Uttarakhand, India during September to November 2020. Contrary to the existing literature, nettle was found growing at 590 metres altitude - a rare occurrence. Location and altitude of samples collected are given in Table 1. Subsequent to collection, they were air dried under shade for 72 hours followed by oven drying at 65°C for four hours. Dried material was finely ground and stored in airtight amber glass bottles at 4°C until analysis to prevent photo degradation of analytes of interest. All the samples were analysed in duplicate for nutritional and anti-nutritional Protein, Crude fibre, compounds Macro Micronutrients, Vitamins – A (as β-carotene and Retinol), Vitamin C, Phytate, Antioxidant activity, Polyphenols, Oxalates, Saponins and energy content. All chemicals and reagents used in the study were of analytical reagent grade. Reference materials used in the study were procured from reputed manufacturers as given in Table 2. For all spectroscopic and chromatographic analyses, instruments were calibrated using five standards and a blank prior to analysis of samples. Calibration curve was considered acceptable only when the correlation coefficient was ≥0.990. Brief methodology followed is described below. For gravimetric analysis, calibrated analytical balance was used.

Protein: It was determined by Kjeldahl method. Hot sulphuric acid is used for digestion to convert protein to ammonia, which is then distilled into a standardized boric acid. It is back titrated to quantitatively determine nitrogen content which is used for protein quantification. To about 2g of the sample in a kjeldahl flask 0.7 gm of Mercuric oxide, 15 gm of Potassium Sulphate and 40 mL of concentrated sulphuric acid was added along with two to three glass beads. Mixture was heated (digested) for about an hour or more until the colour of the digest is pale blue. To the cooled digestate, 200 ml of water was added. Upon cooling, granulated Zinc was added and poured down the side of the flask containing sufficient Sodium Hydroxide solution (450gm/L) before mixing the acid and alkaline layer. Mixture was distilled in a conical flask containing known volume of boric acid. Contents of the digestion flask were mixed and boiled until 150 mL have distilled into the receiver. To the distillate, 5 drops of methyl red indicator was added and titrated with standardized 0.1 N Sodium Hydroxide solution to determine nitrogen content which was multiplied by a factor of 6.25 to determine protein content.

Crude fibre: It was determined as per AOAC method 945.18. About 2g ground sample was taken in a cellulose thimble and extracted for about an hour with petroleum ether in a soxhlet extractor. Extracted material in the thimble was transferred to a 1-litre flask. 200-ml of boiling dilute sulphuric acid was added to the flask containing

sample and immediately connected to a water-cooled reflux condenser and heated. Continued boiling for exactly 30 minutes. Flask was removed and the contents were filtered through a coarse acid washed, hardened filter paper held in a funnel and washed with boiling water until the washings are no longer acid to litmus paper. Transferred the residue on the filter into the flask with 200 ml of boiling sodium hydroxide solution. Immediately connected the flask with the reflux condenser and boiled for exactly 30 minutes. Flask was removed and immediately filtered through the filter paper. Residue was thoroughly washed with hot water and transferred to a gooch crucible prepared with a thin but compact layer of asbestos. Residue was washed thoroughly first with hot water and then with about 15 ml of ethanol followed by three successive washings of petroleum ether. Gooch crucible along with contents was dried in a hot air oven at 105±1°C for three hours. Upon cooling in a desiccator, it was weighed. The process of drying, cooling and weighing was repeated until the difference between two consecutive weights is less than 1-mg. Subsequently, contents of the gooch crucible were incinerated in a muffle furnace at 550±25°C until all carbonaceous matter was burnt. Cooled the gooch crucible in a desiccator and weighed for calculating crude fibre content.

Calorific value: It was determined by using a bomb calorimeter (Make: ARICO, Model: BCM) as per the method ISO 1716. About 1g powdered sample was made into a pellet using pellet maker and oxidized in presence of excess oxygen. Liberated heat increases the water temperature in which the bomb was placed. Based on the "Burn peak time", calorific value was calculated as per the manufacturer instructions.

Elemental analysis: These were performed using Inductively coupled plasma mass spectrometry, ICP-MS (Make: Agilent Technologies, Model: 7800) as per AOAC 2015.01 To about 0.5g of ground sample taken in a dry microwave digestion vessel, 4.0 ml of supra pure nitric acid and 1.0 ml of high purity hydrogen peroxide was added. After about 30 minutes, digestion vessels were closed with caps and loaded into the microwave digestion system (Anton Paar, Multiwave GO) and digested using a temperature-time program (Table 3). After digestion, digestates were transferred to pre-cleaned 20 ml volumetric flask. Digestion vessels were rinsed with ultrapure water and rinsate was added to the digestate. Final volume was made up using ultrapure water and the digestate was transferred to tarson tubes. Calibration curve was prepared using a blank and six standards. Digestates were aspirated into ICP-MS for quantification of elements of interest. Samples were diluted using acidified ultrapure water whenever the concentration in the digestate exceeded calibration range.

Vitamin a (as retinol): To about 5g sample in a 250 ml amber coloured flat bottom flask 50 mg pyrogallol, 10 ml of 50% w/w KOH were added in the order and swirled gently to disperse the contents. Method blank is prepared in a similar manner without the sample. Contents were refluxed at 95°C and hydrolysed for 45 after boiling had started. Upon cooling to room temperature, 10 ml glacial acetic acid was added and cooled to room temperature. Contents were quantitatively transferred to a 100 ml actinic volumetric

flask and made up to volume. It was kept for 60 minutes in the dark at room temperature. Supernatant was filtered through 0.22μ PVDF syringe filter and analysed using UPLC by following the analytical conditions given in Table 4.

Vitamin a (as beta carotene): About 1g sample is dissolved 15 ml petroleum ether and the solvent layer is collected. This process is repeated until a colourless extract is obtained. Extracts are made up to a final volume of 50 ml in a volumetric flask. Extract is transferred into a Separatory funnel and washed thrice — each time with 20 ml ultrapure water. Subsequently, 25 ml of petroleum ether extract was dried in a nitrogen concentrator and reconstituted with 1 ml mobile phase. Such prepared extracts are analysed using UPLC by following the analytical conditions given in Table 4.

Vitamin C: Method as described by Prasad (2014) ^[19] with slight modifications has been followed for quantifying Vitamin C content. To about 0.2 g sample in a 10 ml volumetric flask, 0.5 ml of 0.2% EDTA was added and vortexed for 5 minutes. Subsequently, 0.5 ml of 6% M-Phosphoric acid was added and made up to volume with ultrapure water. Contents were vortexed. Supernatant was filtered through 0.22 μ PVDF syringe filter and analysed using UPLC by following the analytical conditions given in Table 4.

Antioxidant activity: Antioxidant activity was determined by using DPPH radical degradation activity method [20]. DPPH radical was prepared at a concentration of 6×10⁻⁵ M using pure methanol. 2μL methanolic DPPH solution was added into 100μL sample extracts and standard solution. The mixture was kept in dark for 20 minutes followed by measuring absorbance at 515 nm using a UV-Visible spectrophotometer (Make: Agilent Technologies, Model: Cary 60 UV-Vis). Pure methanol was used as blank solution while pure water was used as control. Antioxidant activity of samples was measured against a calibration curve which was prepared by using a series of gallic acid standard solutions.

Polyphenols: Total polyphenolic content analysis of nettle extracts was determined by Folin-Ciocalteau (FC) method [20]. Calibration standards were prepared in 80% methanol using Gallic acid. Quantification was done in terms of gallic acid equivalents. To each of 50µL nettle extract and standard solutions, 250µL FC reactive was added. After keeping the mixture in dark at room temperature for 5 minutes, 750µL of 7% Na₂CO₃ solution was added. Mixture was made-up to 5ml with pure water and kept in dark at room temperature for 2 hours following which absorbance measured at 760nm using **UV-Visible** spectrophotometer (Make: Agilent Technologies, Model: Cary 60 UV-Vis).

Phytates: Method described by Latta and Eskin (1980) [21] was used for phytate determination. To 0.2g of ground sample, 10ml of 2.4% HCl was added. A control sample without sample was also prepared and processed same as that of sample. The mixture was kept on boiling water bath for 5 min and immediately transferred to shaker incubator for 55 min maintained at 37°C and 250 rpm. Reaction

mixture was transferred to a 15 ml centrifuge vial and centrifuged at 1000g for 5 min. Supernatant was transferred to another 15 ml centrifuge vial containing 1g of NaCl; mixed well by keeping in shaker incubator at 250 rpm and 37°C for 20 min followed by 60 minute storage in refrigerator at 4°C. Mixture was centrifuged again at 1000 g for 10 min. From the supernatant 1ml was taken and added to 9 ml of distilled water (using glass pipettes). 3-ml of aliquots from each 10ml volume (Control, standards and samples) were taken in Ria vials in duplicate. To each 3 ml of aliquot 1ml of Wade reagent was added. The reaction mixture was thoroughly shaken and centrifuged at 1000g for 10 min. Supernatants were used to measure absorbance at 500nm using a UV Visible spectrophotometer. Sodium salt of phytic acid was used to prepare calibration standards.

Oxalates: Quantification of oxalates was performed by the method described by Mahlangeni et.al (2015) [22]. About 0.1g of ground sample was mixed with 50mL 3M H₂SO₄ in a conical flask and stirred for 1 h using a magnetic stirrer. The mixture was filtered, and a 25mL aliquot of the filtrate was titrated against a 0.05M KMnO₄ solution until a faint violet colour persisted for at least 30s. Each ml of titrant consumed will be equivalent to 2.2 mg oxalate. Its amount in the sample was quantified using the equation:

Oxalate in mg/100g =
$$\frac{ml\ titrant\ x\ 2.2\ x\ 50}{sample\ wt\ (g)\ x\ 25}x\ 100$$

Saponins: According to the method described by Oshomoh and Uzama (2020) [23], to 20g of ground sample in a conical flask, 100ml of 20% aqueous ethanol was added and heated over a hot water bath at 55°C for 4 h with continuous stirring. The mixture was filtered followed by residue extraction with 200ml of 20% ethanol. Combined extract was reduced to 40ml over a water bath maintained at 90°C. The concentrate was transferred to a 250ml separatory funnel to which 20ml diethyl ether was added with vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. Purification process was repeated followed by addition of 60ml of n-butanol. The combined nbutanol extract was washed twice with 10ml of 5% aqueous sodium chloride. Remaining solution was heated in a water bath until evaporation. Sample was dried in an oven to a constant weight, and saponin content calculated as a percentage.

Saponin content (%) =
$$\frac{Residue\ wt\ (g)}{Sample\ wt\ (g)} \times 100$$

Results and Discussion

All the results are reported at two significant figures except protein and crude fibre which are reported at three significant figures. Results obtained are summarised in Table 5. The results show remarkably higher protein content (27.7%), Vitamin A as Beta Carotene (2900 mg/kg) and Oxalate (3800 mg/100 g) at location G. Whereas, remarkably superior crude fibre (14.3%) was observed at location DG. Calcium (57000 mg/kg) and phosphorus (8400 mg/kg) were remarkably higher at location BD. Similarly, copper (21 mg/kg), sodium (290 mg/kg), zinc (85 mg/kg) and Vitamin A as Retinol (99 mg/kg) was observed higher at location K. However, iron (1200 mg/kg), magnesium (13000 mg/kg), manganese (93 mg/kg), potassium (17000

mg/kg), and energy value (400 Kcal/100g) were observed remarkably superior at location GM. At location VB remarkably superior selenium (0.35 mg/kg), antioxidant (96 DPPH (mg GAE/g)) and polyphenols (230 mg, as GAE/g) were observed compared to other locations. Vitamin C (440 mg/kg) and phytate (6.5 mg/100 g) was remarkably higher at location RB. Whereas, Saponins (<0.5%) and sliver (<0.01 mg/kg) was similar in all locations. However, no significant correlations were observed between studied parameters and the altitude at which they were growing indicating that the altitude didn't had any influence on the nutritional and anti-nutritional parameters. Correlation coefficients were determined between various parameters (Table 6). It is well known that phosphorous complexes with heavy metals such as Iron, Zinc, copper etc to form phytate and makes phosphorus unavailable for absorption. Phosphorus showed a highly positive correlation (0.84) with zinc while it was negatively correlated with Iron (-0.51) and a very weak correlation with copper (0.14). As expected, phytate showed a negative correlation of 0.34, 0.52, and 0.69 with all three heavy metals -Zinc, Iron and Copper, respectively. This is one possible reason for considering phytate as anti-nutritional. Phytate content of stinging nettle is significantly lower than the amount that is present in millets. It is interesting to note that there is almost no correlation (0.02) between phosphorous and phytate content even though phosphorous content ranged from 2700 to 8400 mg/kg which is possibly due to relatively insignificant amount of phytate compared to phosphorous. Despite being considered as anti-nutritional, it is interesting to note that phytate showed a moderately positive correlation of 0.53 and 0.48 with antioxidant activity and polyphenols. respectively, which are considered beneficial for human health. Its positive correlation with nutritionally desirable components supports the hypothesis that it is not necessarily an anti-nutrient, but further studies are needed for better understanding. Phytate also showed a moderate direct correlation (0.45) with another anti-nutritional parameter – oxalates indicating that these two might a directly related. It would be interesting to determine the available forms of minerals such as Iron and Zinc which normally bind with phytate. Polyphenols, which along with various other compounds are known to protect body's tissues against oxidation stress and increase antioxidant properties of a substance showed a high positive correlation of 0.90 with antioxidant activity confirming the role of polyphenols in increasing antioxidant properties of a product. Apart, there are few other significant correlations as can be seen from Table 6. Stinging nettle collected from different elevations were studied for various nutritional and anti-nutritional parameters. It is found to be nutritionally good source of protein, crude fibre, macro & micronutrients, select vitamins, polyphenols and antioxidant activity. However, it also contains oxalates and phytates, which are considered as anti-nutritional factors. However, it has higher nutritional value compared to routinely consumed food products [24] -

Rice, Wheat, Pigeon pea, Millets etc. It contains protein almost at par with Pigeon pea (22%), which is one of the most commonly consumed protein sources amongst vegetarians. It also contains significantly higher amount (11%) of crude fibre compared to commonly consumed rice or wheat which contain 1 - 2% crude fibre. Millets are considered rich source of Calcium, Iron, and Zinc. In contrast to about 400 mg/100g of calcium present in finger millet, nettle contains 5000 mg/100g while the amount of Iron (42 mg/100g) and Zinc (5.5 mg/100g) is almost twice as that present in Barnyard millet and Sorghum, respectively. Phosphorous content of stinging nettle is 490 mg/100g which is about 25% higher than that present in Pearl millet, which is regarded as a good source of phosphorous. However, phytic acid content is significantly lower in stinging nettle (5.6 mg/100g) compared to Pearl millet (172 - 327 mg/100g). Such a high ratio of phosphorous to phytic acid in stinging nettle results in higher amount of available Iron and Zinc. Despite high amounts of beneficial nutrients, Stinging nettle contains significant amounts of oxalates (3100 mg/100gm) which are known to cause kidney stones when consumed in excess. However, cutting-down on the oxalate-rich foods alone will not reduce the likelihood of kidney stone formation. Most kidney stones are formed when oxalate binds to calcium while urine is produced by the kidneys. Oxalate is naturally found in many foods including grains, legumes, fruits & vegetables, nuts etc. Few of the high oxalate foods include beets, spinach, rhubarb, sweet potatoes etc. Spinach, which is considered as a "high oxalate food" contains about 800mg/100g ^[25]. Some soy foods also contain high amounts of oxalate [26]. It is believed that oxalate content of stinging nettle will vary depending upon the harvesting stage. It is highly possible that tender leaves may contain lesser quantity of oxalate. Currently, research is underway to determine the harvesting stage of stinging nettle at which oxalate content is low so as to best reap the nutritional benefits of Stinging nettle.

Tables

Table 1: Details of stinging nettle samples collected in Uttarakhand, India

Sample ID	Location	Altitude (Metres)*
PD	Pataldevi, Almora	1570
VB	Vikas Bawan, Almora	1330
BD	Baldhoti, Almora	1620
GM	Gangnath Mandir, Almora	1600
G	Gagar, Nainital	2180
RB	Ranibagh, Nainital	590
K	Kainchi, Nainital	1270
DG	Dogaon, Nainital	955
KKH	Kunjakhadak, Nainital	2400
PKH	Pandukholi, Almora	2600

* Source: SRTM 30-meter horizontal accuracy data from Shuttle Radar Topography Mission (SRTM). https://earthexplorer.usgs.gov/

Table 2: Reference material manufacturers and catalogue numbers

Reference Material	Manufacturer	Catalogue number				
Selenium	Merck	1.19796.0100				
Potassium	SPEX CertiPrep	CLK2-2Y				
Sodium	SPEX CertiPrep	CLNA2-2Y				
Calcium	SPEX CertiPrep	CLCA2-2Y				

Phosphorus	SPEX CertiPrep	PLP9-2Y
Magnesium	SPEX CertiPrep	CLMG2-2Y
ICP Multi element Solution (23 element)	SPEX CertiPrep	QC-23
Vitamin C	Dr. Ehrenstorfer GmbH	DRE-C10303000
Vitamin A (Retinol)	Sigma-Aldrich	PHR1236
Vitamin A (Beta carotene)	Sigma-Aldrich	PHR1239
Gallic Acid	Sigma-Aldrich	G7384
Phytic Acid sodium salt hydrate	Sigma-Aldrich	P8810

 Table 3: Microwave digestor time-temperature program

Step	Power (W)	Temperature (°C)	Ramp (°C/min)	Hold Time (minutes)	Fan level
1	650	100	10	15	1
2	650	140	10	15	1
3	650	180	10	15	2

Table 4: Liquid chromatography conditions for vitamins determination

Parameter	Vitamin A (Retinol)	Vitamin A (ß Carotene)	Vitamin C
Detector	UV Vis	UV Vis	UV Vis
Column	C18, 3.0x100mm,1.8µm	C18, 3.0x100mm,1.8µm	C18, 3.0x100mm, 1.8µm
Mobile Phase	Methanol: Acetonitrile (1:1)	Acetonitrile: 2 Propanol: Ethyl acetate (40:40:20)	0.25 mM ammonium acetate
Flow Rate (ml/min)	0.50	0.25	0.20
Injection Volume (µl)	10	5.0	20
Column Temperature (°C)	40	35	30
Sampler Temperature (°C)	15	15	10
Detector Wavelength (nm)	325	450	254
Run Time (min)	10.0	5.0	7.0
Mode	Isocratic	Isocratic	Isocratic

Table 5: Nutritional and anti-nutritional values of stinging nettle leaves

Parameter	Units	PD	VB	BD	GM	G	RB	K	DG	KKH	PKH		
			ic Proxii										
Protein	%	25.0	20.7	23.1	23.5	27.7	27.5	25.9	26.4	27.0	21.0		
Crude Fibre	%	9.24	8.96	10.5	10.5	12.4	9.43	13.5	14.3	13.3	12.5		
Energy	kcal/100g	370	340	350	400	390	340	390	390	340	340		
Macro-nutrients													
Calcium	mg/kg	41,000	54,000	57,000	54,000	56,000	55,000	49,000	39,000	54,000	37,000		
Magnesium	mg/kg	7,300	6,800	8,200	13,000	7,600	12,000	6,500	6,100	10,000	6,400		
Phosphorus	mg/kg	2,700	7,000	8,400	3,200	5,000	3,600	6,700	5,700	3,400	2,900		
Potassium	mg/kg	15,000	14,000	14,000	17,000	14,000	9,100	13,000	16,000	12,000	16,000		
Sodium	mg/kg	26	84	85	84	15	37	290	110	13	27		
		Mi	cro-nutri	ients									
Iron	mg/kg	590	230	250	1,200	240	310	380	180	270	520		
Copper	mg/kg	15	12	14	17	13	14	21	16	11	14		
Manganese	mg/kg	43	53	61	93	52	46	47	31	49	66		
Zinc	mg/kg	45	71	79	48	45	50	85	49	32	43		
Selenium	mg/kg	0.22	0.35	0.27	0.32	0.053	0.045	0.070	0.080	0.23	0.22		
Silver	mg/kg	< 0.01	0.011	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01		
			Vitamin										
Vitamin A as Retinol	mg/kg	89	77	79	55	64	35	99	77	44	70		
Vitamin A as Beta Carotene	mg/kg	2,600	1,400	2,000	400	2,900	940	610	900	490	310		
Vitamin C	mg/kg	85	100	65	110	110	440	60	180	170	240		
		A	ntinutrie										
Phytate	mg/100 g	4.4	5.9	5.7	4.9	6.0	6.5	4.3	6.1	6.0	6.0		
Oxalate	mg/100 g	3600	3000	3700	2000	3800	3200	2000	3300	2700	3200		
Saponins	%	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50		
			Others										
Antioxidant Activity	DPPH (mg GAE/g)	36	96	32	44	59	65	46	92	52	50		
Polyphenols	mg, as GAE/g	70	230	94	68	110	130	82	160	78	78		

Table 6: Pearson correlation matrix between various parameters studied

	Protein	CF	Ca	Mg	P	K	Na	Fe	Cu	Mn	Zn	Se	Vit. A (Ret.)	Vit. A (BC)	Vit. C	AoA	PP	Phytate	Oxalate
Protein	1.00																		
CF	0.37	1.00																	
Ca	0.21	-0.32	1.00																
Mg	0.23	-0.33	0.55	1.00															
P	-0.18	0.05	0.36	-0.41	1.00												·		

K	-0.45	0.19	-0.46	-0.25	-0.06	1.00													
Na	-0.01	0.31	-0.02	-0.27	0.53	0.04	1.00												
Fe	-0.26	-0.25	-0.06	0.56	-0.51	0.52	-0.01	1.00											
Cu	0.09	0.27	-0.24	-0.10	0.14	0.27	0.84	0.36	1.00										
Mn	-0.49	-0.27	0.29	0.56	-0.19	0.42	-0.09	0.80	0.07	1.00									
Zn	-0.31																		
Se	-0.78	-0.47	0.11	0.18	0.04	0.38	-0.21	0.42	-0.31	0.59	0.06	1.00							
Vit. A(Ret.)	-0.32	0.12	-0.41	-0.77	0.48	0.46	0.60	-0.10	0.53	-0.23	0.61	0.05	1.00						
Vit. A(BC)	0.19	-0.35	0.20	-0.28	0.21	0.03	-0.30	-0.28	-0.25	-0.27	0.07	-0.11	0.27	1.00					
Vit. C	0.25	-0.11	-0.07	0.36	-0.44	-0.58	-0.36	-0.13	-0.25	-0.16	-0.40	-0.39	-0.70	-0.34	1.00				
AoA	-0.04	0.08	-0.10	-0.26	0.21	-0.12	0.00	-0.44	-0.24	-0.41	-0.02	-0.11	-0.10	-0.13	0.25	1.00			
PP	-0.21	-0.24	0.12	-0.28	0.46	-0.22	0.01	-0.51	-0.32	-0.36	0.26	0.06	0.01	0.09	0.14	0.90	1.00		
Phytate	0.09													-0.08	0.64	$0.\overline{53}$	0.48	1.00	
Oxalate	0.07	-0.20	-0.10	-0.37	0.10	-0.06	-0.60	-0.53	-0.55	-0.41	-0.18	-0.17	0.02	0.74	0.12	0.06	0.20	0.45	1.00

CF-Crude fibre; Ret - Retinol; BC - Beta-carotene; AoA Antioxidant Activity; PP-Polyphenols

Conclusions

The study concludes that stinging nettle is nutritionally very rich compared to most commonly consumed commodities. Apart, it doesn't contain anti-nutritionals such as phytates and saponins though the amount of oxalates can be a concern. Studies are in progress to determine oxalate levels at different stages of plant growth to identify ideal stage at which stinging nettle will contain least amount of oxalates. It might also be possible to reduce oxalate content by certain food processing techniques which need to be further explored.

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