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Proximate analysis, elemental profiling and antioxidant activities of *Tupistra nutans* wall grown in Sikkim Hills, India

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Abstract
Tupistra nutans Wall has been the plant of economic importance in Sikkim located at North eastern hill region of India. The inflorescence is cooked as vegetable or processed as pickle for consumption. Though not fully validated, inflorescence of the plant is reported to have anti diabetic properties and fetch higher price in the market. The present investigation revealed substantially high nutrient profile with 22.59% Crude fat, 6.55% crude fibre, 0.36% total protein, 0.43% ascorbic acid. In analyzing essential and non-essential element, potassium, phosphorus and magnesium were found in abundance. Potassium: sodium ratio was high. *Tupistra nutans* Wall, can be considered as one of the most potent plants to for exploring it as nutraceutical.

Keywords: Sikkim, nutrient, underexploited

Introduction

The North Eastern Hill region of India is a biodiversity rich part of India with abundance of endemic flora and fauna. The diverse communities inhabiting the area have been utilizing different endemic flora as food with high degree of acceptance. They are also used as an ingredient in folk and traditional medicine. One of such flora of Sikkim and Darjeeling Himalayas in India is *Tupistra nutans* Wall (Commonly known as *Nakima* vernacular: Nepali). The plants belonging to Liliaceae family is found growing throughout Sikkim in the altitude range of 3000-7000 ft from Mean Sea Level. In India, other than Sikkim, it is found growing in Meghalaya (Roy *et al*., 2017) [21]. There are reports of availability of this species in Nepal, Bhutan, Myanmar, China, Laos, Thailand, Malaysia and Vietnam. Its flowering spikes (inflorescence) is of most economic importance which is used as a vegetables or processed into pickle by local population. It is highly priced vegetable with considerably high nutrient content like Na, K and Ca (Rai *et al*. 2005) [19]. Its market value was reported to be Rs. 60/ Kg in 2004 (Sudriyal and Sundriyal, 2004) [22] which is never declined. Now it is sold at the exuberant price in the range of Rs. 150- Rs. 200/Kg.

The crop is gaining popularity amongst the consumer because of its medicinal properties. The powdered root and flower decoction (Hussain and Hore [10], 2007, Idrisi *et al*. 2010) [11], dry flower of its inflorescence (Chettri *et al*. 2005) [7] are taken to control diabetes and tonic to relieve pain (Chettri *et al*. 2005, Idrisi *et al*. 2010) [7, 11]. People in Sikkim hills have developed the taste and have gained enough expertise in its organic cultivation. Though underexploited at the national level, it is one of the commercial crops of Sikkim state and has high potential to be included in the diets of people across the globe. For commercial exploitation of underutilised fruits or vegetables, knowing their nutrient content and health benefits is of prime importance. There has been considerable research report in some aspects of the crops viz. production, nutrient management etc. but, scanty report are available where the proximate content, mineral content, phytochemicals and antioxidant profile were searched. This report is one of the maiden for complete nutritional and bioactive profile of *Tupistra nutans* Wall grown in Sikkim.

Material and methods

Flowering spikes as a sample were collected from all the four districts of Sikkim. Five villages from each district were chosen for sampling. The samples from different villages in each district were pooled together to make the final sample size of 1 kg. The collected samples were placed in a polyethene bag to prevent loss of moisture and transported to the Nutrition and Ionome laboratory of the Department of Horticulture, Sikkim University within 24 hours. Samples were washed 2-3 times with running tap water and once with sterile double distilled water and wiped to dry all the water around it as recommended by Badau *et al*. (2013) [5] and Pillai and Nair (2013) [18]. For elemental analysis using ICP- MS, 200g of the sample was dried in an oven at 35 °C and was blended into powder and stored at room temperature under a dry condition in an airtight plastic container. The remaining sample was stored at -20 ^{0}C for further analysis. All the analysis were replicated thrice and data were presented as mean ± SE.

Nutritional analysis

The total carbohydrates, total ash, crude fiber, crude fat, protein and moisture Content were determined using standard methods of the Association of Official Analytical Chemists (AOAC, 2005) [2]. Moisture content was determined by moisture analyser. About 2 g of the blended sample was transferred to a previously dried plate for standard drying at 130 ^{0}C temperature and expressed in weight percentage by measuring the weight loss after drying. About 2 g of the sample placed in a previously weighed crucible and transferred in a furnace (600 ^{0}C) for 2 hours for total ash. The crucible was then removed and cooled. The total ash was expressed as a percentage of the initial weight. Crude fat was determined using Soxhlet extractor. Protein content was determined by Lowry's method using Perkin Elmer, Lamb 35 UV/VIS spectrophotometer.

Crude Fiber Determination

A 2gm of the fresh sample was transferred into a 750 ml Erlenmeyer flask and 0.5 g of asbestos was added. 200 ml of boiling 1.25% H_2SO4 was added immediately and the flask was set on a hot plate and the condenser was connected. After 30 minutes the flask was removed and its content was immediately filtered through a clean linen cloth. The sample was then washed repeatedly with a large volume of water until the washings were no longer acidic. 200 ml of 1.25% of boiling NaOH was added to the filtrate. It was also boiled for 30 minutes and washed several times until it was no longer basic. The residue was then transferred into a weighed crucible. The crucible and its content were dried and ashed for 30 minutes. The cooled crucible was weighed and result was expressed in percentage.

Total Carbohydrate Determination

Total carbohydrate was determined by using Anthrone reagent. 0.1 g of the representative sample was hydrolyzed for 3 hours with 5ml of 2.5 N HCl and cooled down to normal temperature. After neutralisation of acid using sodium carbonate, volume was made to 100ml. A suitable aliquot of the sample was taken and 4ml of Anthrone reagent was added, which was heated for 8 minutes in a water bath and cooled rapidly. Total carbohydrate content was determined by taking absorbance at 630 nm (Perkin Elmer Lambda 35 UV/VIS spectrophotometer) and expressed as a percent.

Total Starch Determination

The total starch content was determined using anthrone reagent. 500 mg of the representative sample was homogenised in hot 80% ethanol to remove sugars until it doesn't give colour with anthrone reagent. The residue was mixed with 5 ml water and 6.5 ml perchloric acid and centrifuged. A suitable volume of supernatant was taken and 4ml of anthrone reagent was added. The mixture was heated for 8 minutes in a water bath and the cooled mixture was subjected for recording absorbance at 630 nm and expressed as a percent.

Estimation of ascorbic acid

Ascorbic Acid content was determined by 2,6-dichlorophenolindophenol visual titration method. 2.5 g of the representative sample was mixed with 3% HPO_3 and made upto 100ml with HPO_3 and centrifuged to obtain a clear solution. A suitable volume of an aliquot of an HPO_3 extract of the sample was titrated with standard dye to a pink end point and ascorbic acid content was expressed in percentage.

Multi-elemental profiling

Dried sample was subjected to microwave digestion with multi-wave digestion system (Anton Par Multi-wave 3000, India) as per following conditions *viz.* power- 1200 W; IR - 190 ^{0}C; rate- 0.3 bar sec^{-1}; ramp- 5 minutes; hold – 7 minutes; sample size- 0.1 g; acids used- HNO_3 - 5 ml and HCl- 1 ml). Digested sample was then cooled and the volume was made up to 50ml with DDW. Analysis of the samples was carried out with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, Nex ION 300 X, USA) system with cross flow nebuliser. The instrument was calibrated using standard reference material (Peach leaves- NIST, 1547. Digested sample was analysed for the ionic constitution using multi elements standards solution.

Extract of the samples

2.0 g of powdered samples of Nakima was mixed with methanol (80%) in a ratio of 1:25 and extracted in a Soxhlet apparatus for 5hr. This process was repeated for 3 times and all extracts were combined. The combined extracted sample was first concentrated using rotatory evaporator. Extracted sample was further concentrated using Eppendorf Speed Vac/Concentrator Plus at 40 ^{0}C and stored at -20 ^{0}C. The concentrated sample was used as a sample extract for estimation of phytochemicals and antioxidant activity.

Antioxidant activity

Free radical scavenging activity using DPPH assay

DPPH (2, 2-diphenyl picrylhydrazyl) assay is based on the scavenging ability of antioxidants towards the stable radical DPPH according to Yu *et al*. (2002) [23] and Aoshima *et al*. (2004) [3] with slight modification. A 2.0 mL of sample extract was added to 5.0 ml of DPPH (0.1 mmol l^{-1}) solution in 95% methanol and vortexed. After 30 minutes change in the absorbance of the sample extract was measured at 517 nm with the help of UV Vis spectrophotometer. The result was expressed as a percentage of inhibition of DPPH which was calculated by the following formula:

Inhibition (%) = $100 \times (A0 - A) / A0$

where, A0 was the beginning absorbance at 517 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 517 nm. Methanol (95%) was used as a blank.

Data Analysis

Data of all measurements were obtained in triplicate and expressed as the mean ± Standard Error (SE). The data were statistically analysed using JMP Pro 11. One-way analysis of variance (ANOVA) was used to evaluate the experimental data. The residual plots were inspected to confirm data confirmed to normality.

Results and Discussion

Nutritional composition

The proximate composition of *Tupistra nutans* Wall collected from different districts of Sikkim is represented in Table 1. Highest value values for all the proximate parameters were obtained in the samples collected from west Sikkim, though there was no significant difference amongst the district. The highest moisture level, ash content, fat and fibre of the test sample was estimated to the tune of 80.68, 3.7, 22.59 and 6.5 percent, respectively (Table 1). The highest protein, ascorbic acid and carbohydrate composition in this experiment was 0.36, 0.43 and 41.83 percent, respectively (Table 1). Low moisture content at harvesting attributes for long storage (Onyeike *et al*., 1995) [17] and is advantageous for drying the products for future use. The highest percentage of ash represents the inorganic content of the vegetable, which is 3.70 percent in Nakima (Table 1). The fat and fibre content in west Sikkim sample was 22.59 and 6.5 percent, respectively and was highest amongst all the sample analysed. High fibre content and low fat recorded during the present investigation substantiate the use of *Tupistra nutans* Wall (*Nakima*) as desired food for obese people. High fibre content may aid to absorption of trace elements in the gut and reduces the absorption of cholesterol (LeVeille and Sauberlich, 1966) [13]. Thus, fibre reduces the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Hanif *et al*., 2006, Jimoh *et al*., 2010) [9, 12]. The protein content was recorded to be 0.36 percent which is much higher than other common vegetables. Carbohydrate content of 41.83% (Table 1) is appreciable. The presence of significant level of important nutrients like carbohydrate, starch, protein, and fat corroborates to support *Nakima* as nutritionally valuable and healthy ingredient to promote health. The high content of Ascorbic acid in plants might link with higher free radical scavenging activity and health benefits like anti carcinogenic and anti-atherogenic (Lui *et al*., 2008) [15].

Table 1: Proximate nutrient content of *Tupistra nutans* Wall

Proximate Composition	***Tupistra nutans* Wall samples collected from different districts**			
	West Sikkim	**East Sikkim**	**North Sikkim**	**South Sikkim**
Moisture (%)	81.68±0.03	80.18±0.03	80.08±0.03	79.98±0.04
Ash content (%)	3.70±0.08	3.65±0.05	3.68±0.08	3.61±0.09
Crude Fat (%)	22.59±0.04	20.51±0.14	21.59±0.29	20.59±1.14
Crude Fibre (%)	6.55±0.10	6.25±0.25	6.05±0.71	6.16±0.81
Crude Protein (%)	0.36±0.02	0.30±0.05	0.37±0.06	0.34±0.09
Carbohydrate (%)	41.83±0.79	43.03±1.91	41.11±0.91	40.81±1.04
Total Starch (%)	0.009±0.001	0.01±0.001	0.01±0.001	0.02±0.001
Ascorbic Acid (%)	0.43±0.07	0.48±0.09	0.41±0.05	0.42±0.08

Data presented are Mean ± Standard error

Elemental profile

The result of the multi-elemental profile of the test samples is presented in Table 2. Among all elements, potassium was the most abundant element followed by phosphorus and magnesium. Other elements, in descending order by quantity were Cu, Fe, Mn, Ca, Mo and Zn. Non-essential elements was also accumulated in the infloresence. Sodium was abundant and others in descending order by quantity, were Ga, Li, Co, U, Ag, Bi, Cs, Ce, and Be. *Nakima* was found to be abundant in potassium content. The higher content of potassium is associated with increased iron utilisation and is also beneficial for people suffering from hypertension (Adeyeye, 2002) [1]. The sodium content was low in comparison with potassium. Thus, a lower sodium/potassium ratio makes *Nakima* a recommended food material to reduce the risk of elevated blood pressure. Calcium is an essential element not only for children but also for lactating, pregnant and menopausal women. The presence of higher level of calcium in *Nakim*a might be useful in preventing diseases such as osteoporosis. Iron is useful in the prevention of anaemia and other related diseases (Arinathan *et al*., 2003) [4] which was present in a very good amount in *Nakima.* Manganese acts as a cofactor in some enzymes and also plays a role in energy production and in supporting the immune system. Zinc is useful for protein and nucleotide synthesis, normal body development and recovery from illness (Oluyemi *et al*., 2006) [16].

Table 2: Elemental profile (mg/100g of dry weight) of *Tupistra nutans* Wall

Essential elements	
Potassium (K)	561.61
Calcium (Ca)	11.30
Magnesium (Mg)	86.82
Phosphorus (P)	110.88
Iron (Fe)	42.33
Manganese (Mn)	26.24
Zinc (Zn)	2.38
Copper (Cu)	52.63
Molybdenum (Mo)	2.70
Non-essential elements	
Sodium (Na)	19.06
Cobalt (Co)	0.52
Silver (Ag)	0.27
Beryllium (Be)	0.10
Bismuth (Bi)	0.17
Caesium (Cs)	0.17
Gallium (Ga)	3.11
Lithium (Li)	1.612

Cobalt plays a role in the metabolism of vitamin B_{12} and increases its absorption; it also functions as an activating ion in some enzymes (Dangoggo *et al*., 2011) [8]. Boron assists and improves retention of minerals like calcium, magnesium, and phosphorus; necessary for brain function, memory and alertness, as well as for the activation of vitamin D (Cabrera *et al*., 1996) [6]. The presence of the above-mentioned macro and micro nutrients in *Nakima* might be essential in preventing diseases related to malnutrition.

Antioxidant activity
In the present study, *Tupistra nutans* Wall was investigated for antioxidant activity by radical scavenging (DPPH) effects, The result of antioxidant activity is presented in Table 3. Scavenging capacity of the $DPPH^+$ of Nakima (878.90%), recorded on the samples collected from west sikkim was very high. Samples from East Sikkim recorded lowest (68%) and A most reactive form of reduced dioxygen *i.e.* Hydroxyl radicals originated from Fenton reaction causes oxidatively induced breaks in DNA and damages cells *in vivo* (Rollet Labelle *et al*., 1998) [20].

Table 3: Antioxidant activities of Tupistra nutans L. collected from different place

Location of sample collection	DPPH Assay (%)
West Sikkim	78.90 ± 0.98^a
East Sikkim	68.00 ± 2.66^c
North Sikkim	76.90 ± 2.18^a
South Sikkim	72.90 ± 3.90^b

Conclusion
From the present study, it can be concluded that Tupistra nutans Wall (*Nakima*) contain an appreciable amount of all the nutrients and minerals, as well as a rich source of natural antioxidants which indicates it should be included in day to day food basket for a healthy life.

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