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Analysis of nutrient content of five non-conventional vegetables of Assam, India

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1. Introduction

The multiple roles of traditional wild vegetables as both food and medicinal sources have been widely documented. Plant foods are rich in macro- and micro-nutrients as well as bioactive compounds, and have been recognized as a major source of dietary antioxidant with therapeutic benefits [1, 2]. Leafy vegetables are the least expensive sources of a number of nutrients [3]. They are rich source of minerals, fibres, vitamins, carbohydrates, proteins and amino acids, both essential and non-essential, which provides important nutrient to human health [4]. Green leafy vegetables are also recognized for their color, flavor and medicinal values. Plant produces a wide range of redox-active secondary metabolites (i.e. antioxidants) such as ascorbic acids, carotenoids, polyphenols and enzymes with antioxidant activity, which protects the cell from oxidative damages [5, 6]. Therefore epidemiological studies have consistently shown that there is a significant positive correlation between the consumption of fruits and vegetables and reduction in the risk of heart diseases mortalities, common cancers, and other degenerative diseases as well as aging [7, 8].

The most serious concern for the survival of humanity is the ever-increasing gap between population growth and food supply [9]. In order to arrest the situation discovery of new sources of plant food have generated great deal of interest among scientist for protein and non-protein calories to human diet [10, 11]. Malnutrition is of major concern for many developing nations. Integrating wild vegetables into diet has been promoted as one of the most practical, sustainable and inexpensive ways to overcome malnutrition since such vegetables are efficient sources of numbers of nutrients [12, 13]. In developing countries numerous types of wild edible plants are exploited as a source of food which provides an adequate level of nutrition to the diet [14].

Assam is one of the richest states of India in flora and fauna. The people of this region are traditionally using different types of wild plants as food and as folk medicine since ancient time. However there is a very little information on the nutrient composition of these wild vegetables. This study was therefore undertaken to assess some nutrient composition of some wild vegetables of Assam.

2. Materials and methods

2.1. Plant materials

Fresh wild plant materials, *Alocasia odora, Typhonium trilobatum, Nymphaea nouchali, Solanum nigram* and *Alternanthera philoxyroydes* were collected from Darrang district Assam and were identified by a taxonomist. Stem part was used for *Alocasia odora and Nymphaea nouchali* while for the other three vegetables leaf part was used.

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They were washed with distilled water and non-edible portions separated and discarded. Moisture was estimated immediately after collection of each sample.

2.2. Estimation of dietary fibre

Dietary fibre content was determined by an enzyme-gravimetric method according to Asp et al. [15]. The method gives sum of insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) as total dietary fibre (TDF).

2.3. Analysis of dietary fibre constituents

Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Hemicellulose, Cellulose, Lignin were determined by method described by Van Soest and Wine [16] modified McQueen and Nicholson [17]. Total pectin was determined by the method Ranganna [18].

2.4. Estimation of proximate composition

AOAC methods [19] were adopted for the determination of moisture, ash, protein ($N \times 6.25$) and lipids content. Moisture content was determined by a gravimetric method by drying the fresh samples to constant weight at 95–100°C in an oven, cooled in a desiccator and weighted. The percentage loss in weight was expressed as percentage moisture content. Ash content was determined by incineration of weighed quantity of vegetables at 550 °C. The weight of the residue after incineration was expressed as percentage ash content. Crude protein was determined by estimating the nitrogen content using the micro Kjeldahl method. Crude lipid was determined by the Soxhlet extraction of a known weight of sample with petroleum ether (bp 40–60 °C). Total carbohydrates were evaluated by the Anthrone method [20].

2.5. Estimation of minerals

For analysis of elements (Ca, P, Na, K, Mg, Zn, Fe) 0.5 g of the calcined ash of each sample was digested with triple acid mixture (1:2:4) HCl-HNO_3-H_2SO_4 to dryness. The residue was dissolved in 2 N HNO_3, the insoluble portion was filtered out with Whatman-42 filter paper; the filtrate was made up to 50 ml and was preserved for analysis of the metals. The concentration of Fe, Mn, Zn, Mg, and Ca was measured by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 200). Na and K were analyzed by flame photometry. Phosphorus was analysed by colorimetric method using molybdovanadate reagent [19].

2.6. Preparation of extracts

The extract was prepared as described by Proestos et al. [21] with some modification. A 0.5 g of homogenized plant material was mixed with 50 ml of aqueous methanol (80:20 v/v) containing 0.1% HCl in a round bottom flask. The mixture was stirred for 30 minutes and then sonicated for 15 minutes. After sonication the mixture was bubbled with nitrogen and refluxed in a water bath at 90 ºC for 2 hours. After cooling it was concentrated in a rotary evaporator and then lyophilized and dried. Dried material was dissolved in the same solvent. The extract was purged with nitrogen and kept in a deep freezer until analyzed.

2.7. DPPH free radical scavenging assay

The radical scavenging activity for the DPPH assay was calculated by the previously described method by Papageorgiou et al. [22]. The radical activity was calculated by the following formula

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B]$$

where, A_A and A_B are the Abs of test sample and control respectively.

The sample providing 50% inhibition, IC_{50} value was calculated from graph plotting % inhibition against extract concentration (200, 100, 50, 25, 10 and 5 mg/L).

2.8. ABTS cation scavenging assay

The ABTS assay described by Re et al. [23] was used to determine antioxidant capacity of the plant extracts. A calibration curve was constructed by using trolox as standard and the antioxidant capacities were expressed as μM trolox/g of dry weight (dw).

2.9. Ferric reducing antioxidant power (FRAP) assay

Total antioxidant capacity was also determined using FRAP assay by Benzine and Strain [24]. A calibration curve was constructed using $FeSO_4.7H_2O$ solution and results were expressed as μM Fe(II) per g dry weight (dw).

2.10. Determination of total phenolic content

Total phenolic content was estimated using Folin-Ciocalteu colorimetric method [22]. Results are expressed as mg of Gallic acid equivalent (GAE)/100 g dry weight (dw).

2.11. Determination of total flavonoid

Total flavonoid content was estimated by colorimetric method using quercetin as reference [25]. The flavonoid content was expressed as mg of quercetin equivalent (QE)/100 g of DW.

2.12. Determination of Vitamin C content

Vitamin C content of the vegetables was determined using the colorimetric method [20].

3. Results and discussion

Dietary fibre plays a significant role in human health. Based on their simulated intestinal solubility, dietary fibres are either classified as soluble dietary fibre (SDF) or insoluble dietary fibre (IDF). IDF includes lignin, cellulose, and hemicelluloses; SDF includes pectins, beta-glucans, galactomanan gums, and a large range of nondigestible oligosaccharides including inulin [26]. IDF seems to be related to the intestinal regulation, whereas SDF is associated with decrease of cholesterol levels and absorption of intestinal glucose [27]. Low blood cholesterol levels have been associated with a reduced risk of coronary disease. As presented in the Table 1, TDF content of the vegetables ranged from 3.12 to 4.82 g/100 g whereas IDF and SDF ranged from 2.62 to 4.21 g/100 g and 0.41 to 0.65 g/100 g respectively. The highest TDF value as well as highest IDF was found in *Solanum nigram* which contains 87% IDF of its TDF. The IDF/SDF ratios in the vegetables studied ranged from 5.1:1 in *Alocasia odora* to 7.1:1 in *Solanum nigram* which also have highest SDF and low SDF respectively. Lower the IDF/SDF ratio, the higher is the dietary fiber-phenolic compound bio accessibility [28]. However, the IDF/SDF ratio is important for both, dietary and functional properties. Table 2 presents the dietary fibre constituents of the vegetables. Neutral detergent fibre (NDF) and acid detergent

Table 1
IDF, SDF and TDF (g/100 g) and IDF/SDF ratio of different vegetables. All data are mean ± SD of three determinations ($n = 3$)

Sample	IDF	SDF	TDF	IDF/SDF
Alocasia odora	2.71 ± 0.21^{a}	0.52 ± 0.21^{a}	3.17 ± 0.78^{a}	5.1:1
Typhonium trilobatum	3.84 ± 0.64^{b}	0.64 ± 0.06^{b}	4.11 ± 0.65^{b}	6.3:1
Nymphaea nouchali	2.62 ± 0.44^{a}	0.41 ± 0.02^{c}	3.12 ± 0.32^{a}	6.5:1
Solanum nigram	4.21 ± 0.32^{c}	0.62 ± 0.07^{b}	4.82 ± 0.21^{c}	7.1:1
Alternanthera philoxyroydes	4.14 ± 0.41^{c}	0.65 ± 0.04^{b}	4.71 ± 0.32^{c}	6.8:1

Mean values in a column with different letters are significantly different at $P < 0.05$.

Table 2
Dietary fibre composition of vegetables. All data are the means ± SD of triplicate experiment

Samples	NDF	ADF	Hemicellulose	Cellulose	Lignin	Pectin
A. odora	2.27 ± 0.12^{a}	1.52 ± 0.05^{a}	0.82 ± 0.01^{a}	1.13 ± 0.02^{a}	0.43 ± 0.01^{a}	0.23 ± 0.01^{a}
T. trilobatum	3.97 ± 0.06^{b}	2.72 ± 0.10^{b}	1.11 ± 0.02^{b}	1.82 ± 0.04^{b}	0.47 ± 0.01^{a}	0.30 ± 0.01^{b}
N. nouchali	3.10 ± 0.21^{c}	1.62 ± 0.02^{a}	0.65 ± 0.01^{a}	1.44 ± 0.02^{c}	0.32 ± 0.02^{b}	0.22 ± 0.02^{a}
S. nigram	4.22 ± 0.17^{d}	2.52 ± 0.7^{c}	1.23 ± 0.01^{b}	2.00 ± 0.05^{b}	0.52 ± 0.01^{a}	0.34 ± 0.02^{b}
A. philoxyroydes	5.17 ± 0.17^{e}	2.15 ± 0.02^{d}	0.97 ± 0.01^{c}	1.76 ± 0.02^{b}	0.57 ± 0.03^{a}	0.29 ± 0.01^{b}

Mean values in a column with different letters are significantly different at $P < 0.05$.

Table 3
Proximate composition of vegetables (g/100 g). All data are the means ± SD of triplicate experiment

	Moisture	Ash	Crude protein	Lipids	Carbohydrate
A. odora	90.8 ± 1.3^{a}	2.13 ± 0.27^{a}	2.21 ± 0.11^{a}	0.12 ± 0.01^{a}	4.13 ± 0.02^{a}
T. trilobatum	87.2 ± 1.7^{b}	2.64 ± 0.08^{b}	2.13 ± 0.11^{a}	0.10 ± 0.00^{a}	6.30 ± 0.03^{b}
N. nouchali	92.2 ± 1.4^{a}	1.81 ± 0.07^{c}	2.63 ± 0.07^{b}	0.09 ± 0.00^{a}	4.18 ± 0.05^{a}
S. nigram	86.3 ± 0.8^{b}	2.55 ± 0.11^{b}	3.34 ± 0.26^{c}	0.07 ± 0.00^{a}	6.46 ± 0.05^{b}
A. philoxyroydes	85.7 ± 2.2^{b}	2.61 ± 0.12^{b}	2.74 ± 0.04^{b}	0.13 ± 0.01^{a}	7.14 ± 0.10^{c}

Mean values in a column with different letters are significantly different at $P < 0.05$.

fibre (ADF) content of vegetables ranged from 2.27 to 5.17 and 1.52 to 2.72 g/100 g respectively. Hemicellulose, cellulose, lignin, pectin content of these vegetables varied from 0.65 to 1.23, 1.13 to 2.00, 0.32 to 0.57 and 0.23 to 0.34 g/100 g respectively.

The proximate composition of the vegetables, as presented in the Table 3, shows the levels to have comparable levels of nutrients reported in literature. The Moisture content of the fresh vegetables ranged from 85.7–92.2 g/100 g. Ash content of the vegetables ranged between 1.81 g/100 g in *N. nouchali* and 2.64 g/100 g in *T. trilobatum*. Higher ash content present in all these vegetables indicates that they are good source of nutritionally important minerals. Protein contents are in the range of 2.13 g/100 g in *T. trilobatum* and 3.34 g/100 g in *S. nigram*. Lipid content in all the vegetables is low, and varies from 0.07 g/100 g in *S. nigram* and 0.13 g/100 g in *A. philoxyroydes*. Carbohydrate concentration ranged from 4.13 g/100 g (*A. odora)* to 7.14 g/100 g (*A. philoxyroydes*).

The results of the analysis for mineral content of the green leafy vegetables are presented in the Table 4. The sodium concentrations of the vegetables range from 6.7 mg/100 g (*T. trilobatum*) to 15.7 mg/100 g (*S. nigram*).The potassium content of the vegetables was found to be higher than that of the sodium content which is in diet necessary to control blood pressure. In the raw samples potassium contents ranged from 132.8 mg/100 g (*A. philoxyroydes*) to 212.4 mg/100 g (*S. nigram*). Consumption of too much Na and less amount of K contributes to high prevalence of hypertension [29]. Calcium along with Phosphorus is important for growth and healthy maintenance of bones, teeth, muscles and blood [30]. Calcium is the most abundant macro-minerals in the studied vegetables, ranging from

Table 4
Mineral content of the vegetables per 100 g of edible portion. All data are the means ± SD of triplicate experiment

Sample	Na	K	Ca	Mg	P	Fe	Zn
A. odora	7.8 ± 0.5^{a}	142.7 ± 5.1^{a}	478.7 ± 15.1^{a}	49.7 ± 2.1^{a}	37.8 ± 0.7^{a}	14.7 ± 1.4^{a}	0.78 ± 0.1^{a}
T. trilobatum	6.7 ± 0.7^{a}	134.9 ± 3.7^{b}	427.3 ± 20.2^{b}	97.1 ± 2.7^{b}	47.8 ± 3.8^{b}	17.8 ± 3.8^{b}	0.47 ± 0.0^{b}
N. nouchali	11.4 ± 1.1^{b}	146.5 ± 3.7^{a}	203.4 ± 12.2^{c}	27.6 ± 0.9^{c}	31.3 ± 3.7^{c}	5.2 ± 0.7^{c}	0.27 ± 0.0^{c}
S. nigram	15.7 ± 2.1^{c}	212.4 ± 5.8^{c}	278.7 ± 7.9^{d}	178.8 ± 8.8^{d}	44.4 ± 2.7^{d}	12.7 ± 1.7^{d}	0.54 ± 0.2^{d}
A. philoxyroydes	9.3 ± 1.3^{d}	132.8 ± 2.2^{b}	178.8 ± 3.7^{e}	167.1 ± 7.9^{d}	47.9 ± 0.9^{b}	14.9 ± 0.7^{a}	0.43 ± 0.1b

Mean values in a column with different letters are significantly different at $P < 0.05$.

Table 5
Potassium/Sodium, Calcium/Phosphorus and Potassium/(Calcium+Magnesium) ratio of the vegetables

Sample	K/Na (wt/wt)	Ca/P (wt/wt)	K/Ca+Mg (meq/meq)
Alocasia odora	18.3	12.6	0.11
Typhonium trilobatum	20.1	8.9	0.12
Nymphaea nouchali	13.1	6.5	0.23
Solanum nigram	13.5	6.3	0.19
Alternanthera philoxyroydes	14.3	3.7	0.14

178.8 mg/100 g (*A. philoxyroydes*) to 478.7 mg/100 g (*A. odora*). The amount of magnesium in these vegetables ranges from 27.6 mg/100 g (*N. nouchali*) to 178.8 mg/100 g (*S. nigram*). Dietary deficiency of magnesium, which is linked with ischemic heart disease [31], can be prevented by the regular consumption of these vegetables as all these vegetables are good source of magnesium. The levels of phosphorus in the vegetables range from 31.3 mg/100 g (*N. nouchali*) to 47.9 mg/100 g (*A. philoxyroydes*).

Amount of iron in these vegetables ranges from 5.2 mg/100 g (*N. nouchali*) to 17.8 mg/100 g (*T. trilobatum*). Iron deficiency may lead to anemia, bone changes, weakness, headache, depressed immunity, behavioral abnormalities and reduced cognitive function [32]. The level of **zinc** of these vegetables ranges from 0.27 mg/100 g (*N. nouchali*) to 0.78 mg/100 g (*A. odora*). Zinc is one of the most important mineral nutrients and is necessary for the proper function of over 200 enzymatic reactions in the body [33].

The values of K/Na, Ca/P and K/(Ca+Mg) ratios are given in the Table 5. The K/Na ratio in our body is of great concern to prevent to prevent high blood pressure as well as for normal retention of proteins during growth [34]. Researchers recommend a dietary K:Na ratio greater than 5:1 to maintain optimal health [35]. The value for all the vegetables ranged from 13.1:1 (*N nouchali*) to 20.1:1 (*T trilobatum*). The **Ca/P** ratio in food is important for good absorption of both [35]. The ratios ranged from 3.7 (*A. philoxyroydes*) to 12.6 (*A. odora*) in the fresh vegetables. A **K/ (Ca+Mg)** ratio of 2.2 or greater, expressed as meq, indicates that consumption of such food makes one susceptible to hypomagnesaemia, an electrolyte disturbance in which there is an abnormally low level of magnesium in the blood [35]. The value for all the vegetables were found to be below this level ranging from 0.11 (*A. odora*) to 0.23 (*N nouchali*).

Table 6 presents the phenolic flavonoid and vitamin C content. Phenolic compounds are the class of phytonutrients that exist in both edible and inedible plants, and they have been reported to have multiple biological effects including antioxidant activity. The total phenolic content of our investigated vegetables ranged from 11.5 to 23.4 mg GAE/g dry weight (dw) of sample. *T. trilobatum* (23.4 mg GAE/g of dw) contains the highest total phenolic content, followed by *A. philoxyroydes* (17.7 mg GAE/g of dw), and *A. odora* (17.5 mg GAE/g of dw). Flavonoids are phenolic compounds occurring ubiquitously in plants which occur as glycosides and contain many phenolic hydroxyl groups in their ring structure, and are very effective antioxidants. Flavonoid content ranged from 2.7 mg QE/g dw (*N. nouchali*) to 7.9 mg QE/g dw (*T. trilobatum*) in the examined vegetables. Vitamin C ranges were 27.8 mg/100 g (*N. nouchali*) to 51.7 mg/100 g (*S. nigram*). Vitamin C is a water soluble antioxidant which is very effective to scavange aqueous peroxyl radicals.

Table 6
Total phenolic content, total flavonoid content and Vitamin C content of the vegetables. All data are the means ± SD of triplicate experiment

Sample	Total phenolic (mg GAE/g dw)	Total flavonoid (mg QE/g dw)	Vitamin C (mg/100 g fw)
A. odora	17.5 ± 2.7^{a}	4.7 ± 0.7^{a}	33.6 ± 3.7^{a}
T. trilobatum	23.4 ± 1.5^{b}	7.9 ± 0.7^{b}	42.7 ± 1.8^{b}
N. nouchali	14.7 ± 0.9^{c}	2.7 ± 0.3^{c}	27.8 ± 2.1^{c}
S. nigram	11.5 ± 1.7^{d}	3.5 ± 0.1^{d}	51.7 ± 2.4^{d}
A.philoxyroydes	17.7 ± 1.3^{a}	5.1 ± 0.3^{a}	43.3 ± 2.1^{b}

Mean values in a column with different letters are significantly different at $P < 0.05$.

Table 7
Antioxidant activity of aqueous methanolic extracts of the vegetables. All data are the means ± SD of triplicate experiment

Sample	DPPH assay (IC_{50}, mg/L)	ABTS assay (μM trolox/g dw)	FRAP assay (μM Fe(II)/g dw)
Alocasia odora	62.7 ± 2.7^{a}	24.7 ± 1.1^{a}	57.7 ± 3.2^{a}
Typhonium trilobatum	51.9 ± 3.1^{b}	36.1 ± 2.3^{b}	78.1 ± 1.3^{b}
Nymphaea nouchali	76.9 ± 4.1^{c}	19.6 ± 0.9^{c}	56.8 ± 3.7^{a}
Solanum nigram	78.1 ± 3.6^{d}	17.8 ± 2.4^{c}	41.4 ± 1.1^{c}
Alternanthera philoxyroydes	65.8 ± 2.4^{a}	28.7 ± 1.7^{d}	67.9 ± 2.1^{d}

Mean values in a column with different letters are significantly different at $P < 0.05$.

In the DPPH assay antioxidant capacity is expressed as IC_{50} value, which is defined as the concentration of antioxidant required for 50% scavenging of DPPH radical in specific time period. IC_{50} value is inversely related to the antioxidant activity. A smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract [36]. The antioxidant capacity measured by DPPH assay and expressed as IC_{50} values ranged from 51.9 to78.1 mg/L (Table 7). The IC_{50} value of *T. trilobatum* was lowest (51.9 mg/L) and gave the highest antiradical activity among all studied vegetables followed by *A. odora* (62.7 mg/L) and *A. philoxyroydes* (65.8 mg/L). In ABTS assay antioxidant capacity is expressed as trolox equivalent antioxidant capacity (TEAC) simply as μM trolox/g of dry weight (dw). TEAC value varied from 17.8 to 36.1 μM trolox/g of dry weight (Table 7). *T. trilobatum* (36.1 μM trolox/g of dw) has the highest TEAC value followed by A.philoxyroydes (28.7 μM trolox/g of dw) and *A. odora* (24.7 μM trolox/g of dw). These findings agree well with those reported for some Thai indigenous medicinal plants [36]. The FRAP assay measures the ability of an antioxidant in terms of activity to reduce $[Fe3\pm (TPTZ)_2]^{3+}$ to intensely blue coloured ferrous complex $[Fe^{2+}\text{- } (TPTZ)_2]^{2+}$ in acidic medium. FRAP results are expressed as μmol of Fe^{2+} per g dry wt of plant (μM Fe(II)/g dw). As indicated in Table 5, the FRAP value ranged from 41.4 to 78.1μM Fe(II) per g dry weight (dw). *T. trilobatum* (78.1 μM Fe(II)/g dw) has the highest FRAP value followed by *A. philoxyroydes* (67.9 μM Fe(II)/g dw) and *A. odora* (57.7 μM Fe(II)/g dw).

4. Conclusion

Based on these findings, the nutrient composition of nov-conventional vegetables revealed them to be good sources of many nutrients like potassium, iron, calcium, ascorbic acid and phenolic. They also had a high fiber content and antioxidant activity and hence would also serve as a natural source of fiber and antioxidants. Systematic Cultivation of these vegetables could be taken up to augment total food supplies also.

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