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Nutritional value, phytochemicals and antioxidant property of six wild edible plants consumed by the Bodos of North-East India

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Abstract.
BACKGROUND: Plants are known to contain minerals and many bioactive compounds which provide several health benefits on consumption.
OBJECTIVE: The aim of present study was to assess the nutritional composition, phytochemical constituents and antioxidant activities of six wild edible plants consumed by the Bodos of North-East India and the plants are *Sphenoclea zeylanica*, *Cardamine hirsuta*, *Natsiatum herpeticum*, *Sphaerantus peguensis*, *Melothria perpusilla*, and *Persicaria chinensis*.
METHODS: Proximate composition of the plant was determined following AOAC method. Mineral contents were analyzed using Atomic Absorption Spectrometer. Phytochemical screening of methanol extracts were performed following standard procedures and several assays were used to evaluate the antioxidant activity of the plants.
RESULTS: The investigation showed that all the six plants have variable quantities of proximate and mineral compositions. The phytochemical screening of methanol extracts revealed the presence of a number of medicinally active secondary metabolites. Among the six wild edible plants, *M. perpusilla* displayed a better antioxidant property showing the strongest DPPH radical scavenging activity, maximum FRAP value, highest phenolic and flavonoid contents.
CONCLUSIONS: The results of this investigation indicate that these wild edible plants are good sources of minerals and natural antioxidants to be incorporated as functional ingredients of food.

Keywords: Wild edible plants, proximate composition, metals, antioxidant, North-East India

1. Introduction

The interest in research and development activities on wild or underutilized plant and fruit species is presently increasing throughout the world because of their positive role against various diseases [1]. Wild plants have been playing a very momentous role in human life for thousands of years. They have been used for food, medicine, fiber and other purposes and also as food for domestic animals. Wild edible plants serve as alternative to staple

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food during periods of food deficit, potential source of nutrient supplements against malnutrition as well as income sources for rural communities. They have occupied a unique place as they are rich sources of essential minerals, vitamins and bioactive compounds which have several health benefits [2, 3].

Plants contain many phytochemicals such as alkaloids and phenolic compounds in addition to nutrients such as minerals, vitamins, proteins and carbohydrates, and several studies have shown that consumption of fruits, vegetables and plant derived food products have health benefits against chronic diseases including cardiovascular disease and certain types of cancer [3–5]. More than 900 different phytochemicals have already been identified in foods and in just one vegetable or plant food, more than 100 different phytochemicals are found to be present [6]. Many of these phytochemicals have antioxidant properties and support in protection of cells against the oxidative damage caused by reactive oxygen species [7, 8]. Antioxidants are the molecules which have the ability to scavenge or inhibit the oxidation of other molecules. Oxidation reactions can generate reactive oxygen species like oxygen free radicals which initiate chain reactions that may lead to formation of unwanted products or cell damage causing many diseases such as cancer, arthritis, diabetes, and other diseases related to humans [3, 9, 10]. Phytochemicals such as polyphenols and other bioactive compounds can prevent these chain reactions by scavenging free radicals and obstruct oxidation of other biomolecules [11, 12]. These phytochemicals provide endless prospects for new drug development due to the unmatched availability of chemical variety and plant derived food products are considered to be less toxic and more free from the side effects than synthetic drugs [13]. According to World Health Organization (WHO), 80% of the world's population still depends on traditional remedies for their medicines which have compounds derived from plants. The massive traditional knowledge of medicinal plants is presently playing a very essential role in the development of new drugs. Nowadays, physicians like Ayurvedic, Homoeo and Unani use various species of medicinal plants that found their way a long time ago into the Hindu Material Media [14].

WHO has strongly recommended for heavy metal analysis in the medicinal plants along with other necessary biological, chemical and environmental analysis [15–17]. Heavy metals are generally taken up and accumulated in plants by absorbing or adsorbing on the fruit and vegetable surfaces, either from the soil or from the polluted environments, or from the use of pesticides and fertilizers in cultivation [18, 19]. There are many factors which play important roles in the absorbance of heavy metals by plants and these are the climatic condition, atmospheric deposition, nature of the soil and maturity of the plant species [19]. Heavy metals found in plant species are of two categories known as essential and toxic heavy metals. The essential heavy metals like Cu, Zn, Cr, Fe, Mn, Co and Ni are required in very trace quantities since they are important for the physiological and biological functions of the human body and the deficiency or excess of minerals are both harmful and may lead to metabolic disorders [20, 21]. Whereas toxic heavy metals like Pb, Cd, As and Hg are nonessential for human beings, animals and plants [17, 21]. Heavy metals are not easily biodegradable and therefore they cause serious health risks in humans and animals even at very low concentration [21, 22].

North-East (NE) India encompasses eight different states and these are Sikkim, Assam, Meghalaya, Arunachal Pradesh, Tripura, Mizoram, Manipur and Nagaland. The region is surrounded in the north by the Himalayas, in the east by Myanmar and in the south by the Bay of Bengal. NE India is well-known for its high ethnic and rich biological biodiversity. Assam is one among the ethnic-cultural variety and rich biodiversity states in NE India with geographical location of E 89°50′ to E 96°10′ and N 24°30′ to N 28°10′ covering the total area of 78,438 sq. km out of which 26,832 sq. km is covered by the forest as per the Forest Survey of India 2011 [10]. The Bodos of Assam are an important tribe and ethnic group in NE of India. They are the major and dominant inhabitant of the Bodoland Territorial Area Districts (BTAD) of Assam which mainly consists of Kokrajhar, Chirang, Baksa and Udalguri Districts. Their main food items are rice, pork, fish and other locally grown vegetables and fruits. Bodos have a huge traditional knowledge of herbal medicines and they have a rich tradition of enjoying the flavor of several wild plants in the form of mixture locally known as "*Gwka-Gwkwi*" (meaning bitter and sour) during the festive season of *Rongjali Bwisagu,* a New Year festival of *Bodo* people known as *Rongali Bihu* in *Assamese*. There is also a traditional belief that this *Gwka-Gwkwi* consumed during the festival act as a medicine for many diseases for the whole year [23]. Hence, encouraging the consumption of wild edible plants may also be

adopted as a policy to discourse some micronutrient problems among rural populations as these are the valuable sources of energy and micronutrients. The aim of present study was to investigate the nutritional composition, phytochemical constituents, and antioxidant activities of these six wild edible plants consumed by Bodos of NE India in order to provide a good reference to the future researchers, health-conscious populaces and to provide the nutritional importance of these plants among the common people.

2. Materials and methods

2.1. Chemicals

DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS (2, 2′-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt) and quercetin were obtained from Himedia Laboratories Pvt. Ltd., Mumbai (India), trolox from Sigma Aldrich, Bangalore (India), H_2O_2 (Hydrogen peroxide), ascorbic acid and Folin-Ciocalteu's reagent from Merck, Mumbai (India) and gallic acid from Central Drug House Pvt. Ltd., New Delhi (India).

2.2. Collection of plants and identification

The six wild edible plants *viz*. *Sphenoclea zeylanica* Gaertn., *Cardamine hirsuta* L., *Natsiatum herpeticum* Buch. -Ham. ex Arn., *Sphaerantus peguensis* Kurtz ex C.B. Clarke, *Melothria perpusilla* (Blume) Cogn., and *Persicaria chinensis* (L) H. Gross were collected from Kokrajhar District of BTAD, Assam during their seasonal availability in the year 2014. Voucher specimens of all the plant species were submitted to Botanical Survey of India, Shillong, Meghalaya and authenticated.

2.3. Sample preparation

The fresh plants were washed thoroughly under tap water followed by distilled water and then moisture and vitamin C contents of the fresh samples were determined on the same day. The remaining samples were then dried at 55°C in a hot-air oven. The dried samples were powdered using a grinder. The powdered materials were extracted with methanol in 1:10 ratio (w/v), shivered, stored for 72 h and filtered (Whatman No. 1). Filtrate was evaporated to dryness using Buchi Rotavapor R-215 (Switzerland) and the dry extracts were kept in air-tight containers at 4°C in a Refrigerator till further analyses.

2.4. Determination of proximate composition

The Association of Official Analytical Chemists methods [24] were used for the estimation of moisture, ash, crude fat, crude protein and crude fiber. Crude fat was determined by extracting with petroleum ether using a Soxhlet apparatus. Crude protein was estimated by the Kjeldhal method. Total protein was calculated by multiplying the evaluated nitrogen by a protein conversion factor of 6.25. Total carbohydrate was determined by the difference method [23] based on traditional carbohydrate determination. Nutritive value or the calorific value in kcal/100 g of the sample was calculated [23] on the basis of data of proximate analysis. Vitamin C was estimated using 2, 6-dichlorophenol indophenol by titration method [23].

2.5. Determination of metals

Samples were digested with concentrated HNO_3. Metals like Ca, Mg, Fe, Cu, Zn, Mn and Ni were determined using Graphite Furnace-Atomic Absorption Spectrometer (GF-AAS, Analytik Jena Vario-6) at Sophisticated Analytical Instrumentation Facility (SAIF), North Eastern Hill University, Shillong (Meghalaya) and metals like

Na, K, Cr, Co, Se, Pb, Cd and As were determined using Atomic Absorption Spectrometer (AAS-ICE 3500, Thermo Scientific, UK) at Sophisticated Analytical Instrumentation Centre (SAIC), Tezpur University (Assam). Results obtained were converted to mg/100 g of dried samples.

2.6. Phytochemical screening

The qualitative phytochemical screening of methanol extracts of plants was performed using the reported procedures [10].

2.7. Determination of antioxidant properties

Using an UV-VIS spectrophotometer (Perkin Elmer, Lambda 35), antioxidant properties of methanol extracts of plants were evaluated based on previously reported procedures of DPPH, ABTS, H_2O_2, and FRAP (Ferric reducing antioxidant power) assays [3].

2.8. Investigation of total phenolic content (TPC) and total flavonoid content (TFC)

TPC using Folin-Ciocalteu's reagent and TFC in methanol extracts of the plant species were investigated spectrophotometrically (Perkin Elmer, Lambda 35) following the reported procedures [3]. The results of TPC and TFC were expressed in mg GAE (gallic acid equivalents)/g DE (dried extract) and mg QE (quercetin equivalents)/g DE, respectively.

2.9. Statistical analysis

The results of all the experiments were expressed as mean of triplicate readings ± standard deviation. Standard deviations were calculated at Microsoft Excel. Relative significant differences among the means were determined by one-way ANOVA *t*-test at $p < 0.05$ using OriginPro 8.5 software (OriginLab Corporation, MA 01060 USA). Pearson's correlation study was performed using SPSS 13.0 software.

3. Results and discussion

3.1. Proximate composition

The proximate analysis results of six wild edible plants are shown in Table 1 which shows that the moisture content of plant species varied from 82.83 ± 1.30 g in *M. Perpusilla* to 92.89 ± 0.44 g in *C. hirsuta* per 100 g of fresh sample. The moisture content of the plant species is close to the values of some underutilized green leafy vegetables reported by Saha et al. [2] and wild edible plants reported by Narzary et al. [23]. Ash content of the plants was found to be highest in *N. herpeticum* (3.42 ± 0.04 g/100 g) and lowest being in *M. perpusilla* (0.661 ± 0.008 g/100 g). The plant species of the present study were found to contain low amount of crude fat content that ranged from 0.124 ± 0.11 g/100 g in *S. zeylanica* to 0.666 ± 0.01 g/100 g in *N. herpeticum* which was less than 1% in all the cases and the values were found to be similar with the works of Saha et al. [2]. Table 1 showed the highest crude fiber in *M. perpusilla* (3.03 ± 0.02 g/100 g) and the lowest being in *S. zeylanica* (1.25 ± 0.21 g/100 g). Foods rich in fibers are required for digestion and effective elimination of wastes. Fibers can lower the serum cholesterol, risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer [23]. The crude protein content of wild edible plant species varied from 2.64 ± 0.13 g/100 g in *M. perpusilla* to 5.37 ± 0.05 g/100 g in *N. herpeticum*. The total carbohydrate content determined was found to be ranged from 1.7 ± 0.51 g/100 g in *C. hirsuta* to 13.64 ± 1.32 g/100 g in *M. perpusilla*. The essential nutrients

Table 1
Proximate analysis of six wild edible plants per 100 g of fresh weight

Plants	Moisture (g)	Ash (g)	Fat (g)	Fiber (g)	Protein (g)	Carbohydrate (g)	Calorific value (kcal)
S. zeylanica	92.81 ± 0.64^{a}	1.41 ± 0.01^{a}	0.124 ± 0.11^{a}	1.25 ± 0.21^{a}	3.08 ± 0.01^{a}	2.55 ± 0.63^{a}	23.70 ± 2.54^{a}
C. hirsuta	92.89 ± 0.44^{a}	1.78 ± 0.07^{a}	0.239 ± 0.01^{a}	$1.64 \pm 0.01^{a,b}$	3.99 ± 0.21^{b}	1.70 ± 0.51^{b}	22.45 ± 2.06^{b}
N. herpeticum	83.72 ± 2.36^{b}	3.42 ± 0.04^{b}	0.666 ± 0.01^{a}	1.99 ± 0.03^{b}	5.37 ± 0.05^{c}	6.80 ± 2.40^{c}	54.72 ± 9.61^{c}
S. peguensis	90.89 ± 2.22^{c}	2.35 ± 0.12^{c}	0.254 ± 0.03^{a}	1.92 ± 0.07^{b}	3.18 ± 0.01^{a}	3.32 ± 2.23^{d}	28.30 ± 8.91^{d}
M. perpusilla	82.83 ± 1.30^{d}	0.661 ± 0.01^{d}	0.208 ± 0.22^{a}	3.03 ± 0.02^{c}	2.64 ± 0.13^{d}	13.64 ± 1.32^{e}	67.03 ± 5.23^{e}
P. chinensis	92.35 ± 0.61^{a}	1.58 ± 0.11^{a}	0.333 ± 0.02^{a}	2.57 ± 0.52^{d}	3.66 ± 0.22^{b}	2.07 ± 0.61^{a}	25.93 ± 2.43^{f}

Results are expressed as mean of triplicate readings ($n = 3$) $\pm$ standard deviation per 100 g of fresh sample. The results with different letters in a column are significantly different from each other at $p < 0.05$.

of life are carbohydrates, fats and proteins. High carbohydrate content indicates high energy content in food. The main function of carbohydrate in the body is to supply energy and it is responsible for doing activities in our daily life [10, 23]. The calorific value per 100 g of fresh sample was found highest in *M. perpusilla* (67.03 ± 5.229 kcal) followed by *N. herpeticum* (54.72 ± 9.61 kcal) and lowest in *C. hirsuta* (22.45 ± 2.06 kcal). Similarly, Narzary et al. [23] studied the calorific value of some wild edible plants and reported to range from 29.48 to 67.42 kcal per 100 g of fresh sample which is close to the values of present study. Foods with a high calorific value can be considered as a good human diet. These wild edible plants with high calorific value can be suggested in the formulation of numerous nutritional supplements.

3.2. Metal contents

Metal contents of six wild edible plants expressed in mg/100 g dried sample are shown in Table 2. The sodium and potassium detected in the plants ranged from 48.73 ± 0.13 mg to 136.71 ± 0.20 mg and 1155.28 ± 0.21 mg to 10462.28 ± 0.13 mg, respectively. The plants are very rich in potassium and the increasing order of potassium content is *P. chinensis* < *M. perpusilla* < *S. zeylanica* < *N. herpeticum* < *S. peguensis* < *C. hirsuta.* Potassium is one of the most important nutrients which plays various biophysical and biochemical roles and plant foods rich in potassium are generally used for the treatment of rheumatoid arthritis and heart disease [12, 21]. In this investigation, low levels of calcium (5.00 ± 0.01 – 6.22 ± 0.03 mg/100 g) and magnesium (4.10 ± 0.02 – 5.60 ± 0.02 mg/100 g) were found to be present. Magnesium is important in the ionic balance and enzyme co-factors, and calcium is required in building of skeletal structures and muscle functioning [25]. Magnesium cooperates with calcium in the muscular contraction and blood coagulation [20]. Iron content was detected highest in *C. hirsuta* (6.10 ± 0.01 mg/100 g) and lowest value in *P. chinensis* (1.60 ± 0.16 mg/100 g). The iron content of lesser known leafy vegetables reported by Bello et al. [26] was comparable to the report of present investigation. Saha et al. [2] reported the iron content of some underutilized green leafy vegetables of Sonitpur district of Assam, India ranging from 29.40 mg/100 g to 241.20 mg/100 g which is higher in comparison to values of present study. Ng et al. [27] also reported the iron content of selected tropical wild vegetables which ranged from 3.6 – 33.1 mg/100 g. The recommended daily intake of iron for adult female is 29 mg per day and for adult male is 8 mg per day. Iron is needed for normal functioning of the central nervous system and for the synthesis of haemoglobin in red blood cells which is required for oxygen transportation to all parts of the body. Iron deficiency is linked to anaemia and causes immune system dysfunction which is associated with increased risk of infection [1, 10, 27]. When dietary iron consumption is not sufficient, about 15% of the body's iron stored for future supplies is mobilized [28]. The level of copper ranged from 1.62 ± 0.03 mg in *C. hirsuta* (lowest) to 2.60 ± 0.41 mg in *P. chinensis* (highest) per 100 g of dried sample. Almost similar value of copper was detected in *S. zeylanica*, *M. perpusilla* and *P. chinensis*. Copper plays an important role in biological electron transport and is essential for the production of enzyme in the body [2]. Deficiency of copper leads to reduced energy

Table 2
Metal analysis of six wild edible plants (mg/100 g dried sample)

Metals	Plant species					
	S. zeylanica	*C. hirsuta*	*N. herpeticum*	*S. peguensis*	*M. perpusilla*	*P. chinensis*
Na	48.73 ± 0.13^{a}	100.48 ± 0.20^{b}	92.15 ± 0.02^{c}	136.71 ± 0.20^{d}	55.35 ± 0.30^{e}	53.35 ± 0.06^{f}
K	7684.29 ± 0.15^{a}	10462.28 ± 0.13^{b}	8436.10 ± 0.09^{c}	10164.25 ± 0.10^{d}	5886.17 ± 0.16^{e}	1155.28 ± 0.21^{f}
Ca	6.22 ± 0.03^{a}	6.20 ± 0.14^{a}	5.47 ± 0.02^{b}	5.00 ± 0.01^{b}	6.14 ± 0.04^{a}	5.11 ± 0.02^{b}
Mg	4.90 ± 0.01^{a}	5.60 ± 0.02^{b}	$4.58 \pm 0.03^{a,c}$	4.10 ± 0.02^{c}	5.10 ± 0.01^{b}	4.20 ± 0.06^{c}
Fe	1.83 ± 0.05^{a}	6.10 ± 0.01^{b}	2.48 ± 0.01^{c}	2.40 ± 0.06^{c}	1.70 ± 0.07^{a}	1.60 ± 0.16^{a}
Cu	2.58 ± 0.07^{a}	1.62 ± 0.03^{b}	2.10 ± 0.01^{a}	2.11 ± 0.02^{a}	$2.60 \pm 0.01^{a,c}$	$2.60 \pm 0.41^{a,c}$
Zn	0.70 ± 0.05^{a}	0.30 ± 0.01^{a}	0.70 ± 0.02^{a}	0.20 ± 0.01^{a}	0.20 ± 0.03^{a}	0.30 ± 0.05^{a}
Mn	0.54 ± 0.04^{a}	0.64 ± 0.03^{a}	0.70 ± 0.01^{a}	0.62 ± 0.03^{a}	0.80 ± 0.14^{a}	0.60 ± 0.02^{a}
Ni	2.60 ± 0.02^{a}	3.60 ± 0.03^{b}	4.70 ± 0.06^{c}	4.20 ± 0.02^{c}	3.80 ± 0.02^{b}	4.70 ± 0.08^{d}
Cr	0.58 ± 0.03^{a}	0.87 ± 0.02^{a}	0.74 ± 0.09^{a}	2.36 ± 0.01^{b}	1.32 ± 0.12^{c}	2.38 ± 0.19^{b}
Co	0.39 ± 0.11^{a}	0.57 ± 0.02^{a}	0.59 ± 0.12^{a}	0.57 ± 0.16^{a}	0.26 ± 0.09^{a}	0.36 ± 0.07^{a}
Se	0.81 ± 0.40^{a}	1.83 ± 0.60^{b}	0.78 ± 0.41^{a}	0.45 ± 0.90^{a}	0.37 ± 0.53^{a}	0.84 ± 0.24^{a}
Pb	0.59 ± 0.11^{a}	0.86 ± 0.31^{a}	0.75 ± 0.51^{a}	2.36 ± 0.21^{b}	1.32 ± 0.16^{c}	2.39 ± 0.31^{b}
Cd	BDL					
As	BDL					

BDL, Below detection level; Results are expressed as mean of triplicate readings ($n = 3$) ± standard deviation per 100 g of dried sample. The results with different letters along a row are significantly different from each other at $p < 0.05$.

production, abnormal glucose and cholesterol metabolism, and increased oxidative damage [10, 29]. The zinc content of the plant species varied from 0.20 ± 0.01 mg to 0.70 ± 0.05 mg with the highest being in *S. zeylanica* and the lowest value in *S. peguensis*. However, a higher level of zinc was reported in some underutilized green leafy vegetables by Saha et al. [2] that ranged from 1.50 mg/100 g to 7.50 mg/100 g. The recommended dietary allowance for zinc is 8 mg and 11 mg per day for adult women and men respectively [30]. Zinc is an essential element for human growth which increases resistance to infection and excessive intake of zinc has been reported to be toxic [10, 31]. Zinc is a cofactor for the antioxidant enzyme super oxide dismutase. It is required for the functioning of over 300 different enzymes, and many enzymatic reactions involved in carbohydrate and protein metabolism [28]. The manganese content found in the present study (Table 2) varied from 0.54 ± 0.04 mg in *S. zeylanica* to 0.80 ± 0.14 mg in *M. perpusilla*. Manganese is an important component of metalloenzymes and plays essential roles in a number of physiological processes as a constituent or activator of some enzymes which are essential for the metabolism of carbohydrate, cholesterol and amino acid [29]. The highest nickel content was detected in *P. chinensis* (4.70 ± 0.06 mg) and lowest in *S. zeylanica* (2.60 ± 0.02 mg). Chromium, cobalt and selenium content varied from 0.58 ± 0.03 mg (*S. zeylanica*) to 2.38 ± 0.19 mg (*P. chinensis*), 0.26 ± 0.09 mg (*M. perpusilla*) to 0.59 ± 0.12 mg (*N. herpeticum*) and 0.37 ± 0.53 mg (*M. perpusilla*) to 1.83 ± 0.60 mg (*C. hirsuta*), respectively. *S. zeylanica* (0.59 ± 0.11 mg) had the lowest lead content and the highest level was detected in *P. chinensis* (2.39 ± 0.31 mg). Cadmium and arsenic were not detected (below detection level) in the plants.

3.3. Phytochemical screening

The preliminary screening of phytochemical constituents present in six wild edible plants was performed using methanol extract. The phytochemical constituents investigated were alkaloids, saponins, cardiac glycosides, steroids, anthraquinones, coumarins, phenols, tannins, flavonoids, anthocyanins, phlobatannins, carbohydrates, starch, proteins, and lignin. The results were summarized in Table 3 which showed the presence of various

Table 3
Qualitative phytochemical analysis with methanol extracts of plants

Phytochemicals	Name of Test	SZ	CH	NH	SP	MP	PC
Alkaloids	Wagner's reagent	+	+	+	+	+	+
	Dragendroff's reagent	+	+	+	+	+	+
Saponins	Frothing test	+	+	+	+	+	+
Cardiac glycosides	Keller-Killiani's test	+	+	+	+	+	+
Steroids	Liebermann-Burchard test	+	+	+	+	+	+
	Salkowski's test	+	+	+	+	+	+
Anthraquinones	Modified Borntrager's test	–	+	–	+	+	+
Coumarins		+	+	+	+	+	+
Phenols	$FeCl_3$ test	–	–	+	+	+	+
Tannins	Gelatine test	–	+	+	+	+	+
Flavonoids	Shinoda's test	+	+	+	+	+	+
Anthocyanin		–	–	+	–	+	+
Phlobatannins		–	–	+	–	–	+
Carbohydrates	Molish's test	+	+	+	+	+	+
	Felhing's test	–	+	+	+	+	+
Starch	Iodine test	–	–	–	–	–	+
Proteins	Ninhydrin test	–	–	+	–	–	+
	Millon's test	+	+	+	+	+	+
Lignin		–	+	+	+	+	+

Sphenoclea zeylanica (SZ), *Cardamine hirsuta* (CH), *Natsiatum herpeticum* (NH), *Sphaerantus peguensis* (SP), *Melothria perpusilla* (MP), *Persicaria chinensis* (PC); (+), present; (–), absent.

bioactive compounds. Fruits and vegetables are very important sources of bioactive compounds that differ widely in terms of structure, biological properties, and mechanisms of actions. Many phytochemical constituents found in plants are known to be responsible for antioxidant, antimicrobial, anti-larvicidal, and anti-inflammatory activities [10, 25].

3.4. Antioxidant property

DPPH free radical scavenging activity of methanolic extract of six wild plants is shown in Table 4. The DPPH method is widely used for screening antioxidant activity of plant extracts. DPPH is a stable free radical having a characteristic absorption at 517 nm. Antioxidants in the extracts react with DPPH and convert 1, 1-diphenyl-2-picrylhydrazyl (deep violet color) to 1, 1-diphenyl-2-picrylhydrazine, a stable molecule (yellow color or bleached product) by accepting an electron or hydrogen radical at a very rapid rate resulting in a decrease in absorbance at 517 nm [10]. IC_{50} value is defined as the inhibitory concentration of the crude extract that scavenges 50% of reactive oxygen species or inhibits the process of oxidation by 50%. It is inversely related to antioxidant capacity and lower IC_{50} value signals better antioxidant activity. In this investigation, all the plant extracts were compared with ascorbic acid as standard reference. The methanol extract of all the wild edible plants showed DPPH free radical scavenging activity and the antioxidant activity increases with increasing concentration of sample extract (Table 4). The results showed that *M. perpusilla* ($97.54 \pm 0.15\%$) had the highest DPPH radical scavenging activity with an IC_{50} value of 134.96 ± 0.35 μg/mL and *N. herpeticum* ($24.85 \pm 0.07\%$) had the lowest activity with IC_{50} value of 1658.47 ± 2.72 μg/mL. While the standard ascorbic acid showed $98.84 \pm 0.10\%$ inhibition with an IC_{50} value of 25.01 ± 0.52 μg/mL.

Table 4
DPPH, ABTS and H_2O_2 scavenging activities of methanolic extracts of plants

Conc. (μg/mL)	*S. zeylanica*	*C. hirsuta*	*N. herpeticum*	*S. peguensis*	*M. perpusilla*	*P. chinensis*	Standard**
			Inhibition (%) of plants for DPPH assay				
2	12.60 ± 0.28^{a}	12.73 ± 0.09^{a}	14.48 ± 0.07^{b}	17.18 ± 0.07^{c}	14.59 ± 0.12^{b}	8.75 ± 0.09^{d}	15.94 ± 0.14^{e}
5	13.82 ± 0.05^{a}	13.20 ± 0.09^{b}	15.14 ± 0.07^{c}	18.28 ± 0.34^{d}	19.71 ± 0.23^{e}	14.65 ± 0.13^{f}	26.93 ± 0.19^{g}
10	14.91 ± 0.14^{a}	14.04 ± 0.09^{b}	16.15 ± 0.04^{c}	19.47 ± 0.41^{d}	25.17 ± 0.15^{e}	16.33 ± 0.09^{f}	36.57 ± 0.28^{g}
50	17.19 ± 0.35^{a}	15.63 ± 0.09^{b}	17.18 ± 0.11^{a}	22.61 ± 0.12^{c}	41.09 ± 0.28^{d}	17.62 ± 0.27^{a}	83.11 ± 0.23^{e}
100	20.31 ± 0.09^{a}	16.63 ± 0.10^{b}	18.67 ± 0.51^{c}	24.54 ± 0.15^{d}	47.92 ± 0.19^{e}	21.14 ± 0.22^{f}	90.04 ± 0.23^{g}
200	34.61 ± 0.42^{a}	22.00 ± 0.18^{b}	23.28 ± 0.22^{c}	36.81 ± 0.15^{d}	91.00 ± 0.27^{e}	30.08 ± 0.31^{f}	93.03 ± 0.47^{g}
500	46.25 ± 0.33^{a}	31.42 ± 0.19^{b}	24.85 ± 0.07^{c}	51.43 ± 0.16^{d}	97.54 ± 0.15^{e}	45.21 ± 0.27^{f}	98.84 ± 0.10^{g}
IC_{50}	519.90 ± 2.88^{a}	989.98 ± 6.07^{b}	1658.47 ± 2.72^{c}	455.76 ± 0.87^{d}	134.96 ± 0.35^{e}	550.68 ± 2.86^{f}	25.01 ± 0.52^{g}
			Inhibition (%) of plants for ABTS assay				
20	15.46 ± 0.14^{a}	2.33 ± 0.09^{b}	8.74 ± 0.14^{c}	16.24 ± 0.24^{d}	10.81 ± 2.01^{e}	14.81 ± 0.23^{f}	11.41 ± 0.22^{g}
50	31.40 ± 0.08^{a}	3.60 ± 0.18^{b}	16.19 ± 0.22^{c}	16.74 ± 0.23^{c}	18.24 ± 0.22^{d}	16.64 ± 0.23^{c}	47.98 ± 0.14^{e}
100	47.44 ± 0.08^{a}	6.62 ± 0.23^{b}	20.00 ± 0.14^{c}	24.37 ± 0.24^{d}	23.82 ± 0.16^{e}	26.38 ± 0.08^{f}	69.10 ± 0.22^{g}
150	67.63 ± 0.14^{a}	7.36 ± 0.24^{b}	28.34 ± 0.08^{c}	34.48 ± 0.23^{d}	41.58 ± 0.22^{e}	33.07 ± 0.24^{f}	87.89 ± 0.29^{g}
200	79.91 ± 0.14^{a}	8.42 ± 0.15^{b}	35.01 ± 0.22^{c}	49.58 ± 0.23^{d}	58.95 ± 0.14^{e}	44.44 ± 0.15^{f}	92.17 ± 0.17^{g}
250	87.86 ± 0.14^{a}	15.73 ± 0.15^{b}	44.98 ± 0.23^{c}	60.84 ± 0.15^{d}	80.54 ± 0.22^{e}	50.43 ± 0.23^{f}	94.76 ± 0.14^{g}
300	91.71 ± 0.08^{a}	23.74 ± 0.18^{b}	54.27 ± 0.22^{c}	63.99 ± 0.16^{d}	90.29 ± 0.23^{e}	59.25 ± 0.23^{f}	96.46 ± 0.22^{g}
IC_{50}	115.99 ± 0.12^{a}	746.46 ± 1.90^{b}	283.23 ± 0.49^{c}	214.86 ± 0.65^{d}	165.18 ± 0.25^{e}	244.36 ± 0.50^{f}	73.67 ± 0.74^{g}
			Inhibition (%) of plants for H_2O_2 assay				
5	0.76 ± 0.03^{a}	2.27 ± 0.06^{b}	7.71 ± 0.07^{c}	3.99 ± 0.09^{d}	9.63 ± 0.13^{e}	3.40 ± 0.07^{f}	10.73 ± 0.02^{g}
10	1.33 ± 0.04^{a}	2.89 ± 0.03^{b}	9.80 ± 0.91^{c}	6.81 ± 0.07^{d}	15.23 ± 0.11^{e}	5.82 ± 0.07^{f}	27.91 ± 0.04^{g}
15	3.95 ± 0.03^{a}	4.74 ± 0.06^{b}	16.02 ± 0.07^{c}	7.46 ± 0.07^{d}	23.51 ± 0.06^{e}	14.96 ± 0.09^{f}	41.96 ± 0.07^{g}
20	6.95 ± 0.03^{a}	10.39 ± 0.03^{b}	18.77 ± 0.07^{c}	16.46 ± 0.08^{d}	25.7 ± 0.14^{e}	22.56 ± 0.05^{f}	51.42 ± 0.07^{g}
25	8.51 ± 0.04^{a}	14.49 ± 0.06^{b}	37.12 ± 0.02^{c}	21.91 ± 0.07^{d}	28.75 ± 0.13^{e}	27.29 ± 0.07^{f}	64.86 ± 0.08^{g}
IC_{50}	123.19 ± 0.54^{a}	82.34 ± 0.53^{b}	38.54 ± 0.19^{c}	57.49 ± 0.26^{d}	45.2 ± 0.09^{e}	42.26 ± 0.04^{f}	19.02 ± 0.01^{g}

Conc., concentration; IC_{50} value in μg/mL; Standard**, Standard used was ascorbic acid for both DPPH and H_2O_2 assays and trolox for ABTS assay; Results are expressed as mean of 3 replicates ± standard deviation. The results with different letters along a row are significantly different from each other at $p < 0.05$.

ABTS radical scavenging activity of methanolic extract of six wild plants is shown in Table 4. The ABTS radical scavenging activity was found highest in methanol extract of *S. zeylanica* ($91.71 \pm 0.08\%$) followed by *M. perpusilla* ($90.29 \pm 0.23\%$) with an IC_{50} value of 115.99 ± 0.12 μg/mL and 165.18 ± 0.25 μg/mL respectively, whereas *C. hirsuta* ($23.74 \pm 0.18\%$) exhibited the lowest activity among the selected plants with IC_{50} value of 746.46 ± 1.9 μg/mL. Trolox was used as standard in ABTS assay and displayed an IC_{50} value 73.67 ± 0.74 μg/mL (Table 4). ABTS IC_{50} value is the effective concentration of methanol extract in μg/mL which inhibits the ABTS activity by 50%. $ABTS^{+}$ radical is stable free radical and accepts an electron or hydrogen radical from antioxidant compounds to become a stable molecule which inhibits initiation or propagation of free-radical chain of oxidation. This study showed that the extracts of *S. zeylanica* and *M. perpusilla* were potent antioxidants. It has been reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{+}$) and their effectiveness depends on the molecular weight, number of aromatic rings, and nature of hydroxyl group's substitution than the specific functional groups [25].

Table 5
Ferric reducing antioxidant power (FRAP) and phytochemical contents of six wild edible plants

Plants	FRAP (μM TE/g DE)	Phenolic (mg GAE/g DE)	Flavonoid (mg QE/g DE)	Vitamin C (mg/100 g FW)
S. zeylanica	38.57 ± 7.14^{a}	36.39 ± 5.31^{a}	0.23 ± 0.10^{a}	40.37 ± 1.63^{a}
C. hirsuta	50.47 ± 5.45^{b}	71.77 ± 5.81^{b}	0.47 ± 0.23^{a}	35.62 ± 3.25^{b}
N. herpeticum	60.01 ± 3.57^{c}	10.45 ± 0.68^{c}	0.29 ± 0.11^{a}	85.71 ± 5.71^{c}
S. peguensis	115.95 ± 5.45^{d}	54.08 ± 7.21^{d}	0.77 ± 0.19^{a}	15.24 ± 1.65^{d}
M. perpusilla	855.23 ± 10.91^{e}	239.62 ± 5.4^{e}	1.66 ± 0.10^{b}	57.07 ± 1.59^{e}
P. chinensis	88.57 ± 7.14^{f}	52.12 ± 2.35^{f}	0.65 ± 0.17^{a}	27.78 ± 0.10^{f}

DE, Dried extract; FW, Fresh weight; Results are expressed as mean of 3 replicates ± standard deviation. The results with different letters in a column are significantly different from each other at $p < 0.05$.

H_2O_2 scavenging activity of methanolic extract of six wild plants is shown in Table 4 and compared with the standard ascorbic acid. The percentage of scavenging activity was found highest in *N. herpeticum* extract with an IC_{50} value of 38.54 ± 0.19 μg/mL, while standard ascorbic acid exhibited an IC_{50} value of 19.02 ± 0.01 μg/mL. *S. zeylanica* plant extract exhibited lowest H_2O_2 scavenging activity showing an IC_{50} value of 123.19 ± 0.54 μg/mL. Hydrogen peroxide, a non-radical reactive oxygen species in living organisms, itself is not very reactive but sometimes it is toxic to cells because it has the ability to penetrate cell membranes which may give rise to hydroxyl radicals and singlet oxygen, and initiate oxidation in the cells [32]. Therefore, removing of H_2O_2 by natural antioxidant sources is very important for protection of biological systems. Food polyphenols have been shown to defend mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structure, quercetin, catechin, gallic acid ester, and caffeic acid ester [25].

Ferric reducing antioxidant power (FRAP) assay was another method used to investigate the antioxidant activity of the wild plants in this study. The FRAP assay is a simple, inexpensive and widely employed method for the assessment of antioxidant activity and is based on the capacity of antioxidants to reduce ferric (III) ions to ferrous (II) ions [33]. Higher FRAP value indicates higher antioxidant activity. In this study, FRAP values (Table 5) ranged from 38.57 ± 7.14 to 855.23 ± 10.91 μM TE/g DE showing the strongest antioxidant activity in *M. perpusilla* extract while *S. zeylanica* had the lowest activity. The high activity may be due to the antioxidant compounds present in the methanol extracts of plants which could react with free radicals to stabilize and prevent radical chain reactions. Free radical scavenging is a very important function of antioxidants as free radicals encourage oxidation of proteins, DNA, and lipids which results in disturbances and functional loss of biological membranes and enzymes, and ultimately produces toxins [34].

3.5. Phenolic, flavonoid and vitamin C contents

Phenolic, flavonoid and vitamin C contents of six wild edible plants are presented in Table 5. The total phenolic content in the methanol extract of plants ranged from 10.45 ± 0.68 to 239.62 ± 5.4 mg GAE/g DE. *M. perpusilla* extract showed the highest phenolic content and the lowest being in *N. herpeticum.* While the flavonoid content varied from 0.23 ± 0.1 to 1.66 ± 0.1 mg QE/g DE, the highest being in *M. perpusilla* extract and the lowest being in *S. zeylanica*. It is seen from the results (Table 5) that the total phenolic content is higher than the flavonoid content. The total phenolic content of selected wild edible plants reported by Wong et al. [35] ranged from 0.69 to 19.65 mg GAE/g dried weight and the flavonoid content from 0.19 ± 0.02 to 8.37 ± 2.62 mg catechin equivalent per gram of dried weight. Xia et al. [36] reported higher phenolic contents in six edible wild plants that ranged from 278.7 ± 24.4 to 417.3 ± 38.3 mg GAE/g dried weight. Saikia et al. [37] reported phenolic content in some non-conventional green leafy vegetables of North-East India that ranged from 4.62 – 14.74 mg GAE/g dry weight and flavonoid content varying from 0.65 – 7.72 mg QE/g dry weight. Ng et al. [27] also reported phenolic content

of selected tropical wild vegetables that ranged from 1.8 to 4.1 mg GAE/g fresh weight and flavonoid content varied from 0.4 to 1.4 mg rutin equivalents/g fresh weight.

Phenolic compounds are secondary metabolites and widely distributed in plants. Natural antioxidants are generally derived from plants in the form of phenolic compounds such as phenolic acids, flavonoids, tocopherols *etc*. Phenolics are essential components of many vegetables and fruits not only for their major influence on physical qualities of the vegetables and fruits like color, flavor, and taste, but also for their antioxidant, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory properties [38]. Several literatures have revealed that the antioxidant activity of phenolic compounds is due to their redox properties, hydrogen donating abilities, and singlet oxygen quenchers [3, 25]. Therefore, they have important roles in decreasing the risk of many human diseases. Flavonoids which are known as secondary natural metabolites are a group of phenolic compounds and free radical scavengers which prevent oxidative cell damage through their water soluble property and also possess anti-cancer, antimicrobial, antithrombotic, and antimutagenic activities [8, 34, 39].

The vitamin C content (Table 5) varied from 15.24 ± 1.650 to 85.71 ± 5.710 mg/100 g of fresh weight and was detected highest in *N. herpeticum* (85.71 ± 5.710 mg/100 g) followed by *M. perpusilla* (57.07 ± 1.59 mg/100 g) and *S. zeylanica* (40.37 ± 1.626 mg/100 g) and the lowest being in *S. peguensis* (15.24 ± 1.650 mg/100 g). Similarly, the vitamin C content in some wild edible plants reported by Narzary et al. [23] ranged from 11.39 ± 0.0002 to 79.06 ± 0.02 mg/100 g fresh weight which is close to the values of present report. Vitamin C is also known as ascorbic acid and it the main vitamin provided by fruits and vegetables in the human diet. Fruits and vegetables generally can supply about 90% of a person's dietary vitamin C requirement. The average vitamin C requirement for an adult human being is about 70 mg per day. Vitamin C is required during various growth stages of human life and being a powerful reducing agent, it plays an essential role in absorbing and deactivating free radicals and thus protects the body from harmful effects [10, 40].

In this study, more than one assay was used to investigate antioxidant activity of plant extracts because of the more complex nature of phytochemicals that may reveal several mechanisms of antioxidant action. *M. perpusilla* revealed strong antioxidant activity among the six wild edible plants. It is interesting that *M. perpusilla* methanol extract which showed the highest FRAP value, highest phenolic and flavonoid contents (Table 5) also exhibited the strongest DPPH radical scavenging activity (Table 4). Olszewska et al. [41] reported that there is a high correlation between phenolic compounds and antioxidant activity, and the phenolic compounds are the main constituents for the antioxidant property. Xia et al. [36] reported that plant having the highest phenolic content showed the similar ability to scavenge DPPH free radical and similar reducing power. Samappito et al. [42] also reported in their work that the sample with the highest amount of phenolics had a very high level of scavenging activity. The consumption of plant foods with high content polyphenols can lessen the risk of heart disease by slowing the development of atherosclerosis due to their antioxidant properties [43, 44].

3.6. Pearson's correlation

Pearson's correlation study of the plant species showed that FRAP assay is strongly correlated with TPC and TFC, and TPC also displayed a strong positive correlation with TFC significantly at $p < 0.01$ (Table 6). A strong positive correlation of FRAP assay with TPC and TFC was also reported by Islary et al. [45] which is in agreement with this study. Moreover, this study also presented a positive correlation of DPPH assay with ABTS and Vitamin C, ABTS assay with H_2O_2, FRAP assay with Vitamin C, and TPC with Vitamin C. Similar types of reports on some wild plants and fruits from Assam of North-East India were also presented by Narzary et al. [3] and Islary et al. [12].

4. Conclusion

The investigation revealed that all the six wild edible plants have variable quantities of proximate composition and mineral contents. The phytochemical analysis showed the presence of many medicinally active secondary

Table 6
Pearson's correlation coefficients of antioxidant activity (DPPH, ABTS, H_2O_2, FRAP), TPC, TFC and vitamin C in the plant species

	DPPH	ABTS	H_2O_2	FRAP	TPC	TFC	Vitamin C
DPPH	1						
ABTS	0.412	1					
H_2O_2	–0.164	0.055	1				
FRAP	–0.559	–0.298	–0.344	1			
TPC	–0.640	–0.131	–0.236	0.969[a]	1		
TFC	–0.660	–0.238	–0.455	0.947[a]	0.947[a]	1	
Vitamin C	0.606	–0.077	–0.279	0.220	0.059	–0.033	1

[a]Correlation is significant at $p < 0.01$.

metabolites. Methanol extracts of the plants exhibited good antioxidant properties with the strongest activity in *Melothria perpusilla*. The analyses of phenolic, flavonoid, and vitamin C contents established the food values of plants which are associated to free radical scavenging activities. Hence, the plants could be good sources of nutritional value and natural antioxidants in improving malnutrition problems, combating many human deficiency diseases and could also be developed as drugs for the prevention and treatment of diseases especially caused by oxidative stress.

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Conflict of interest

None to report.

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