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Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants

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Abstract

The present study aimed to investigate the nutritional properties and antioxidant activities of six underutilized wild edible plants *viz. Ipomoea aquatica, Achyranthes aspera, Aasystasia ganjetica*, *Enhydra fluctuans, Oldenlandia corymbosa* and *Amaranthus viridis* that are commonly consumed as food in the India The antioxidant properties of the plants were evaluated by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, ABTS radical scavenging ability, reducing power capacity, metal chelating activity, lipid peroxidation assay, estimation of total phenolic content and flavonoids content in different solvent extraction system like benzene, chloroform, methanol and 70% aqueous (aq.) ethanol. The quantitation of phenolic acids and flavonoids and

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water soluble vitamins in these plants were carried out by HPLC using Acclaim C 18 column (5 μm particle size, 250 × 4.6 mm), Dionex Ultimate 3000 liquid chromatograph and detection was carried out in photo diode array (PDA) detector. The results of investigation showed that these plants are rich sources of protein, carbohydrate minerals and vitamins, especially the B group of vitamins that can contribute immensely to nutrition, food security, and health and therapeutic benefits. The different levels of antioxidant activities were found in the solvent systems used. The HPLC analysis also showed the presence of phenolic acids and flavonoids in various amounts in these plants which could be utilized as natural antioxidant.

Keywords: Nutrition, Food science, Natural product chemistry

1. Introduction

The wild edible vegetables contribute to the major portion of human diet that provides the essential biochemical's like carbohydrate, protein and lipids. These wild edibles not only contribute to the essential biochemical's and energy requirement but also act as supplementary sources of vitamins and minerals that are indispensable requirement of the body to maintain the proper physiological homoeostasis of the body. Nutritional qualities of wild edibles are comparable and sometimes superior to the domesticated variety (Ebert, 2014). More recently, several researches are focussed on wild edibles as source of food (Narzary et al., 2015; Abdus Satter et al., 2016; Seal et al., 2017a) and medicine, used for the treatment of different diseases like diabetes, jaundice, wounds, cancer etc. as established from different ethno pharmacological studies (Mir, 2014). It has been established that oxidative stress is associated with induction of many chronic and degenerative diseases including atherosclerosis, diabetes, cancer, immunosuppression, neurodegenerative diseases and others (Young and Woodside, 2001). The study of antioxidant activity of medicinal plants and vegetables strongly supports the idea that plant constituents can exert protective effects against oxidative stress in biological systems (Cao et al., 1996). The use of synthetic antioxidants like butylated hydroxyl toluene, butylated hydroxyl anisole and tertiary butyl hydroquinone appeared more effective but continuous usage is associated with cancer and other side effects (Branen, 1975). Therefore there is an increased interest in the search of natural antioxidant sources. Plants based secondary metabolites such as phenolic acids, tannins, alkaloids, flavonoids, terpenoids, lignin, quinones, coumarins, amines and others are the best potential antioxidants (Zheng and Wang, 2001; Cai et al., 2003).

Therefore, the present study was designed to analyse the nutritional composition, minerals content and antioxidant analysis and vitamin content of the traditionally used wild vegetables *viz. Ipomoea aquatica, Achyranthes aspera, Aasystasia*

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ganjetica, *Enhydra fluctuans, Oldenlandia corymbosa* and *Amaranthus viridis* as nutritional supplement for maintenance of a better health.

The leaves of *I. aquatica* are consumed to treat diabetes and the juice is applied to cure wounds (Majumder and Mukherjee, 2015). The paste of aerial parts of *A. aspera* is applied on boils and wounds (Singh and Maheshwari, 1994) and consumed as vegetables by people suffering from piles. *A. ganjetica* has been reported to be used as medicine for the treatment of diabetes (Senthilkumar et al., 2006) and its paste is applied to get relief from rheumatism (Shanmugam et al., 2012). The decoction of *E. fluctuans* is prescribed as antidiabetic (Khan and Yadava, 2010). The aqueous extract of *O. corymbosa* is used for treatment of hepatitis and gastric complaints (Deka and Devi, 2015). The leaves of *A. viridis* are diuretic and prescribed in urinary complaints (Majumder and Mukherjee, 2015). These selected plants apart from being edible are also used medicinally and hence selected for analysis in detail.

2. Materials and methods

2.1. Plant materials

The fresh plant materials *viz. Ipomoea aquatic* Forssk. (family: Convolvulaceae), *Achyranthes aspera* L (family: Amaranthaceae), *Aasystasia ganjetica* (L.) T. Anderson (family: Acanthaceae), *Enhydra fluctuans* Lour.(family: Asteraceae), *Oldenlandia corymbosa* Aiton (family: Rubiaceae) and *Amaranthus viridis* L. (family: Amaranthaceae) were collected from various locations of Kolkata, India and identifications were authenticated from Botanical Survey of India, Howrah. The voucher specimens were preserved in our office. One portion of the plant samples were stored at 15 °C and processed for vitamin estimation. The other part was dried in shade, pulverized and stored in an airtight container to evaluate the antioxidant properties and quantitation of phenolics and polyphenolics by HPLC.

2.2. Estimation of proximate composition

The nutritional composition of the powdered vegetable sample was analyzed as follows in our laboratory following the standard food analysis methods described in the Association of Official Analytical Chemists (AOAC, 2000).

Ash content was estimated by heating plant sample in a muffle furnace for about 5−6 h at 500 °C whereas moisture content were determined by heating plant sample in an air oven at 100−110 °C. The crude lipid was extracted from moisture free sample with petroleum ether (60−80 °C) in a Soxhlet apparatus for about 6−8 h. Estimation of crude fibre content in the plant materials were carried out by treating the fat and moisture free materials with 1.25% dilute acid and 1.25% alkali followed by washing with water and ignition of the residue. The crude protein was determined using micro

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Kjeldahl method as described in AOAC procedures (AOAC, 2000). The total carbohydrate content was estimated as described in the method of Hedge and Hofreiter (1962). The energy content of each plant samples were determined by multiplying the values obtained for protein, fat and available carbohydrate by 4.00, 9.00 and 4.00, respectively and adding up the values (AOAC, 2000).

2.3. Estimation of minerals

Plant material was taken in a pre-cleaned and constantly weighed silica crucible and heated in a muffle furnace at 400 °C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600 °C till the weight of the content was constant (~2–3 h). One gram of sulphated ash obtained above was dissolved in 100 ml of 5 % hydrochloric acid (HCl) to obtain the solution ready for determination of mineral elements through atomic absorption spectroscopy (AAS) (AA 800, Perkin-Elmer Germany). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS (Indrayan et al., 2005).

2.4. Antioxidant activity in different solvent extraction system

2.4.1. Extraction of plant material

One gram of plant material was taken and 20 ml each solvent (benzene, chloroform, methanol and 70% aq. ethanol) and extraction was achieved with agitation for 18–24 h at ambient temperature. The extracts were filtered, diluted up to 25 ml and antioxidant properties were analyzed.

2.4.2. Antioxidant properties of plant materials

The total phenolic content of the different plant extracts was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). The total phenolic content was calculated as gallic acid equivalent (GAE) in mg/g dry weight of extract (DE).

Total flavonoids content was estimated using the method of Ordonez et al. (2006). Total flavonoids contents were calculated as rutin equivalent (RE) mg/g dry weight of extract.

The reducing power of the extracts was determined according to the method of Oyaizu (1986). The reducing power was calculated as ascorbic acid equivalent (AAE) in mg/g of dry extract.

Ferric reducing antioxidant power (FRAP) assay was carried out using the method described by Benzie and Strain (1996). The ability of the plant extract to reduce

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ferric ions of sample was the base of its antioxidant capacity and it was calculated as trolox equivalent (TE) in mg/g dry extract.

The free radical scavenging activity of the plant samples was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) (Blois, 1958). The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Re et al. (1999). The capability to scavenge the DPPH/ABTS radical was calculated, using the following equation:

$$\text{DPPH/ABTS scavenged } (\%) = \{(\text{Ac} - \text{At})/\text{Ac}\} \times 100$$

where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts.

For determination of metal chelating activity, the process of Lin et al. (2009) was followed. The inhibition percentage of ferrozine−Fe^{+2} complex formations was calculated by using the formula given below:

$$\text{Chelating ability } (\%) = \{(\text{Ac} - \text{At})/\text{Ac}\} \times 100$$

where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts.

Anti-lipid peroxidation was assayed following method of Amabye (2015), with modifications. A negative control was maintained that contained all the reagents except the extract. Inhibition of peroxidation was calculated using the formula

$$\text{Inhibition of lipid peroxidation } (\%) = \{(\text{Ac} - \text{At})/\text{Ac}\} \times 100$$

where Ac is the increase of absorbance of the control reaction and At is the increase of absorbance in presence of the sample of the extracts.

2.5. Estimation of phenolic acids and flavonoids by HPLC

2.5.1. HPLC equipment

HPLC analyses were performed using Dionex Ultimate 3000 liquid chromatograph including a diode array detector (DAD) with 5 cm flow cell and with Chromeleon system manager as data processor. Separation was achieved by a reversed-phase Acclaim C18 column (5 micron particle size, 250 × 4.6 mm). 20 μL of sample was introduced into the HPLC column.

2.5.2. Preparation of standard solutions

The stock solution of 1 mg/ml concentration the of standard phenolics acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid,

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vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid) and flavonoids (catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin and kaempferol) was prepared in methanol. The working solutions were prepared by diluting the standard solution with the mobile phase solvent system. The standard and working solutions were filtered through 0.45 μm PVDF-syringe filter before injecting in the HPLC instrument.

2.5.3. Chromatographic analysis of phenolic acids and flavonoids

HPLC analyses for the quantification of phenolic acids and flavonoids in the extract was performed following method of Seal et al. (2017a) using Dionex Ultimate 3000 liquid chromatograph including a diode array detector (DAD) with 5 cm flow cell and with Chromeleon system manager as data processor. Separation was achieved by a reversed phase Acclaim C18 column (5 micron particle size, 250 × 4.6 mm). 20 μL of sample was introduced into the HPLC column. The method was validated according to the USP and ICH guidelines. The mobile phase contains methanol (Solvent A) and 0.5% aq. acetic acid solution (Solvent B) and the column was thermostatically controlled at 25 °C and the injection volume was kept at 20 μl. A gradient elution was performed by varying the proportion of solvent A to solvent B. Total analysis time per sample was 105 min. HPLC chromatograms were detected at three different wavelengths (272, 280 and 310 nm) using a photo diode array UV detector. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of phenolic acids and flavonoids in the extracts were carried out by the measurement of the integrated peak area and the contents were calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in triplicate.

2.6. Estimation of water soluble vitamins by HPLC

2.6.1. Preparation of mixture standard vitamin solutions

The stock standard solutions of vitamin C, B1, B3, B5 and B6 and were prepared by dissolving 25 mg of the each standard in 1 ml 0.1M hydrochloric acid in 25 ml standard volumetric flask. For preparation of standard stock solutions of vitamin B9 and B2, 25 mg of the each standard were dissolved in one ml 0.1 M sodium hydroxide in 25 ml standard volumetric flask. The standard solution was stored in amber-glass bottles in the refrigerator at 4 °C. The working standards were prepared by diluting with phosphate buffer (1M, pH 5.5).

2.6.2. Preparation of sample solution

Plant materials were washed with distilled water. The washed plant materials were cut into very small pieces, frozen in liquid nitrogen and kept at −20 °C until analysis.

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1 g each of freeze-dried sample was soaked in 10 ml water and extracted with 1 ml 0.1M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in dark for 24 hours. The solution was first filtered through a Whatman No. 1 filter paper and the resulting filtrate was taken in a 25 ml volumetric flask and solution was topped up to the mark with HPLC grade water. The sample solution was filtered through 0.45 μm membrane filter before injection into LC system. The stock solutions of sample were kept in a refrigerator for further use.

2.6.3. Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by Seal et al. (2017a) with minor modifications. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (Solvent B), the column was thermostatically controlled at 220 C and the injection volume was kept at 20 μl. A gradient elution was performed by varying the proportion of solvent A to solvent B. Total analysis time per sample was 35 min. HPLC Chromatograms of all vitamins were detected using a photo diode array UV/detector at four different wavelengths (210, 245, 275 and 290 nm) according to absorption maxima of analysed compounds. Detection of compound was done in same manner that followed in detection of phenolic acids and flavonoids. The data were reported as means ± standard error of means of three independent analyses.

3. Results

3.1. Proximate composition

The fresh leaves and stems of the wild edible vegetables were used for nutritional analysis represented in Table 1. The highest moisture content of the fresh vegetables was found in *I. aquatica* (69.11%) and the lowest was in *A. aspera* (53.34%). The ash

Table 1. Proximate composition of edible plants.

Plants	Moisture (%)	Fibre (%)	Ash (%)	Protein (%)	Carbohydrate (%)	Lipid (%)	Energy (kcal/100 g)
I. aqatica	69.11 ± 0.72^{b}	$7.44 \pm 0.27^{d,e}$	$16.37 \pm 0.67^{b,c}$	13.82 ± 0.08^{a}	$10.51 \pm 0.08^{d,e,f}$	2.19 ± 0.08^{a}	$117.27 \pm 0.24^{b,c}$
A. aspera	53.34 ± 0.58^{f}	16.89 ± 0.34^{a}	23.26 ± 0.65^{a}	12.60 ± 0.11^{b}	14.35 ± 0.14^{b}	$1.196 \pm 0.01^{d,e,f}$	118.62 ± 0.06^{b}
A. ganjetica	70.21 ± 0.98^{a}	8.14 ± 0.55^{c}	17.35 ± 0.26^{b}	7.84 ± 0.12^{e}	$10.63 \pm 0.23^{c,d,e}$	$2.04 \pm 0.03^{b,c}$	92.27 ± 0.27^{e}
E. fluctuans	67.69 ± 0.78^{c}	15.37 ± 0.21^{b}	$15.15 \pm 0.44^{c,d}$	$8.00 \pm 0.06^{d,e}$	$9.64 \pm 0.06^{e,f}$	$1.10 \pm 0.01^{e,f}$	80.53 ± 0.16^{f}
O. corymbosa	60.28 ± 0.40^{d}	$7.26 \pm 0.30^{e,f}$	8.34 ± 0.39^{g}	10.52 ± 0.10^{c}	9.08 ± 0.37^{f}	$2.16 \pm 0.06^{a,b}$	97.94 ± 0.04^{d}
A. viridis	55.80 ± 0.23^{e}	6.54 ± 0.28^{g}	$13.31 \pm 0.40^{e,f}$	13.99 ± 0.12^{a}	19.84 ± 0.07^{a}	$1.40 \pm 0.02^{b,c,d}$	148.02 ± 0.28^{a}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM).
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

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content was found highest in *A. ganjetica* (17.35 %) and lowest in *O. corymbosa* (8.34%), the lipid content did not show much variation in these wild edibles studied and ranged between 1-2%. The protein content in *I. aquatica* (13.82 %) and *A. viridis* (13.99%) was comparable. The lowest protein content was observed in the leaves of *A. ganjetica* (7.84 %). The fibre content ranged between 6-8% in *I. aquatica, A. ganjetica, O. corymbosa* and *A. viridis.* The *A. aspera* had the highest (16.89%) fibre content amongst the wild edibles under study followed by *E. fluctuans* (15.37%). The carbohydrate content of the wild vegetables represented in Table 1 was found between 9.08% and 19.84%, which was lowest in *O. corymbosa* and highest in *A. viridis*.

According to the results obtained for the caloric value, *A. viridis* had the highest energy content (148.02 kcal/100 g). *I. aquatica* and *A. aspera* also showed significant calorific value as represented in Table 1.

3.2. Mineral content

The mineral content of the six wild edibles are shown in Table 2. The macro minerals analysed included sodium (Na), potassium (K) and calcium (Ca). The micro minerals included magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn). The mineral content is expressed as mg/g dry plant material, except for Cu which is expressed as μg/g dry plant material. *E. fluctuans* contained the highest amount of K and Ca. *O. corymbosa* contained highest amount of Na and Mg. Highest amount of Fe, Cu, Mn and Zn was observed in *A. viridis, I. aquatica, A. ganjetica* and *A. aspera* respectively.

3.3. Antioxidant activities

3.3.1. Extractive yield

The percentage yields of the four different solvent extracts of the experimental plants under study are shown in Table 3. The extraction yield of these plants varied from 0.9% to 9.88 %. The 70 % hydro-ethanol followed by methanol proved to be better solvent for extraction of antioxidant compounds from the plants compared to chloroform and benzene.

3.3.2. Antioxidant properties of the edible plants

Total phenolic content is expressed as mg gallic acid equivalent (GAE)/g dry extract and is represented in Table 4. The aq. ethanol and methanol extract showed maximum phenolic content as compared to the chloroform and benzene extract in all the plant studied. *A. ganjetica* contained maximum amount of phenolics in the 70% aq. ethanol extract (91.797 ± 0.295 mg/g GAE). The aq. ethanol extracts of

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Table 2. Mineral content in studied plant materials (mg/g dry plant material) Cu (μg/g dry plant material).

Plants	Na	K	Ca	Cu	Mg	Fe	Zn	Mn
I. aquatica	0.840 ± 0.004^{b}	4.406 ± 0.001^{b}	5.983 ± 0.002	6.338 ± 0.004^{b}	$0.044 \pm 0.001^{c,d}$	$0.144 \pm 0.02^{c,d}$	$0.042 \pm 0.003^{a,b,c}$	$0.012 \pm 0.001^{b,c,d}$
A. aspera	0.050 ± 0.001^{f}	3.405 ± 0.003^{c}	5.375 ± 0.003	3.554 ± 0.060^{d}	$0.049 \pm 0.001^{b,c}$	0.054 ± 0.007^{e}	0.050 ± 0.001^{a}	$0.013 \pm 0.002^{b,c,d}$
A. ganjetica	0.427 ± 0.005^{c}	2.740 ± 0.052^{d}	5.661 ± 0.003	4.553 ± 0.073^{c}	$0.042 \pm 0.002^{c,d}$	0.408 ± 0.007^{a}	$0.044 \pm 0.004^{a,b}$	0.021 ± 0.002^{a}
E. fluctuans	0.204 ± 0.004^{d}	4.870 ± 0.067^{a}	6.216 ± 0.055	3.002 ± 0.002^{a}	0.038 ± 0.001^{d}	0.123 ± 0.003^{d}	$0.043 \pm 0.002^{a,b}$	$0.009 \pm 0.001^{b,c,d}$
O. corumbosa	1.111 ± 0.007^{a}	1.216 ± 0.060^{f}	4.926 ± 0.047	2.806 ± 0.067^{e}	$0.053 \pm 0.003^{a,b,c}$	0.319 ± 0.004^{b}	$0.036 \pm 0.001^{b,c,d}$	$0.016 \pm 0.002^{b,c}$
A. viridis	0.104 ± 0.003^{e}	3.316 ± 0.050^{c}	5.736 ± 0.012	1.860 ± 0.049^{c}	0.039 ± 0.001^{d}	0.415 ± 0.002^{a}	$0.019 \pm 0.001^{d,e}$	$0.019 \pm 0.001^{a,b}$

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

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Table 3. Extractive yield (%) of edible plants in different solvent extract.

Plants	70% aq. ethanol	Methanol	Chloroform	Benzene
I. aquatica	9.886 ± 0.060^{a}	5.994 ± 0.117^{b}	2.006 ± 0.094^{c}	1.495 ± 0.058^{b}
A. aspera	$5.258 \pm 0.080^{b,c}$	3.980 ± 0.160^{d}	2.982 ± 0.133^{a}	2.465 ± 0.053^{a}
A. ganjetica	2.559 ± 0.082^{e}	3.980 ± 0.106^{d}	0.999 ± 0.054^{e}	0.993 ± 0.011^{c}
E. fluctuans	6.192 ± 0.044^{b}	2.461 ± 0.104^{e}	0.983 ± 0.038^{e}	0.995 ± 0.032^{c}
O. corymbosa	4.270 ± 0.713^{c}	6.693 ± 0.060^{a}	$1.835 \pm 0.061^{c,d}$	0.898 ± 0.057^{d}
A. viridis	3.246 ± 0.554^{d}	$5.451 \pm 0.178^{b,c}$	2.458 ± 0.086^{b}	0.997 ± 0.057^{c}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

E. fluctuans and *A. Aspera* were also rich in their phenolic content. Methanol, chloroform and benzene extract of *E. fluctuans* showed higher phenolic content than the other wild edibles under study.

Total flavonoids content are expressed as equivalent mg rutin equivalent (RE)/g dry extract and are represented in Table 4. The aq. ethanol and methanol were observed to be more effective in extracting flavonoids than benzene or chloroform in case of the plants studied. The aq. ethanol and methanol extract of *A. aspera* and *A. ganjetica* contained good amount of flavonoids and did not show much variation in the flavonoids content. The maximum flavonoids content were observed in aq. ethanol extract of *E. fluctuans* (21.759 ± 0.039 mg RE).

FRAP is expressed as μ mole Trolox equivalent (TE)/g dry extract and is represented in Table 4. *A. aspera* (2.408 ± 0.002 mg μ mole TE) and *A. ganjetica* (2.057 ± 0.004 mg μ mole TE) showed maximum reducing property in aq. ethanol extract. The methanol extract of these plants showed promising FRAP activity. The FRAP values decreased with the decreasing polarity of the solvent.

Radical scavenging activity using DPPH radical and ABTS radical are shown in Table 4 and expressed as % radical scavenged/g dry extract. The aq. ethanol extract of all the plants studied showed maximum radical scavenging property. Maximum DPPH radical scavenging ability was observed *A. ganjetica* (39.140 ± 0.221%) and *A. aspera* (28.051 ± 0.057%). The chloroform and benzene extract showed minimal radical scavenging activity. Similar results were obtained using ABTS radical, however these plants showed more prominent activity against ABTS radical. Maximum radical scavenging activity was exhibited by aq. ethanol extract of *A. ganjetica* (69.048 ± 0.221%) and *E. fluctuans* (60.797 ± 0.418 %). *A. aspera* also showed promising radical scavenging activity. All the plants under study exhibited better results with aq. ethanol extract followed by methanol extract.

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Table 4. Antioxidant properties of the edible plants.

Antioxidant parameters	Solvent	*Plants*					
		I. aquatica	***A. aspera***	***A. ganjetica***	***E. fluctuans***	***O. corymbosa***	***A. viridis***
Total phenolic content (GAE, mg/g DE)	70% Aq.ethanol	45.449 ± 0.130^{f}	74.831 ± 0.243^{b}	91.797 ± 0.295^{a}	70.338 ± 0.103^{c}	47.184 ± 0.060^{e}	50.700 ± 0.079^{d}
	Methanol	13.953 ± 0.534^{e}	37.276 ± 0.321^{c,d}	34.487 ± 0.321^{d}	63.744 ± 0.925^{a}	39.992 ± 0.266^{c}	48.858 ± 0.418^{b}
	Chloroform	12.692 ± 0.110^{f}	31.325 ± 0.931^{c,d}	26.026 ± 0.282^{e}	57.436 ± 0.696^{a}	33.472 ± 0.039^{c}	42.718 ± 0.681^{b}
	Benzene	7.094 ± 0.427^{f}	26.051 ± 0.256^{c}	10.000 ± 0.110^{e}	35.641 ± 0.641^{a}	24.644 ± 0.884^{d}	33.718 ± 0.696^{b}
Total flavonoids content (RE, mg/g DE)	70% Aq.ethanol	13.941 ± 0.040^{f}	20.793 ± 0.122^{b}	20.132 ± 0.093^{b,c}	21.759 ± 0.039^{a}	15.848 ± 0.125^{e}	19.970 ± 0.252^{d}
	Methanol	10.856 ± 0.013^{f}	22.019 ± 0.020^{a}	20.412 ± 0.143^{b}	16.750 ± 0.066^{d}	14.884 ± 0.157^{e}	18.591 ± 0.577^{c}
	Chloroform	8.943 ± 0.040^{f}	14.172 ± 0.081^{b}	20.174 ± 0.200^{a}	12.848 ± 0.121^{d}	12.266 ± 0.043^{d,e}	13.637 ± 0.032^{b,c}
	Benzene	6.855 ± 0.081^{e}	10.542 ± 0.073^{c,d}	17.518 ± 0.121^{a}	11.978 ± 0.079^{b}	10.561 ± 0.088^{c,d}	10.604 ± 0.079^{c}
FRAP assay (TE, mg/g DE)	70% Aq.ethanol	1.338 ± 0.004^{e}	2.408 ± 0.002^{a}	2.057 ± 0.004^{b}	1.999 ± 0.002^{c}	1.454 ± 0.011^{d,e}	1.553 ± 0.004^{d}
	Methanol	1.296 ± 0.003^{f}	2.178 ± 0.002^{a}	2.051 ± 0.004^{b}	1.934 ± 0.003^{c}	1.340 ± 0.004^{e}	1.510 ± 0.004^{d}
	Chloroform	0.618 ± 0.005^{f}	1.529 ± 0.002^{b}	1.805 ± 0.002^{a}	1.285 ± 0.002^{c}	0.723 ± 0.004^{e}	0.853 ± 0.002^{d}
	Benzene	0.456 ± 0.003^{c}	0.488 ± 0.002^{b}	0.822 ± 0.002^{a}	0.434 ± 0.003^{d}	0.419 ± 0.002^{f}	0.425 ± 0.001^{e}
DPPH (% of inhibition)	70% Aq.ethanol	12.172 ± 0.066^{e}	28.051 ± 0.057^{b}	39.140 ± 0.221^{a}	19.173 ± 0.289^{c}	12.937 ± 0.125^{d,e}	13.126 ± 0.263^{d}
	Methanol	7.831 ± 0.257^{f}	12.907 ± 0.075^{c}	29.117 ± 0.300^{a}	15.831 ± 0.278^{b}	10.476 ± 0.095^{d}	8.671 ± 0.261^{e}
	Chloroform	3.166 ± 0.072^{d}	4.756 ± 0.176^{c}	14.916 ± 0.477^{a}	6.404 ± 0.221^{b}	2.619 ± 0.126^{e}	4.216 ± 0.199^{c}
	Benzene	3.594 ± 0.257^{d,e}	4.837 ± 0.363^{a}	4.535 ± 0.119^{b}	3.779 ± 0.105^{d}	1.571 ± 0.082^{f}	4.177 ± 0.182^{c}
ABTS (% of inhibition)	70% Aq.ethanol	40.028 ± 0.783^{c}	59.782 ± 0.207^{b}	69.048 ± 0.221^{a}	60.797 ± 0.418^{b}	41.727 ± 0.128^{c}	42.644 ± 0.048^{c}
	Methanol	33.461 ± 0.444^{d}	52.745 ± 0.069^{b}	53.801 ± 0.328^{a}	44.714 ± 0.968^{c}	30.089 ± 0.366^{e}	29.060 ± 0.064^{f}
	Chloroform	12.788 ± 0.074^{f}	28.759 ± 0.138^{a}	24.966 ± 0.234^{b,c}	23.887 ± 0.206^{c}	17.059 ± 0.704^{e}	18.444 ± 0.119^{d}
	Benzene	5.627 ± 0.256^{e}	0.676 ± 0.143^{f}	9.312 ± 0.412^{b}	8.322 ± 0.274^{c}	8.059 ± 0.926^{c,d}	11.156 ± 0.162^{f}
Reducing power (AAE, mg/g DE)	70% Aq.ethanol	14.786 ± 0.133^{d}	48.112 ± 0.586^{a}	41.242 ± 0.991^{b}	18.808 ± 0.101^{c}	13.270 ± 0.179^{f}	15.955 ± 0.160^{e}
	Methanol	13.521 ± 0.121^{c}	35.223 ± 0.087^{a}	21.721 ± 0.181^{b}	12.594 ± 0.145^{d}	12.952 ± 0.270^{c,d}	13.426 ± 0.132^{c}
	Chloroform	8.14 ± 0.15^{e}	23.37 ± 0.19^{a}	18.15 ± 0.14^{b}	9.28 ± 0.32^{d}	9.35 ± 0.26^{d}	10.24 ± 0.55^{c}
	Benzene	6.25 ± 0.06^{f}	15.25 ± 0.12^{a}	11.16 ± 0.04^{b}	8.33 ± 0.12^{d}	7.35 ± 0.16^{e}	9.18 ± 0.75^{c}
Metal chelating activity (% of inhibition)	70% Aq.ethanol	17.936 ± 0.284^{f}	47.912 ± 0.142^{b}	52.416 ± 0.164^{a}	35.217 ± 0.217^{c}	20.229 ± 0.357^{e}	22.359 ± 0.491^{d}
	Methanol	15.366 ± 0.745^{e}	35.203 ± 0.293^{b}	37.805 ± 0.141^{a}	21.138 ± 0.215^{c}	14.390 ± 0.141^{f}	20.081 ± 0.215^{d}
	Chloroform	11.24 ± 0.05^{e}	25.12 ± 0.09^{b}	31.25 ± 0.04^{a}	14.58 ± 0.22^{d}	10.23 ± 0.16^{f}	16.44 ± 0.35^{c}
	Benzene	7.15 ± 0.09^{f}	18.55 ± 0.02^{b}	21.06 ± 0.14^{a}	9.28 ± 0.02^{d}	8.44 ± 0.06^{d,e}	11.08 ± 0.25^{c}
Lipid peroxidation assay (% of inhibition)	70% Aq.ethanol	18.268 ± 0.057^{f}	49.449 ± 0.088^{a}	45.039 ± 0.072^{b}	24.094 ± 0.273^{c}	22.677 ± 0.067^{d}	21.102 ± 0.085^{e}
	Methanol	13.540 ± 0.11^{d,e}	37.094 ± 0.038^{a}	30.606 ± 0.035^{b}	14.104 ± 0.168^{d}	5.501 ± 0.058	16.079 ± 0.036^{c}
	Chloroform	9.230 ± 0.05^{d}	16.15 ± 0.04^{a}	13.45 ± 0.02^{b}	8.30 ± 0.14^{e}	3.45 ± 0.07^{f}	11.06 ± 0.09^{c}
	Benzene	4.12 ± 0.09^{d}	7.06 ± 0.08^{a}	6.25 ± 0.06^{b}	3.20 ± 0.11^{e}	1.55 ± 0.09^{f}	5.11 ± 0.08^{c}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

Since 70% aq. ethanol followed by methanol extracts gave promising activity both in polyphenolic content and total antioxidant capacity these two extracts were chosen for further study.

Reducing property is expressed as mg ascorbic acid equivalent (AAE)/g dry extract and is represented in Table 4. *A. aspera* (48.112 ± 0.586 mg AAE) and *A. ganjetica* (41.242 ± 0.991 mg AAE) showed maximum reducing property in aq. ethanol extract. *A. aspera* also showed promising reducing activity in methanol extract. The reducing activity decreased with the decreasing polarity of the solvent.

Metal chelating activity is expressed as % inhibition of metal ions/g dry extract and represented in Table 4. Maximum chelating activity is observed in aq. ethanol extract followed by methanol extract for all the plants under study. Aq. ethanol extracts of *A. ganjetica* (52.416 ± 0.164 %) and *A. Aspera* (47.912 ± 0.142 %) showed highest chelating activity. Methanol extract of these plants also showed promising metal chelating activity compared to the other wild edibles under study. *I. aquatica* and *O. corymbosa* showed lowest chelating ability.

Anti-lipid peroxidation assay was performed using 70% aq. ethanol and methanol extract and is expressed as % inhibition of lipid peroxidation/g dry extract and is represented in Table 4. *A. aspera* (49.449 ± 0.088 %) and *A. ganjetica* (45.039 ± 0.072%) showed maximum inhibition of lipid peroxidation in aq. ethanol extract. The methanol extract of these two plants also showed greater efficacy in inhibiting lipid peroxidation than other plant extracts. Inhibition of lipid peroxidation is more effective using aq. ethanol extract over methanol extract.

3.4. Quantitative profiling of phenolic acids and flavonoids in the 70 % aq. ethanol extract of different plant materials under study by RP-HPLC

A total of 13 phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*- hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid) and 8 flavonoids (catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin and kaempferol) were analyzed, expressed as mg/100 g dry plant material (DPM) and is represented in Table 5. All investigated compounds had responses at 260 nm, where they were successfully separated. The constituents under investigation were also identified by the recorded absorption spectra, which were comparable both for plant extracts and standard substance. *p*-hydroxy benzoic acid and *p*-coumaric acid was reported only from *I. aquatica,* gallic acid from *A. viridis* and salicylic acid from *A. ganjetica. A. aspera* contained maximum amount of vanillic acid (0.690 ± 0.006 mg/100 g DPM). *E. fluctuans* contained maximum amount of ferulic acid

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Table 5. Quantitative estimation of phenolic acids and flavonoids in the 70 % aq. ethanol extract of edible plants by HPLC (mg/100 g dry plant material).

Phenolic acids/flavonoids	Plants					
	I. aquatica	*A. aspera*	*A. ganjetica*	*E. fluctuans*	*O. corumbosa*	*A. viridis*
Gallic acid	ND	ND	ND	ND	ND	0.145 ± 0.001^{a}
Protocatechuic acid	ND	ND	ND	ND	ND	ND
Gentisic acid	ND	ND	ND	ND	ND	ND
p-Hydroxy benzoic acid	0.033 ± 0.003^{a}	ND	ND	ND	ND	ND
Chlorogenic acid	1.827 ± 0.001^{b}	ND	ND	ND	ND	3.807 ± 0.001^{a}
Vanillic acid	0.360 ± 0.002^{c}	0.690 ± 0.006^{a}	ND	ND	0.500 ± 0.002^{b}	ND
Caffeic acid	ND	ND	ND	ND	ND	ND
Syringic acid	0.404 ± 0.001^{b}	ND	ND	ND	ND	6.065 ± 0.002^{a}
p-Coumaric acid	0.374 ± 0.01^{a}	ND	ND	ND	ND	ND
Ferulic acid	ND	0.194 ± 0.004^{d}	0.249 ± 0.007^{c}	0.375 ± 0.008^{a}	0.281 ± 0.002^{b}	0.104 ± 0.002^{e}
Sinapic acid	0.055 ± 0.001^{b}	ND	ND	ND	0.267 ± 0.002^{a}	ND
Salicylic acid	ND	ND	1.518 ± 0.012^{a}	ND	ND	ND
Ellagic acid	ND	ND	ND	0.366 ± 0.007^{a}	0.025 ± 0.002^{c}	0.063 ± 0.001^{b}
Naringin	ND	ND	ND	ND	ND	ND
Rutin	0.727 ± 0.001^{a}	0.058 ± 0.005^{b}	ND	ND	ND	ND
Myricetin	1.787 ± 0.002^{a}	ND	0.414 ± 0.008^{b}	ND	ND	ND
Quercetin	ND	0.147 ± 0.006^{c}	1.239 ± 0.005^{a}	0.184 ± 0.003^{b}	0.091 ± 0.002^{d}	ND
Naringenin	ND	ND	ND	ND	ND	ND
Apigenin	ND	3.592 ± 0.061^{b}	0.466 ± 0.012^{c}	0.194 ± 0.003^{d}	0.121 ± 0.002^{e}	4.231 ± 0.002^{a}
Kaempferol	ND	0.489 ± 0.006^{a}	0.196 ± 0.001^{c}	0.244 ± 0.005^{b}	0.098 ± 0.001^{d}	ND
Catechin	ND	ND	9.447 ± 0.009^{a}	ND	ND	ND

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM ND: Not detected.
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

(0.375 ± 0.008 mg/100 g DPM) and ellagic acid (0.366 ± 0.007 mg/100 g DPM). *O. corymbosa* contained maximum amount of syringic acid (0.267 ± 0.002 mg/100 g DPM). *A. viridis* contained high amount of chlorogenic acid (3.807 ± 0.001 mg/100 g DPM) and syringic acid (6.065 ± 0.002 mg/100 g DPM). Gentisic acid, protocatechuic acid, caffeic acid, naringin and naringenin were not recorded from any of the plant sample. Maximum amount of rutin (0.727 ± 0.001 mg/100 g DPM) and myricetin (1.787 ± 0.002 mg/100 g DPM) were recorded from *I. aquatica*, apigenin (3.592 ± 0.061 mg/100 g DPM) and kaempferol (0.489 ± 0.006 mg/100 g DPM) from *A. aspera,* quercetin (1.239 ± 0.005 mg/100 g DPM) and catechin (9.447 ± 0.009 mg/100 g DPM) from *A. ganjetica.*

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3.5. Quantitative and qualitative profiling of water soluble vitamins of different plant materials under study by RP-HPLC

Water soluble vitamin analysed included Ascorbic acid (vitamin C), thiamine (vitamin B1), niacin (vitamin B3), pyridoxine (vitamin B6), pantothenic acid (vitamin B5), folic acid (vitamin B9), riboflavin (vitamin B2) and is expressed as mg/100 g dry plant material (DPM) in Table 6. All investigated compounds had responses at 275 nm, where they were successfully separated. The constituents under investigation were identified by the recorded absorption spectra, which were comparable both for plant extracts and standard substances.

Vitamin B1, B2, B6 and B9 is ubiquitously present in all studied plant samples. Vitamin B5 was not recorded in any of the wild edibles. Ascorbic acid was present in *A. ganjetica*, *O. corymbosa* and in highest amount in *A. aspera* (151.75 mg/100 g dry plant material). Vitamin B3 was found in *A. aspera*, *E. fluctuans, A. viridis* and *I. aquatica* contained highest amount of vitamin B1, B6 and B9.

4. Discussion

4.1. Proximate composition

Moisture content is the quantity of water in a material. Water is an essential compound of many foods. 20% of the total water consumption is through food moisture (FNB, 2005). When foods are eaten, the water content in them is absorbed by the body. All the plant under investigation had moisture content ranging from 53−70%. The relatively high moisture contents reveal that the studied leafy vegetables need care for appropriate preservation as they are more liable to microbial degradation (Kwenin et al., 2011), and would also exhibit a greater activity of water soluble enzymes (Iheanacho and Udebuani, 2009). The moisture content is largely dependent on humidity, temperature and harvest time of the species.

Table 6. Water soluble vitamin content in the edible plants by HPLC (mg/100 g DPM).

Plants	C	B1	B2	B3	B5	B6	B9
I. aqatica	ND	0.454 ± 0.007^{a}	$0.710 \pm 0.005^{c,d}$	ND	ND	2.419 ± 0.005^{a}	0.174 ± 0.006^{b}
A. aspera	151.75 ± 0.333^{a}	0.131 ± 0.003^{c}	$0.384 \pm 0.013^{d,e}$	0.113 ± 0.006^{c}	ND	1.227 ± 0.003^{d}	0.074 ± 0.002^{c}
A. ganjetica	1.507 ± 0.001^{c}	0.007 ± 0.001^{f}	2.511 ± 0.006^{a}	ND	ND	0.759 ± 0.010^{e}	0.077 ± 0.001^{c}
E. fluctuans	ND	0.404 ± 0.003^{b}	1.043 ± 0.002^{b}	0.604 ± 0.002^{a}	ND	1.850 ± 0.004^{b}	0.050 ± 0.005^{d}
O. corumbosa	3.791 ± 0.005^{b}	$0.049 \pm 0.001^{d,e}$	0.472 ± 0.011^{d}	ND	ND	$1.278 \pm 0.001^{c,d}$	0.245 ± 0.002^{a}
A. viridis	ND	$0.044 \pm 0.003^{d,e}$	0.012 ± 0.001^{f}	0.258 ± 0.003^{b}	ND	1.308 ± 0.011^{c}	0.038 ± 0.004^{e}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM ND: Not detected.
Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c d, e, f denotes the significance of various parameters. Letter a is significant to b, c, d, e, f.

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Ash contains inorganic material of the plant which includes oxides and salts containing anions such as phosphates, sulphates, chlorides and other halides and cations such as sodium, potassium, calcium, magnesium, iron, and manganese (Gopalan et al., 2004). The ash content indicates the amount of minerals in that food. The ash content ranged between 7−23% for the plant materials under study. The ash content of these wild vegetables corroborates the results reported for some commonly used edibles of Bangladesh, Arunachal Pradesh and Meghalaya of India (Abdus Satter et al., 2016; Seal et al., 2013, 2017b) and higher than the commonly consumed leafy vegetables of Bodo tribe in Assam, India (Narzary et al., 2015). This variation may be due to ecological factors or age of the plant samples under study. Food fibres are advantageous in increasing dietary bulk due to their ability to absorb water therefore easing the intestinal transit (Jenkin et al., 1986). The RDA of dietary fibre for adult males and females is 38 and 25 g/day, respectively (Trumbo et al., 2002). Fibres can lower the risk of coronary heart disease, serum cholesterol, hypertension, diabetes, and breast and colon cancer (Rehman et al., 2014; Vadivel and Janardhanan, 2005). So, the content of fiber in the wild vegetables used in our study can encourage their use in the human diet to fulfill the fibre RDA. The total lipid content ranged between 1−2 % in these plants, which was in congruence to the findings of many works surmised that leafy vegetables are poor sources of lipids (Ejoh et al., 1996). Diet providing 1−2 % of its caloric energy as lipid is said to be sufficient to human beings, as excess lipid consumption results in cardiovascular disorders (Kris-Etherton et al., 2002). The lipid content of these plants under study similar to different studies made on wild edible plants of Bangladesh and India (Abdus Satter et al., 2016; Seal et al., 2013, 2017b). However the lipid content in wild edibles of Cameroon was found to be higher, ranging between 4−14% (Mih et al., 2017), than that reported from this study. Fats also provide essential fatty acids like linoleic and linolenic acid, which can only be obtained from food. They are important for controlling inflammation, blood clotting, and brain development. The absorption of lipid soluble vitamins like vitamin A and carotene in the body is also enhanced by the presence of fat (Gopalan et al., 2004).

The protein content ranged from 7−13 % and the value obtained in this study was almost similar to the values of some underutilized green leafy vegetables reported by Gupta et al. (2005) and also to some edible invasive species of West Bengal, India reported by Datta et al. (2018). Foods which provide more than 12 % of their calorific value from proteins have been shown to be good source of proteins (Ali, 2009). Therefore these plants if included as a part of diet can play a significant role in providing cheap and easily available proteins for rural communities.

Total carbohydrate content ranged from 9−19%. The carbohydrate contents of some green leafy vegetables of Sonitpur district of Assam, India reported by Saha et al. (2015) ranged from 5 − 11% and carbohydrate content from wild edibles consumed by Bodo tribe of Assam, India, also indicated similar carbohydrate content which

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ranged between 4 − 12% (Narzary et al., 2015) which are close to the values obtained in this study. Vishwakarma and Karma (2011) also reported similar values of carbohydrate contents in wild edible herbs used in Eastern Chattisgarh, India. The carbohydrate content of the vegetables was generally low compared to those reported by Abdus Satter et al. (2016) for species in Bangladesh and Seal and Chaudhuri (2016); Seal et al., (2017b) for NE India and the results were considerably higher than the reported values when compared to some wild edible plants (3%) of Pakistan (Khan et al., 2013). The recommended carbohydrate values for children and adults are 130 g. It implied that 6.9−14.61 % of the daily requirement could be reached when 100 g of these dried plants are consumed.

In the present study, the calorific value ranged from 80−148 kcal/100 g of dry sample. Ullah et al. (2013) studied the calorific value of selected medicinal plants of Tank and South Waziristan Area of Pakistan and reported to be ranged from 261.33 to 485.70 kcal per 100 g of dry sample. Similar calorific value has been obtained on studies of wild edibles of Bangladesh (Abdus Satter et al., 2016) and NE India (Seal and Chaudhuri, 2016; Seal et al., 2017b). The calorific values of these wild edibles are higher than some edible invasive species which ranged between 70−124 kcal/100 g (Datta et al., 2018). The plants with a high calorific value can be considered as a good diet. High calorific value is an obvious indication towards the fact that that these wild plants can be used as food or may be included as a part of dietary supplements. Thus, the calorific value agreement with general observation that vegetables have low energy values (Lintas, 1992). The outcome of investigation revealed that these plants had greater nutritive potential than the common leafy vegetables like cabbage (27 kcal/100 g), spinach (26 kcal/100 g) and lettuce (21 kcal/100 g) (Gopalan et al., 2004).

Leaves of *E. fluctuans* were evaluated for its nutritional attributes and showed to be a rich source of carbohydrate (61%) and protein (16%). It is also a rich reserve of minerals like calcium, potassium, sodium, magnesium and iron (Abdus Satter et al., 2016). Our study shows lesser amount of protein and carbohydrate content. Nutritional analysis of different parts of the *O. corymbosa* was evaluated separately. Carbohydrate content (62%) was maximum in roots whereas protein content (13%) was maximum in leaves. Level of fat was relatively the lowest in all parts (Ezeabara and Egwuoba, 2016). Our study also corroborates the above finding. The seeds of *A. viridis* possess 14−16% protein and 4.7−7% fat. The leaves are a rich source of carbohydrate (44%), protein (34%) and ash (16%) and vitamin A, riboflavin, vitamin C, niacin (Duke and Ayensu, 1985), which is much higher than that obtained from our study. Seeds of *A. aspera* were evaluated for protein, fibre and ash content which was found to be 18.91%, 21.03% and 2.86% (Talreja et al., 2015), seeds appeared to be richer source of food value compared to the leaves. Study by Saha et al. (2015) reported the moisture and ash content of the fresh aerial parts of the plant as 87.63% and 10.55% respectively, our finding is in congruence with the above study. The

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variation in the nutritional composition may be attributed to the differences in edaphic and other environmental conditions in the different study areas, time of harvesting and age of the plant.

4.2. Mineral content

The essential minerals K, Na, Ca, Mg and trace minerals Fe, Zn, Cu and Mn of the wild vegetables on dry weight basis are shown in Table 2. Sodium (Na) concentration ranged between 0.050−1.111 mg/g dry materials. The sodium levels of some cultivated vegetables and fruits vary between 0.030-1.249 mg/g (Gopalan et al., 2004). The potassium (K) ranged between 1.216 − 4.870 mg/g. Na and K maintains the ionic balance of the human body and maintain tissue excitability. Na plays an important role in the transport of metabolites and K is important for its diuretic nature. The ratio of K/Na in any food is an important factor associated with hypertension and arteriosclerosis. Na enhances and K depresses blood pressure (Saupi et al., 2009). The ratio of K/Na in *A. aspera* (67.019), *E. fluctuans* (24.047), *A. viridis* (32.476) are comparable to some common fruits (*Castanea sativa* − 56.67, Amla − 45, ripe papaya − 11.5, tomato − 11.31) (Sundriyal and Sundriyal, 2004). The Na/K ratio (lower than 1) revealed that the vegetables could be valuable in ameliorating sodium-related health risks (Appiah et al., 2011). The Ca levels varied within 1.860−6.338 mg/g whereas that in of some cultivated vegetables (lettuce, cabbage and spinach) varies between 0.39 −0.73 mg/g (Gopalan et al., 2004). The results from this study were almost similar to the wild leafy vegetables consumed in Bangladesh (Abdus Satter et al., 2016), lower compared to the wild green leafy vegetables consumed in North-East India which ranged between 12.52 − 27.91 mg/g (Seal and Chaudhuri, 2016) but much higher than the vegetables consumed as food in Pakistan (9.00−181) mg/100 g (Hussain et al., 2011), and Cameroon (2.12−60.02 mg/100 g) (Mih et al., 2017). Based on the data it can be concluded that these wild plants could provide a good source of Ca to our diet. It is also important for blood coagulation and the normal functioning of the cardiac muscles (Sundriyal and Sundriyal, 2004).

The iron content of these plants ranged between 0.054 − 0.415 mg/g which compared favourably to most of the values reported from 21.30 mg/100 g to 33.40 mg/100 g for some commonly and wildly consumed leafy vegetables in Kano, Nigeria (Mohammed and Sharif, 2011), and 0.240−1.396 mg/g for wild leafy vegetables of Meghalaya, India (Seal et al., 2013). Iron is essential in oxygen binding to haemoglobin and also acts as catalyst for many enzymes like cytochrome oxydase (Geissler and Powers, 2005). Thus, the selected leaves of this study could be recommended in diets for reducing anaemia. Magnesium helps to prevent muscle degeneration, growth retardation, cardiomyopathy, immunologic dysfunction, impaired spermatogenesis and bleeding disorders (Chaturvedi et al., 2004). Saikia

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and Deka (2013) have reported that the Mg content was highest (201.20 ± 10.50 mg/100 g) and lowest (30.30 ± 2.30 mg/100 g) in the twenty one wild green leafy vegetables of North-East India. *Echinops giganteus, Capsicum frutescens, Piper guineense* and *Piper umbellatum* from Cameroon were evaluated for Mg content and it ranged between was found 89−490 mg/100 g, respectively (Bouba et al., 2012). The magnesium content of the plants under study, represented in Table 3 is much lower than these previous reports. The recommended dietary allowance (RDA) for calcium (1000 mg/day), magnesium (400 mg/day) and iron (8 mg/day) suggest that these plants contribute substantially in improving the diet in terms of mineral requirement. Manganese (Mn) acts as the cofactor for the enzymes like arginase, and glycosyl transferase. There are other enzymes like phosphoenol pyruvate carboxy kinase and glutamine synthetase, which are activated by Mn ions. Mn is also essential for haemoglobin formation (Indrayan et al., 2005). The Mn concentration ranged between 0.009−0.021 mg/g, which is lower than the values from wild edible of Meghalaya (Seal and Chaudhury, 2016) and Arunachal Pradesh (Seal et al., 2017b). Zinc has a role in stabilizing macromolecular structure and synthesis. The role of the metal ion in the DNA and RNA synthesis is well documented and both DNA and RNA polymerases are zinc-dependent enzymes. Zn concentration ranged between 0.019−0.050 mg/g, which is similar to the levels reported in some wild and leafy vegetables in India (Salkia and Deka, 2013), Bangladesh (Abdus Satter et al., 2016) and Nigeria (Mohammed and Sharif, 2011) and higher than the reports from Cameroon (Mih et al., 2017). Copper (Cu) acts as an important part of copper protein. Cytochrome C oxidase, lysyl oxidase and tyrosine oxidase are the major Cu containing metalloenzymes. The recommended intake of copper is 1.35 mg/day and keeping this in mind all the plants under study is devoid of copper toxicity. The Cu content reported in this study was lower than earlier reports of wild edible from Meghalaya, Arunachal Pradesh of India (Seal et al., 2013, 2017b) and Cameroon (Mih et al., 2017). The bioavailability of these minerals is largely affected by the presence of antinutrients but their effects can be the considerably reduced after processing such as soaking, boiling or frying (Ekop and Eddy, 2005).

4.3. Antioxidant activity

4.3.1. Extractive value

The process of extraction solubilises the phenolic compounds of the plant sample studied and hence it is an essential part of estimation of antioxidant activity. The effect of solvent system on extraction has been variously studied in wild edibles (Seal, 2014; Fatiha et al., 2012; Sultana et al., 2007). The amount of the antioxidant components extracted from a plant is affected by the plant parts used, extraction procedure and efficiency of the extracting solvent to dissolve the antioxidant compounds and finally the availability of extractable components (Siddhuraju and Becker, 2003; Hsu

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et al., 2006; Sultana et al., 2007). In this study four different solvent systems of varying polarity *viz*. 70% hydro-ethanol, methanol, chloroform and benzene were used as extracting solvent. Based on the results reported here, the highest extraction yield was found with hydro-alcoholic solvents. This indicates that most of the components in these plants are water-soluble. Addition of water to organic solvent increases polarity and facilitates the extraction of antioxidant compounds of wide range of polarity (Uma et al., 2010). Hydro-alcohol has revealed to be more efficient in extracting phenolic constituents than compared to pure solvent system (Spingo et al., 2007). High content of antioxidant compounds was obtained with 80 % aq. methanol from plant materials like rice bran, wheat bran, coffee beans, citrus peel and guava leaves (Anwar et al., 2006). Methanol proved to be a better extracting solvent in case of wild leafy vegetables (Seal et al., 2013a) and wild edible fruits (Seal et al., 2013b).

4.3.2. Total phenolic content

Methanol, ethanol and acetone are commonly used as solvents for extraction of phenolic compounds (Bimakr et al., 2011). Results obtained confirm that extracting solvent largely affects the phenolic content. Phenolics are secondary metabolites of plants that are very important for their antioxidant activity by chelating metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversions into reactive oxyradicals (Sahreen et al., 2010; Florence et al., 2011). Inhibitory effects of polyphenolic compounds on mutagenesis and carcinogenesis has also been established (Gursoy et al., 2009). Among all the solvent extracts; the hydro-ethanol extracts had the highest phenolic content followed by methanol and it decreased with decreasing polarity. Phenolics are often extracted in higher amounts in more polar solvents such as aqueous methanol/ethanol as compared with absolute methanol/ethanol (Anwar et al., 2006; Seal et al., 2013a, 2013b; Siddhuraju and Becker, 2003; Sultana et al., 2007) which are in accordance to the finding reported here. Often phenolics remain associated with different biomolecules like carbohydrate, pigments, terpenes, therefore 100% recovery of a compound cannot be achieved using a single solvent system. High yield using methanol may be due to the ability of the solvent to dissolve the associated biomolecules. Moreover methanol has the ability to inhibit phenol oxidase and hence is suitable for extraction of polyphenols. This may be the reason that methanol extract of the selected plants in this study showed high phenolic content. In general, 40−80% ethanol or methanol, had greater efficacy in the extraction of polyphenolic compounds compared to water or pure ethanol or methanol (Jayaprakasha et al., 2008).

4.3.3. Total flavonoids content

Flavonoids are widely distributed group of plant phenolic compounds responsible for the antioxidant activity of the plants. It has been proven to display a wide range

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of pharmacological and biochemical activities including radical scavenging properties (Bravo, 1998; Pourmorad et al., 2006; Prasad et al., 2009). The flavonoids content in the plants under study was highest in 70% ethanol. Similar observation was made in case of *Torilis leptophylla* where the highest amount of flavonoids content was analyzed in ethanolic extract, followed by that in methanol and benzene (Saeed et al., 2012). The flavonoids content from the methanol extract was comparable to the flavonoids content in the 70% aq.ethanol extract, in this study. This data is in congruence to earlier report of maximum flavonoids content from methanol extract of wild edible fruits of Meghalaya (Seal et al., 2013b).

4.3.4. Estimation of total antioxidant capacity assessed in terms of FRAP

In the FRAP assay, the presence of antioxidants in the sample results in the reduction of Fe^{+3} to Fe^{+2} and it reflects the antioxidant potential of the sample. They are electron donors and can reduce the intermediates of lipid peroxidation processes (Yen and Chen, 1995). The results indicate that FRAP for both hydro ethanol and methanol extract of all the plants under study were comparable. Highest FRAP is observed in the hydro alcoholic extracts of all the plants which can be attributed to its high content of phenolics in those extracts. By observing the results from the present study it can be confirmed that the extraction solvent used in the preparation of samples has significantly affected the total phenolic content which affected the FRAP. A similar observation was made while evaluating the antioxidant activity of *Brugeiera gymnorrhiza* and *Aegialitis rotundifolia* (Reddy and Grace, 2016).

4.3.5. Estimation of total antioxidant capacity assessed in terms of DPPH and ABTS radical scavenging property

The methods used to measure antioxidant activity are dependent on the reaction conditions and the substrates or products, for which all methods do not show the same values for the activity (Abaza et al., 2011) but a similar trend is expected to be observed. The antioxidant capacity was assessed using DPPH and ABTS assays of various extracts. Maximum radical scavenging activity was observed with 70% ethanol and the activity decreased with decreasing polarity. The result is in accordance with the study by Sultana et al. (2007) on medicinal plants where aqueous ethanol exhibited maximum DPPH radical scavenging activity over absolute ethanol. Flavonoids possess higher antioxidant activity because of double bonds in C-ring. The arrangement of hydroxyl group also affects the radical scavenging activity of flavonoids. The highest radical scavenging activity is exhibited by compounds that have an *ortho* 3, 4,-dihydroxy structure at B ring (eg., quercetin), hydroxyl groups in position *meta* e.g. 5, 7, dihydroxy at ring A (eg., kaempferol),

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as well as those that have a double bond between the C2 and the C3 hydroxyl group at ring C. It has been further reported that no single compound was able react with all kinds of radicals (Kondo et al., 2000; Rice-Evans et al., 1996). *A. ganjetica, A. aspera* and *E. fkuctuans* showed the highest radical scavenging activity in 70% aq. ethanol extract and both these extract contain the high amount of flavonoids. The methanolic extract of these plants are also rich in their flavonoids content and exhibited good scavenging activity. Hagerman et al. (1998) have reported free radicals scavenging property increases with the increase in molecular weight of the phenolic compounds and it depends on the number of aromatic rings and nature of hydroxyl groups substitution.

4.3.6. Estimation of reducing property

The reducing property of the antioxidant is based on the ability of donating a hydrogen atom to the free radical and thereby stabilizing them and breaking the free radical chain (Jamuna et al., 2011). The reducing property of the solvent extract can be ranked as 70% ethanol > Methanol > Chloroform > Benzene for all the plants under study. Similar observation was made by Sultana et al. (2007) on medicinal plants where the hydro ethanol extract showed maximum reducing power and also contained the maximum phenolic content. Different studies confirm that the plant extracts with higher levels of total phenolics also exhibit greater reducing power (Saeed et al., 2012; Siddhuraju and Becker, 2003; Sultana et al., 2007).

4.3.7. Metal chelating property

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon, 1990). The catalysis of metal ions also correlates with etiology of cancer and arthritis (Halliwell et al., 1995). In addition, lipid peroxidation and oxidative damage of protein systems ferrous ions catalyze the conversion of hydrogen peroxide to hydroxyl radical with the production of ferric ion via Fenton reaction. Ferrous ions, the most effective pro-oxidants, are commonly found in food systems (Yamaguchi et al., 1998). Chelating capacity of the investigated plant extracts decreased with the decreasing polarity. A similar trend is followed as recorded for reducing property, and radical scavenging property. Chelating ability can be attributed to the high phenol and flavonoid content in the 70% ethanol extracts. Polyphenols with dihydroxy groups can conjugate metals, preventing metal catalyzed free radical formation (Gua et al., 1996).

4.3.8. Anti-lipid peroxidation activity in linoleic acid system

Of the four different extracts compared maximum activity was observed in 70% hydro ethanol and methanol extract for all the plants studied. Based on this

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anti-lipid peroxidation assay was studied in hydro ethanol and methanol extracts. Lipid peroxidation leads to rapid development of rancid and stale flavours, and it is considered as a primary mechanism of quality deterioration in lipid foods (Gutensperger et al., 1998). Food additives such as synthetic antioxidants, e.g., BHA, are added in food during processing to suppress lipid peroxidation and resulting improved food quality and stability. In addition, lipid peroxidation of cell membrane is associated with various pathological events such as atherosclerosis, inflammation and liver injury (Singh et al., 2012). The phenolic compounds and other chemical components may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition or radical recombination (Mathew and Abraham, 2006). All the plants studied yielded highest phenolic content in the 70% aq. ethanol extract so their anti-lipid peroxidation activity was also higher in their 70% aq. ethanol extract. This is in accordance with several other investigations on fruits and vegetables where a significant relationship between total phenolic content and peroxidation activity is evident (Velioglu et al., 1998; Loganayaki et al., 2013). The flavonoids rich extract of *Hypericum perforatum* also inhibited the peroxidation of linoleic acid during 84 h incubation (Zou et al., 2004).

4.4. Quantitative and qualitative profiling of phenolic acids and flavonoids in the 70 % aq. ethanol extract of different plant materials under study by RP-HPLC

The 70% aq. ethanol extracts of the plants appeared to have significant antioxidant properties and were further investigated for identification of 13 phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid) and 8 flavonoids (catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin and kaempferol) using RP-HPLC.

The gallic acid was detected only from *A. viridis* (0.145 mg/100 g dm) which is lower to that in common vegetable like chilli pepper (3.33 mg/g), lemon (2.03 mg/g), spinach (1.82 mg/g), onion bulb (1.55 mg/g), cabbage (0.49 mg/g) etc. (Romaric et al., 2011). Chlorogenic acid, an ester of caffeic acid and quinic acid is found in coffee and coffee beans and also found in higher plants. It has been reported to reduce blood sugar levels and potentially exert an anti-diabetic effect (Uma et al., 2010). Chlorogenic acid content in *I. aquatica* (1.827 mg/100 g dm) and *A. viridis* (3.807 mg/100 g dm) is lower to *Solanum gilo* and *Solanum kurzii* (Seal et al., 2016b). The consumption of plants containing chlorogenic acid is associated with a lower risk of liver cirrhosis and liver cancer (Tverdal and Skurtveit, 2003). *p*-Hydroxybenzoic acid possesses antifungal, anti-mutagenic, anti-sickling, estrogenic, and anti-microbial activities. It has been isolated from many sources

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viz. Daucus carota, *Paratecoma peroba*, *Tabebuia impetiginosa*, *Vitex negundo*, *Roystonea regia* and *Mespilus germanica* (Khadem and Marles, 2010). The *p*-Hydroxybenzoic acid content is *I. auatica* is lower than that in a wild edible fruit *Cucumis hardwickii* (Seal et al., 2017a) and also justifies the use of this plant on cuts and wound because of its antimicrobial properties. Vanillic acid is used as a flavouring agent. Hepatoprotective activity of vanillic acid in liver inflammation by concanavalin A-induced liver injury has been proved (Kim et al., 2010). The vanillic acid content of *A. aspera, O. corymbosa* and *I. aquatica* is lower to that of *Solanum kurzii* (Seal et al., 2016b) but is comparable to *Perilla ocimoides* of north eastern India (Seal et al., 2016a). The anti-cancer, anti-proliferative, and hepato-protective actions of syringic acid is well known (Periyannan and Veerasamy, 2018). Syringic acid has been detected in *A. viridis,* which is lower than that from some wild vegetables like *Viburnum foetidum, Houttuynia cordata* and *Perilla ocimoides* of north eastern India (Seal et al., 2016a). *p*-coumaric acid exhibit strong antioxidant activity and is widely distributed in barley, peanuts, tomato, carrots etc. and reduce the formation of carcinogenic nitrosamines in the stomach (Ramadoss et al., 2015). *p*-coumaric acid has been quantified from *I. aquatica* which indicates their protective value as food. Ferulic acid is known for its anti-microbial, anti-inflammatory, anti-cancer activities etc (Mussatto et al., 2007). It is found in almost all the plants under study. Sinapic acid showed antioxidant, anti-microbial, anti-inflammatory, anticancer, and anti-anxiety activity (Seal et al., 2016a). Presence of sinapic acid in *O. coymbosa* justifies its application on cuts and wounds because of its antimicrobial property. Ellagic acid possesses antioxidant and antiproliferative activity (Seeram et al., 2005) and acts as a chemopreventive agent (Vattem and Shetty, 2005) too. *E. fluctuans* contain good amount of ellagic acid which justifies its folklore use as an anti-inflammatory medicine.

Catechins, a flavanols that are found only in foods and drinks derived from plants (Seal and Chaudhuri, 2015). High amount of catechin is detected in *A. ganjetica* which might contribute to its antioxidant property. The catechin content is however much lower than that detected from *Oenanthe linearis* (Seal, 2016a) and *Bauhinia purpurea* (Seal and Chaudhuri, 2015). Rutin is a flavonol with biological effects, such as antidiabetic effect (Srinivasan et al., 2005) and anticancer activity (Lin et al., 2012) and can potentially be used as a therapeutic agent. Appreciable amount of rutin in *I. aquatica* suggests their potential use as therapeutic agents and justifies the folklore application. Myricetin has been proven to posses' anticancer, antidiabetic and anti-inflammatory activity (Ong and Khoo, 1997). *I. aquatica* contain high amount of myricetin and can be exploited for its antidiabetic potential. Quercetin display anti-cancer (D'Andrea, 2015) anti-histamine, as also anti-inflammatory activities (Seal, 2016b) which mostly follow its antioxidant traits. The dietary sources of quercetin include citrus fruits, apples, onions, parsley, sage, tea and red wine. Majority of the plants under study contained quercetin but

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in much lower amount compared to *Oenanthe linearis* (Seal, 2016a) and *Bauhinia purpurea* (Seal and Chaudhuri, 2015). Apigenin, a flavone, reduce the risk of cardiac ailments, neurological syndromes, mutagenesis (Mohammad and Elham, 2013) and is detected in most of the plants under study. The amount of apigenin in *A. viridis* and *A. aspera* is comparable to *Oenanthe linearis* (Seal, 2016a) and *Bauhinia purpurea* and *Clerodendron colebrookianum* (Seal and Chaudhuri, 2015). Kaempferol can prevent the oxidation of low-density lipid proteins indicating a potential protective role in atherosclerosis. Consumption of kaempferol enriched food with reduces the chances of gastric cancer (Calderon-Montaño et al., 2011). Good amount of kaempferol is detected in *A. aspera* and suggests that consumption of these plants would render protective action.

4.5. Quantification of water soluble vitamins by RP-HPLC

The vitamin content in the selected plants under study is depicted in Table 6. Vitamin C is well known for its antioxidant properties and it helps inhibiting infection, and toxicity. It is also required for the prevention of scurvy and maintenance of healthy skin. The vitamin C content was comparable to values obtained by Blessing et al. (2011) for pumpkin (3.47−4.39 mg/100 g), Igile et al. (2013) for *Vernonia calvoana* (11.33 mg/100 g) and by Misra and Misra (2014) for *Moringa oleifera* and *Ipomoea aquatica* (2.17 mg/100 g and 0.34 mg/100 g respectively). When compared to some common edible vegetable like tomato (23 mg/100 g), spinach (51 mg/100 g) and onion (190 mg/100 g) (Zennie and Dwayne, 1977), the vitamin C content was relatively high in *A. aspera* (151.75 mg/100 g). The results indicates that this indigenous vegetables are potentially good sources of vitamin C. In this study, vitamin C content was below detection level for *I. aquatica,* which can be because of the edaphic factors affecting the growth of this plant. Apart from being a potent antioxidant vitamin C iron transport and uptake at the intestinal mucosa, synthesis of cortisol and minimize the intermediates of folic acid synthesis. It also aids in the purification of blood (Igile et al., 2013).

Thiamine (B1) is essential for energy production, carbohydrate metabolism and nerve cell function. Thiamin content ranged between 0.007 and 0.454 mg/100 g (0.46−59%, RDA of 1.5 mg/day), and is comparable to some common vegetables like beans (0.132 mg/100 gm), cauliflower (0.073 mg/100 gm), spinach (0.076 mg/100 gm) (Watada and Tran, 1987). These plants could provide adequate thiamin content to all groups of people except in pregnancy and lactation.

Riboflavin (B2) content ranged between 0.012 and 2.511 mg/100 g. It was higher than that of fruits, 0.01−0.05 mg/100 g, common vegetables (0.01−0.3 mg/100 g), and most common cereals (0.11−0.18 mg/100 g) (FAO, 1972), but comparable to some common leafy vegetables of Bangladesh (Hasan et al., 2013). The vitamin B2 content of *A. viridis* in this study (0.012 mg/100 g) was much lower to that

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recorded by Hasan et al. (2013) (0.523 mg/100 g), this variation can be attributed to the soil condition and the age of the plant.

Vitamin B3 plays an important role in DNA repair and metabolism. The B3 content in the plants studied were much higher than that recorded by Hasan et al. (2013) and comparable to some wild edible fruits like *Docynia indica* (0.38 mg/100 g), *Elaeagnus latifolia* (0.29 mg/100 g) and *Myrica esculenta* (0.46 mg/100 g) (Seal et al., 2017c).

Pantothenic acid (B5) is a component of CoA required in fatty acid metabolism (FAO/WHO, 2001). RDA for pantothenate is 5 mg/day, from the results obtained in this study it can surely be confirmed that these plant materials could fulfil the required vitamin B5 supplement to the diet. Pantothenic acid remained undetected in the green leafy vegetables studied by Hasan et al. (2013).

The vitamin B6 (pyridoxine) content of these wild edibles ranged from 0.759 mg/100 g to 2.419 mg/100 g. These values were comparable with the values obtained by Igile et al. (2013) for the leafy vegetable, Akah and Onweluzo (2014) for elephant grass (2.40 mg/100 g), *Vernonia calvaona* (0.56 mg/100 g), and Hasan et al. (2013) for *Lagennaria vulgaris* (0.755 mg/100 g) and *Amaranthus viridis* (0.07 mg/100 g). The B6 level for *A. viridis* in our study is much higher than the above record which may be due to the age associated with other demographic conditions. The level of vitamin B6 in the vegetables in this study provides more than the 1.3 mg/day RDA requirement for both adult men and women between 19 and 50 years respectively. Vitamin B6 is one of the essential amino acids, that is only available via food. It boosts the metabolism of protides and unsaturated fatty acids (Igile et al., 2013). Based on the RDA and the level of vitamin B6 observed in the leafy vegetable samples analyzed in this study, these vegetables, therefore, may be considered good sources of vitamin B6.

Folic acid (B9) plays an important role in DNA synthesis and repair. Folic acid is one of the commonest deficiencies seen worldwide. It has been found to affect several groups of people including pregnant mothers, elderly and children are affected by its deficiency. In this study, folic acid content (0.050−0.174 mg/100 g) is adequate for all age groups. The amount recorded is comparable to the wild some edible fruits studied by Seal et al. (2017c).

5. Conclusion

Based on the above findings it can be ascertained that these wild edibles can be exploited as a source of natural antioxidant and vitamin B. This knowledge of antioxidant and nutraceutical potential of these plants will be useful in selecting plants as nutritional supplements as well in developing antioxidant based drugs.

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Declarations

Author contribution statement

Tapan Seal: Analyzed and interpreted the data; Wrote the paper.

Sudeshna Datta: Performed the experiments.

B. K. Sinha: Contributed reagents, materials, analysis tools or data.

Soumen Bhattacharjee: Conceived and designed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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