Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 654-663
Received: 04-01-2018
Accepted: 05-02-2018

Sagari Chaudhury
Botanical Survey of India, Central Botanical Laboratory, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

Chowdhury Habibur Rahaman
Department of Botany, Visva-Bharati University, Santiniketan, West Bengal, India

Harish Singh
Botanical Survey of India, Central Botanical Laboratory, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

K Chaudhuri
Plant Chemistry Department, Botanical Survey of India, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

B Pillai
Plant Chemistry Department, Botanical Survey of India, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

Tapan Seal
Plant Chemistry Department, Botanical Survey of India, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

Correspondence
Tapan Seal
Plant Chemistry Department, Botanical Survey of India, AJC Bose Indian Botanic Garden, Shibpur, Howrah, West Bengal, India

Dioscorea alata: A potent wild edible plant consumed by the *Lodha* Tribal community of West Bengal, India

Sagari Chaudhury, Chowdhury Habibur Rahaman, Harish Singh, K Chaudhuri, B Pillai and Tapan Seal

Abstract
The present study designed to evaluate the proximate composition, minerals content and *in vitro* antioxidant properties, of the tuber of *Dioscorea alata*. The quantitation of polyphenolics and water-soluble vitamins in the plant were carried out by High Performance liquid chromatography (HPLC) method. The HPLC analysis were carried out using Dionex Ultimate 3000 liquid chromatograph attached with Acclaim C 18 column (5 µm particle size, 250 x 4.6 mm), and photo diode array (PDA) detector. The toxic heavy metals such as lead, cadmium, arsenic and mercury of the plant were also estimated. The present study showed the presence of very good amount of protein (18.653±0.035%), carbohydrate (43.13±0.031%) and various amount of minerals and heavy metals. The total phenolic and flavonoid content were found 63.846 ± 1.83 mg/gm and 8.213 mg/gm dry extract (DE) respectively in the plant. The plant showed the presence of water- soluble vitamins ranged between 0.004 to 1.548 mg/100gm dry plant material (DPM). The HPLC analysis also indicated the presence of phenolic acids and polyphenolics in various amounts in this wild edible plant. The abundance of protein, fat, carbohydrate, minerals, water soluble vitamins and natural antioxidant components in this plant makes them as considerable sources of nutrition and could be consumed as a regular diet to the human being.

Keywords: *Dioscorea alata*; Proximate composition; Minerals content; Antioxidant activity; Vitamin and Polyphenolics by HPLC

1. Introduction
Wild edible plants have always been critical components in the diet among the people in developing countries. Due to the rapid growth of population, scarcity of fertile land for cultivation and high prices of available staples, the poor people frequently collect wild edible plants and other plants from natural habitats to meet their adequate level of nutrition. The different biochemical methods have been developed to cultivate some desired plant species in large scale in the garden and fields to meet the caloric necessities of human being [1]. Recently, a lot of interest has been concentrated to evaluate various wild edible plants because they contribute the human being with minerals, vitamins and certain hormone precursors, in addition to protein and energy. It has been reported in several communications that the nutraceutical value of unconventional plants foods could be comparable to or even sometimes superior to the common vegetables [1]. In this context, the analysis of wild edible plants from nutritional and antioxidant aspect is important to identify the potential sources which could be exploited as alternative food.
Dioscorea alata, belonging to the Dioscoreaceae family, found in the tropical regions of the World. In India, it is reported from different states like West Bengal, Assam, Arunachal Pradesh, Sikkim and Mizoram etc. as an important food plant. The tubers are cooked with arums, mushrooms, vegetables and mixed with rice. The tuber paste of this plant is reported to be applied tropically on cancerous wounds and skin diseases. It has also been used in the treatment of piles and leprosy in Western Mizoram of India [2-5]. Though the nutritional potential of *D. alata* grown at Kerala was reported by Joy and Siddhuraju [6] but the detail study has not been described in any literature. Therefore, the present study aimed to determine the nutritional composition, minerals content, vitamin content, antioxidant properties of the tubers collected from *Lodha* tribal populated area of Paschim Mednipur, West Bengal. The quantitation of phenolics and flavonoids using HPLC in this plant established its potent antioxidant properties as well as the result of investigation will encourage the common people for domestication.

2. Materials and Methods

2.1. Collection and identification of plant

The fresh tubers of *Discorea alata* Linn were collected from Paschim Medinipur district of West Bengal and the identification was authenticated by the Botanical Survey of India, Howrah and the herbarium specimens has been kept in the Department. The fresh tubers were taken in our laboratory at refrigerated temperature in ice cold container. The refrigerated plant samples were stored at -15^{0}C and one part processed for vitamin estimation. The other parts were dried at room temperature, powdered and stored in an airtight container to carry out the nutritional and antioxidant properties.

2.2. Chemicals

The standard 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), flavonoids (catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol), water soluble vitamins (C, B1, B2, B3, B5, B6 and B9), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteus's phenol reagent, potassium ferricyanide, potassium per sulphate, aluminium chloride, ferric chloride, anthrone, sodium carbonate, HPLC-grade solvents (acetonitrile, methanol, water and trifluoroacetic acid), sodium dihydrogen phosphate were purchased from Merck (Germany). All the chemicals and solvents used were of analytical grade.

2.3. HPLC equipment

Dionex Ultimate 3000 liquid chromatograph attached with a diode array detector (DAD) was taken for HPLC analysis. The separation of components was achieved by a reversed-phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). 20 μL of sample was injected into the HPLC column. The Chromeleon system manager was used for analyzing the data.

2.4. Estimation of ash content

Five gms of powdered tubers were taken in a silica crucible and heated for about 5-6 h in a muffle furnace controlled at 500 °C in. The crucible was cooled, weighed and heated again in the furnace for half an hour. This process was repeated consequently, until the weight of the crucible along with sample became constant (ash became white or grayish white). Weight of ash gave the ash content [7]. The ash obtained was preserved for mineral analysis.

Ash content (%) = Weight of ash × 100/Weight of sample

2.5. Estimation of moisture content

The moisture in the plant sample was carried out by heating a known amount of fresh tubers in an air oven at 100–110°C and weighed. The loss in weight was considered as a measure of moisture content in the sample [7].

Moisture (%)=[(Weight of original sample−Weight of dried sample)]x100 / Weight of original sample

2.6. Estimation of crude fat content

Two gm moisture free tubers were soxhleted with petroleum ether (40-60°C) for about 6-8 h. The petrol ether extract was filtered and evaporated in a pre-weighed beaker. Increase in weight of a beaker determines crude fat content. Percentage of fat content was calculated using the following formula [7].

Crude fat (%) = Weight of fat in sample × 100/Weight of dry sample.

2.7. Estimation of crude fibre content

The crude fibre content in the plant sample was carried out by warming two gm of moisture and fat-free tubers with 200 ml of 1.25 % H_2SO_4 followed by 1.25 % NaOH solution and with 1 % HNO_3. The solution was filtered and the residue was washed with boiling water and then the residue was dried in an oven at 130 °C to constant weight. The residue was heated in muffle furnace at 550 °C for two hours, cooled in a desiccator and weighed. The crude fibre content was expressed as percentage loss in weight on ignition [7].

Crude fibre (%) = (Weight of residue − Weight of ash) x 100/ Weight of the sample.

2.8. Estimation of crude protein content

The micro Kjeldahl method was adopted for the estimation of crude protein content in the plant was where two gm of samples were digested with concentrated sulphuric acid in a Kjeldahl flask in the presence of a catalyst, until a clear solution was obtained. The digested solution cooled and was diluted with distilled water and an excess of sodium hydroxide solution (40%) was added to the diluted reaction mixture, the liberated ammonia was distilled in steam and absorbed in 25 ml N/20 sulphuric acid. The excess mineral acid was titrated with known strength of sodium hydroxide and from this, the percentage of nitrogen in the sample was calculated. The amount of protein content determined by multiplying the amount of nitrogen with 6.25 [7].

2.9. Estimation of carbohydrate content

100 mg of tubers were hydrolysed with 5 ml 2.5 N HCl, cooled to room temperature and neutralised with solid sodium carbonate until the effervescence ceases. The solution filtered in a 100 ml volumetric flask and make up the volume with distilled water. To one ml of this solution, 4 ml freshly prepared anthrone reagent (200 mg anthrone dissolved in 100 mL of ice-cold 95% H_2SO_4) were added and heated in a water bath for eight minutes. The mixture was cooled rapidly, a dark green colour appeared and the absorption at 630 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800). The total carbohydrate content was expressed as glucose equivalents using the following equation based on the calibration curve $y = 0.0081x + 0.2475$, $R^2 = 0.9993$ where y was the absorbance and x concentration of glucose in mg/ml [8].

2.10. Estimation of energy content

The energy (kcal/100gm) content of plant sample was 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 [7, 9].

2.11. Estimation of minerals in plant material

One gram of ash of the plant obtained above was dissolved in 30 ml of 5 % HCl solution, filtered and volume make up to 50 ml with double distilled water and minerals were estimated in atomic absorption spectrophotometer (AAS) (AA 800, Perkin-Elmer Germany). The standard solution of each element was prepared and calibration curves were drawn for each element using AAS [10]. All assays were carried out in

triplicate and values were obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

2.12. Quantification of water soluble vitamin by HPLC

2.12.1. 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 one ml 0.1 M hydrochloric acid in 25 ml standard volumetric flask and topped up to mark with double distilled water. 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 and made up to mark with double distilled water. The standard solution was stored in amber-glass bottles in the refrigerator at 4^0C. The working standards were prepared from the stock standard solutions by mixing 100 µl mixed vitamins standard (vitamin B9, B5 and B2), 800 µl phosphate buffer (1 M, pH 5.5) and 100 µl mixed vitamins standard (vitamin C, B1, B6 and B3) which represent 100 µg/ml mixed working standards. The working standard solutions of concentrations 20, 40, 60 and 80 µg/ml were prepared accordingly [11].

2.12.2. Preparation of sample solution

Plant materials were cleaned and the inedible portions were removed. The edible parts were rinsed thoroughly with tap water and then with distilled water. The washed plant materials were dried with clean cloth, were cut into very small pieces, frozen in liquid nitrogen, freeze-dried and kept at -20 ^{0} C until analysis [11].

One gm of freeze-dried tubers were soaked in 10 ml water. Then 1 ml 0.1 M 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 HPLC system. The stock solutions of sample were kept in a refrigerator for further use [11].

2.12.3. Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by Seal and Chaudhuri [11] with minor modification. 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 22^0 C and 20 µl solution was injected. A gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elusion was 1 % A and 99 % B with flow rate 0.5 ml/min in 5 min, from 1 % to 25% A with flow rate 0.5 ml/min for 16 min, 45 % A, with flow rate 0.5 ml/min for 8 min. from 45 to 1 % A with flow rate 0.5 ml/min in 5 min. The mobile phase composition back to initial condition (solvent A: solvent B: 1: 99) in 34 min and allowed to run for another 1 min, before the injection of another sample. The total run time of mobile phase per sample was 35 min.

The various concentrations of (20, 40. 60, 80 and 100 µg/ml) vitamin working standards were injected into the HPLC column separately. The retention times and absorption maxima of each standard vitamin at four different wavelengths (210, 245, 275 and 290 nm) using DAD detector were noted and used to identify the vitamins in the sample.

The quantification of the vitamins was carried out by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported as means ± standard error means of three independent analyses and the method was validated according to the USP and ICH guidelines [12-13].

2.13. Antioxidant activities of *D. alata*

2.13.1. Extraction of plant material

One gram of dried tubers were soaked in 20 ml 80 % aq. ethanol with constant stirring for 18 -24 h at room temperature. The extract was filtered and diluted to 50 ml and aliquot was analyzed for their total phenolic, flavonoid and flavonol content, reducing power and free radical scavenging capacity.

2.13.2. Estimation of total phenolic content

The Folin-Ciocalteu method [14] was followed to estimate the total phenolic content in the crude extract of *D. alata*. The tested extracts in varying concentrations were mixed with 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%). The mixtures were allowed to stand for 30 min and the absorption of the solution were measured at 765 nm in a UV-VIS spectrophotometer (Shimadzu UV 1800). The total phenolic content in the sample was expressed as gallic acid equivalents (GAE) in miligram per 100 gram (mg/100gm) of plant material using the following equation based on the calibration curve $y = 0.0013x + 0.0498$, $R^2 = 0.999$ where y was the absorbance and x was the Gallic acid equivalent (mg/g).

2.13.3. Estimation of total flavonoids

The flavonoid content in *D. alata* was estimated according to method described by Ordonez *et al*., 2006 [14]. In a test tube, 0.5 ml of plant extract was mixed with equal volume of 2% $AlCl_3$ (in absolute ethanol) solution. The mixture was allowed to keep one hour at room temperature and the absorbance of the solution was measured at 420 nm (UV-visible spectrophotometer Shimadzu UV1800). The development of a yellow color in the mixture indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin equivalent (mg/100gm plant material) using the following equation based on the calibration curve: $y = 0.0182x - 0.0222$, $R^2 = 0.9962$, where y was the absorbance and x was the Rutin equivalent (mg/g).

2.13.4. Estimation of total flavonols

The amount of flavonols present in the tuber extract was estimated following the method of Kumaran and Karunakaran, 2006 [14]. 2.0 ml of tuber extract was mixed with 2.0 ml of 2% $AlCl_3$ (in absolute ethanol) and 3.0 ml (50 g/L) sodium acetate solution. The absorption of the mixture at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20°C. Total flavonol content was calculated as quercetin equivalent (mg/100gm plant material) using the following equation based on the calibration curve: $y = 0.0049x + 0.0047$, $R^2 = 0.9935$, where y was the absorbance and x was the quercetin equivalent (mg/g).

2.13.5. Measurement of reducing power

The reducing power of the extract was assessed following the method of Oyaizu, 1986 [14]. 100 µl extract of the tuber of plant was mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was

incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%) was added to the mixture. The absorbance of the solution was measured at 700 nm. The reducing power was determined ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry material using the following equation based on the calibration curve: $y = 0.0023x - 0.0063$, $R^2 = 0.9955$ where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

2.13.6. Determination of DPPH free radical scavenging activity

The free radical scavenging activity of the tuber extract and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [14]. The different amount of extracts (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. The absorbance of the solution was measured at 517 nm after 30 mins (UV-visible spectrophotometer, Shimadzu UV 1800). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

2.13.7. Scavenging activity of ABTS radical cation

ABTS radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Miller [14]. ABTS was dissolved in water to a 7 mM concentration and radicals were produced by adding 2.45 mM potassium persulphate in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. The radical scavenging activity was determined by adding 1 ml of diluted $ABTS^{\cdot+}$ solution to 10 µl of plant extract (water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

ABTS scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$

where A_{cont} and A_{test} are the absorbencies of the control and of the test sample, respectively. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material that inhibits the formation of ABTS radicals by 50%. Each value was determined from regression equation.

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and IC_{50} value of plant material was calculated by using Linear Regression analysis.

2.14. Quantification of phenolic acids and flavonoids in the 80 % aq. ethanol extract of *D. alata* by HPLC

2.14.1. Preparation of standard solutions

The stock solution of gallic acid of concentration 1mg / ml was prepared by dissolving 10 mg gallic acid in 1 ml HPLC-grade methanol followed by sonication for 10 min and the resulting volume was made up to 10 ml with the solvent for the Mobile phase (methanol and 0.5% aq. acetic acid 1:9). The same method was followed to prepare the standard stock solutions of the phenolic acids and the flavonoids *viz*. 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, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol. The working standard solutions of concentrations 20, 40, 60, 80 and 100 µg/ml were prepared by further dilution of the standard solution with the mobile phase solvent system. The standard and working solutions were filtered through 0.45 µm PVDF-syringe filter and the mobile phase was degassed before the injection of the solutions.

2.14.2. Chromatography analysis for quantification of phenolic acids and flavonoids

HPLC analyses for the quantification of phenolic acids and flavonoids in the plant extract were performed following the method described by Seal 2016 with minor modification [15]. The analysis were carried out 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. The separation was achieved by a reversed phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). 20 μL of sample was introduced into the HPLC column. The method was validated according to the USP and ICH guidelines [12-13]. The mobile phase contains methanol (Solvent A) and 0.5% aq. acetic acid solution (Solvent B) and the column was thermostatically controlled at 25 ^{0}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. The gradient elusion was 10 % A and 90% B with flow rate 1ml/min to 0.7 ml/min in 27 min, from 10 to 40 % A with flow rate 0.7 ml/min for 23 min, 40% A and 60% B with flow rate 0.7 ml/min initially for 2 min and then flow rate changed from 0.7 to 0.3 ml/min in 65min, from 40 to 44% A with flow rate 0.3 to 0.7ml/min in 70 min, 44% A with flow rate 0.7 to 1ml/min for 10 min duration, solvent A changed from 44% to 58 % with flow rate 1ml/min for 5 min, 58 to 70% A in 98 min at constant flow rate 1 ml/min. The mobile phase composition back to initial condition (solvent A: solvent B: 10: 90) in 101 min and allowed to run for another 4 min, before the injection of another sample. Total analysis time per sample was 105 min.

HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analysed compounds. 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 tubers of the plant 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.

3. Results

3.1. Proximate composition of *D. alata*

The fresh tubers of *D. alata* were taken for the analysis of proximate composition. The proximate composition of these plants has been presented in Table 1.

Table 1: Proximate composition, minerals and vitamin content in the tubers of *D. alata*

Proximate composition	Amount (%)	Minerals	Amount (mg/g)	Vitamin	Amount mg/100gm dry plant material
Ash	8.817 ± 0.055	Sodium (Na)	0.754 ± 0.015	Vitamin C	1.110 ± 0.038
Moisture	80.693 ± 0.043	Potassium (K)	39.329 ± 0.155	Vitamin B1	0.004 ± 0.0012
Protein	18.653±0.035	Calcium (Ca)	30.321± 0.250	Vitamin B2	0.682 ± 0.0141
Fat	0.970±0.023	Copper (Cu)	0.01216±0.0008	Vitamin B3	0.967 ± 0.0036
Carbohydrate	43.130±0.031	Zinc (Zn)	0.057 ± 0.0014	Vitamin B5	0.892 ± 0.0033
Crude fibre	2.853±0.035	Magnesium (Mg)	1.207 ± 0.0051	Vitamin B6	1.548 ± 0.0026
Energy (kcal/100gm)	255.86±0.26	Iron (Fe)	1.2425 ± 0.0040	Vitamin B9	0.004 ± 0.0001
		Manganese (Mn)	0.0133 ± 0.0006		
		Heavy metals	Amount (µg/g)		
		Lead (Pb)	0.261±0.0008		
		Arsenic (As)	1.221±0.0008		
		Cadmium (Cd)	BDL		
		Mercury (Hg)	0.493±0.004		

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The proximate analysis of the tubers of *D. alata* showed that 100gm of dry plant contain 8.817 ± 0.055 gm ash and 80.693 ± 0.043 gm moisture. The tubers of the plant were found to contain protein, fat, fibre and carbohydrate 18.653±0.035 %, 0.970±0.023, 2.853±0.035 % and 43.130±0.031 respectively. The energy content of the plant was calculated at 255.86 ± 0.26 kcal/100gm.

3.2. Minerals content in *D. alata*

Table 1 shows the minerals content in mg per gm of the air-dried tuber. Sodium content of the plant was found to be 0.754 ± 0.015 mg/gm. The tubers were found to be rich in minerals like potassium (39.33 ±0.155 mg/gm), calcium (30.32 ±0.25 mg/gm), magnesium (1.207 ±0.0051 mg/gm) and iron (1.24± 0.004 mg/gm) respectively. An appreciable amount of copper, zinc and manganese were also detected in the tuber of *D. alata*. The amount of lead, arsenic and mercury were noticed in the plant were 0.261±0.0008 1.221± 0.0008 and 0.493 ± 0.004 µg/gm respectively whereas other toxic metal like cadmium was not found in the plant under investigation.

3.3. Identification and quantification of water soluble vitamins in the tuber of *D. alata* by HPLC

A typical HPLC chromatogram of the all standard vitamin mixture recorded at 210 nm is presented in fig. 1.

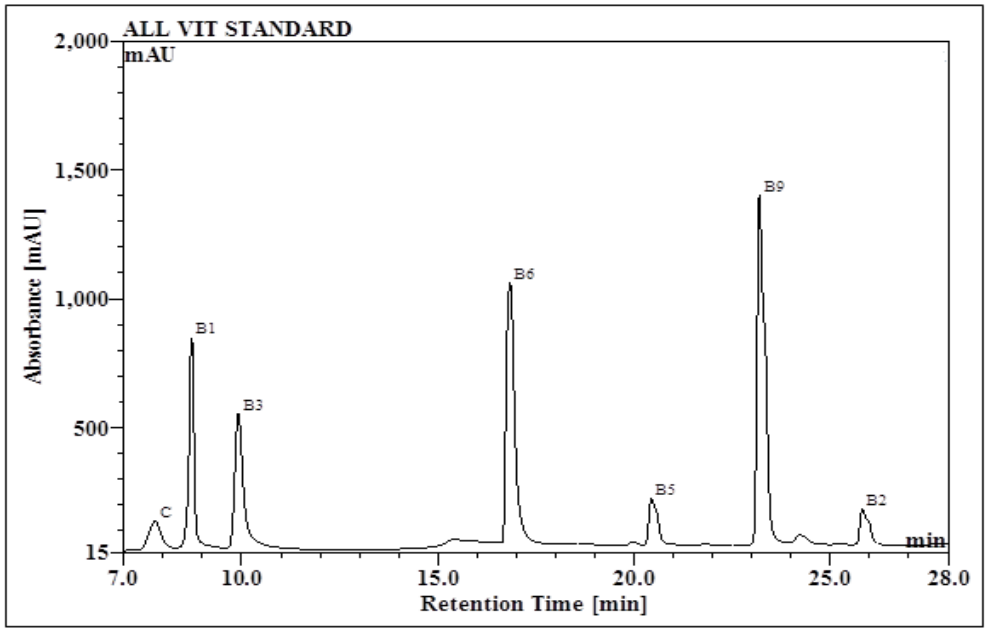


Fig 1: HPLC Chromatogram of mixture of Standard vitamin
(C) Ascorbic acid ; (B1) Thiamine ; (B3) Niacin ; (B6) Pyridoxine ; (B5) Pantothenic acid ; (B9) Folic acid ; (B2) Riboflavin

The HPLC method was successfully performed for the estimation of water soluble vitamin C and B vitamins. The quantity of all vitamins of in *D. alata* has been expressed as mg/100gm dry plant material (DPM) and data presented in table 1.

The HPLC chromatogram of the tubers *D. alata* (Fig.2.) showed the presence of vitamin C (1.11±0.04), B1(0.004±0.0012), B2 (0.682±0.0141), B3 (0.967±0.0036) B5 (0.892±0.0033), B6 (1.548±0.0026) and B9 (0.004±0.0001).

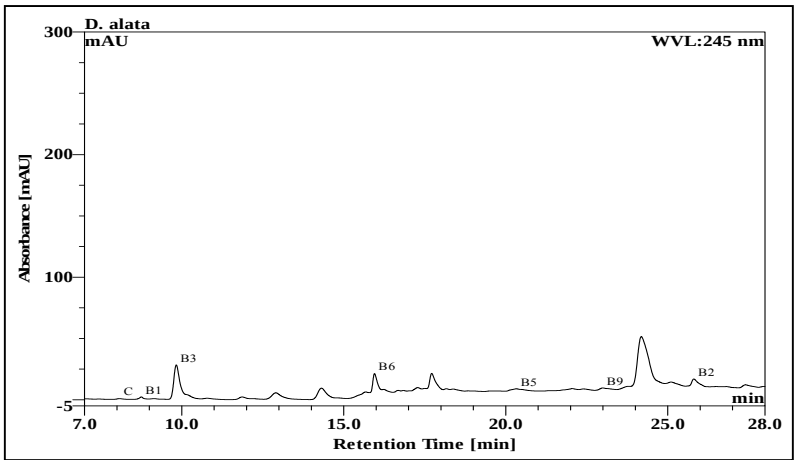


Fig 2: HPLC chromatogram of tubers of *D. alata* showing water soluble vitamins

3.4. Antioxidant activities of the tuber of *D. alata*

In this study, estimation of total phenolic, total flavonoid & total flavonol content, reducing power, ABTS and DPPH methods were employed to evaluate the *in vitro* antioxidant activities of the 80 % aq. ethanol extract of the tubers of *D. alata* and result has been presented in table 2. The result showed total phenolic content in the plant was 63.846 ± 1.832 mg GAE/gm dry extract (DE). The flavonoid and flavonol amount were detected in the tubers were 8.213±0.020 and 17.231±0.198 mg/gm DE respectively. The reducing power (AAE) of the 80% aq. ethanol extract of the edible parts was observed 14.064 ±0.32 mg/gm DE of the plant.

Table 2: Antioxidant properties of the tubers of *D. alata*

Antioxidant parameters	Amount mg/gm dry extract
Total phenolic content (Gallic acid equivalent)	63.846±1.832
Total flavonoid content (Rutin equivalent)	8.213±0.020
Total flavonol content (Quercetin equivalent)	17.231±0.198
Reducing power (Ascorbic acid equivalent	14.064±0.320
DPPH Radical scavenging activity (IC_{50})	0.603± 0.010
ABTS Radical scavenging activity (IC_{50})	0.136 ± 0.001

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The study also revealed that the ABTS radical scavenging activity showed higher antioxidant capabilities with IC_{50} value 0.136±0.001 mg DE than DPPH assay (IC_{50} 0.603±0.010 mg DE).

3.5. Identification and quantification of phenolic acids and flavonoids in *D. alata* by HPLC

A typical HPLC chromatogram of the mixture of all standard phenolic acids and flavonoids recorded at 280 nm is presented in fig. 3.

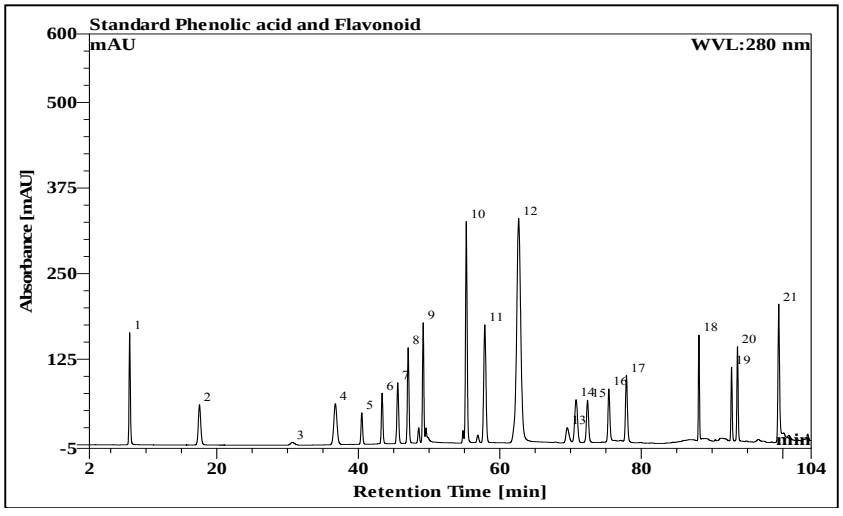


Fig 3: HPLC Chromatogram of mixture of Standard phenolic acids and flavonoids

1. Gallic acid 2. Protocatechuic acid 3. Gentisic acid 4.p-Hydroxy benzoic acid 5.Catechin 6. Chlorogenic acid 7.Vanillic acid 8. Caffeic acid 9. Syringic acid 10. p-Coumaric acid 11. Ferullic acid 12. Sinapic acid 13. Salicylic acid14. Naringin15. Rutin16. Ellagic acid 17. Myricetin 18.Quercetin.19. Naringenin 20. Apigenin 21. Kaempferol

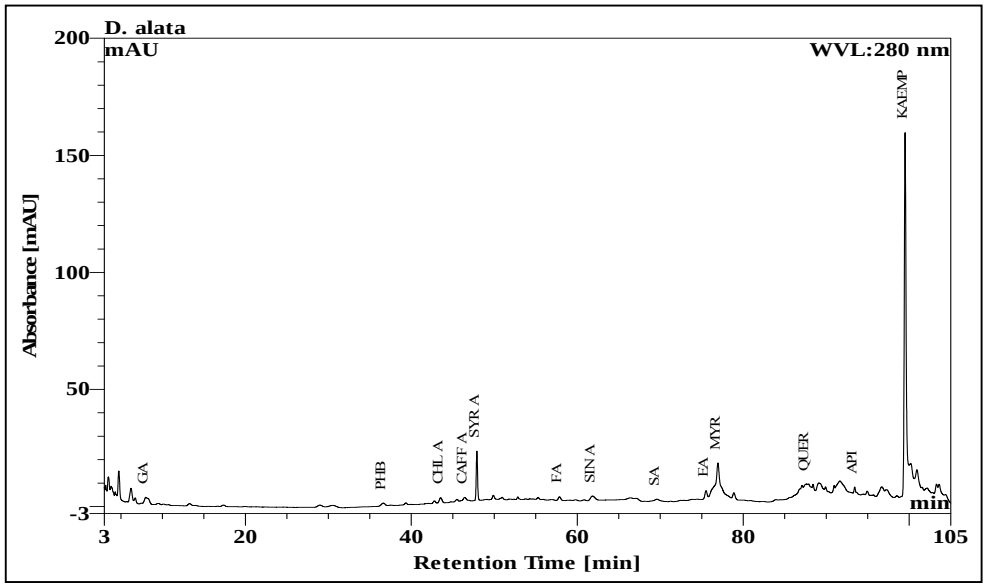
The HPLC method was successfully employed for the identification and quantification of phenolic acids and flavonoids e.g gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol in the 80 % aq.ethanol extract of *D. alata*. The quantity of all phenolic acids and flavonoids in this plant has been expressed as mg/100gm dry plant material and data presented in table 3.

Table 3: Phenolic acid and flavonoid content in *D. alata* by HPLC

Phenolic acids/flavonoids	Amount (mg/100gm dry plant material)	Phenolic acids/flavonoids	Amount (mg/100gm dry plant material)	Phenolic acids/flavonoids	Amount (mg/100gm dry plant material)
Gallic acid	0.482 ± 0.057	Caffeic acid	0.215± 0.0023	Rutin	ND
Protocatechuic acid	ND	Syringic acid	0.899± 0.0022	Ellagic acid	0.341±0.0038
Gentisic acid	ND	p-Coumaric acid	ND	Myricetin	4.613±0.0030
p-Hydroxy benzoic acid	0.192± 0.0024	Ferulic acid	0.089± 0.0005	Quercetin	0.687±0.0030
Catechin	ND	Sinapic acid	0.202± 0.0501	Naringenin	ND
Chlorogenic acid	0.451± 0.0038	Salicylic acid	ND	Apigenin	0.210 ± 0.0041
Vanillic acid	ND	Naringin	ND	Kaempferol	9.219±0.0043

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The HPLC chromatogram of the tubers *D. alata* (Fig.4.) showed the presence of gallic acid (0.482 ± 0.057), *p*-hydroxy benzoic acid (0.192± 0.0024), chlorogenic acid (0.451± 0.0038), caffeic acid (0.215± 0.0023), syringic acid (0.899± 0.0022), ferulic acid (0.089± 0.0005), sinapic acid (0.202± 0.0501), myricetin (4.613±0.0030), quercetin (0.687±0.0030) and apigenin (0.210 ± 0.0041 mg/100gm DPM).



GA: Gallic acid; PHB: p-Hydroxy benzoic acid; CHLA: Chlorogenic acid; CA: Caffeic acid ; SYRA: Syringic acid ; FA: Ferulic acid; SINA: Sinapic acid; MYR: Myricetin ; QUER: Quercetin; API: Apigenin, KAEMP: Kaempferol

Fig 4: HPLC chromatogram of tuber of *D. alata* showing phenolic acids and flavonoids

4. Discussion

The estimation of moisture content is necessary to determine the freshness, duration of storage and physical properties of the food before consumption [16]. The moisture content in *D. alata* was found to be 80.69± 0.043 % which was very close to sweet potato (68.5%), potato (74.7%), colocasia (73.1%) etc, grown in India [17]. The wild tuber *D. alata* contained high amounts of ash (8.817 ± 0.05%) indicating that this plant was rich in minerals and could provide a substantial amount of mineral elements in our diet and able to play an important role from nutritional point of view [18]. Fat is an important component of diet and serves a number of functions in the body. The fat content in the tubers of *D. alata* (0.97±0.023 %) was particularly high and well compared to that reported for some common vegetables like spinach (0.7 %), lettuce (0.20 %) [19] which indicates that the consumption of the plant would be helpful for the absorption of fat soluble vitamins like vitamin A and carotene in the body. The tubers of *D. alata* contained a good amount of crude fibre (2.853 ± 0.035%) and comparable to commercial fruits and vegetables like apple (3.2 %), broad beans (8.9 %), cabbage (2.8 %), potato (1.7 %), spinach (2.5 %) [17]. So, this plant might play an important role in decreasing the risks of many disorders such as constipation, diabetes, serum cholesterol, heart diseases, breast and colon cancer, hypertension, etc [20] and could be used in the human diet to fulfil WHO recommendation of intake of 22-23 kg of fibre for every 1000 k. cal. of diet which is necessary for digestion and effective elimination of wastes [21]. The carbohydrate content in this tubers (43.13±0.031%) considerably higher than the reported values when compared to some wild edible vegetables like bitter gourd (10.6 %), beans (29.1 %), potato (20.9 %), jack fruit seeds (25.8%) etc [17] and could be a good source of carbohydrate for human consumption. The crude protein

content in *D. alata* (18.65± 0.035%) was very much higher than some common roots and tubers such as beet root (1.7%), carrot (0.9%), potato (1.6%), yam wild (2.5%) etc [17]. So, the data (Table1) showed that the plant are rich sources of protein which can encourage their use in human diets and would be helpful for the proper functioning of antibodies resisting infection [18]. The energy value of foods is often more easily calculated from the analysis of foods for proteins, fat and carbohydrates and multiplication of the content of these components with appropriate factors. The results obtained from systematic chemical analysis of *D. alata* established that caloriefic value of this tubers (255.86±0.26 kcal/100gm) were higher than potato (97 kcal/100gm), beans (158 kcal/100gm), jack fruit (133 kcal/100gm) etc. [17].

Fruits, and vegetables, are important sources of macro-minerals (Na, K, Ca Mg) and micro-minerals (Fe, Zn, Cu, Mn, Zn, Pb, Cr) which are responsible in maintaining physiological and biological functions of the human body. The tubers contain a very good amount of sodium and potassium. The significant K/Na ratio (52.14) of this plant would be responsible to control the high blood pressure of our body. The tubers of this plant containing 25.34 ±0.18 mg/g calcium might be beneficial to build strong and healthy bones and also for the normal functioning of the cardiac muscles [10]. The consumption of this vegetable might be helpful for preventing anaemia and nucleic acid metabolism due to presence of sufficient amount of Cu and Zn (0.01216 ±0.0008 and 0.057 ±0.0014 mg/g respectively) in this plant [22]. A very good amount of Fe (1.24 ±0.004 mg/gm) and Mn (0.0133 ±0.0006 mg/gm) were detected in the tuber of *D. alata*, for which it might be helpful to play an important role in the metabolism of protein, carbohydrate and fats of human being [20]. The sufficient quantity of Mg (1.207 ± 0.0051 mg/g) in the plant would be beneficial to control the blood- glucose levels and support a healthy immune system [23].

The heavy metals content of *D. alata* has been given listed in table 1. The concentrations of Pb, As and Hg detected in this plant were below the WHO permissible limit of 0.3 µg/g, 5 µg/g and 0.23 µg /g respectively. Therefore, the consumption of this plant containing these heavy metals would not be responsible for acute and chronic poisoning of the human being [24-25].

In the present investigation, the water-soluble B vitamins and vitamin C content were determined using HPLC. This plant might be beneficial for the prevention of scurvy and maintenance of healthy skin and also to reduce the risk of atherosclerosis and some forms of cancer due to having appreciable amount of vitamin C (1.11 ±0.038) [26-27].

Thiamine (B1), is an essential nutrient required by the body for maintaining cellular function and consequently a wide array of organ functions whereas riboflavin (B2) is the counterpart to thiamine used in the strengthening of food products [28].

The amount of other water soluble vitamins (B1, B2, B3, B5, B6 and B9) detected in the tuber of *D. alata* were sufficiently high and comparable with some common fruits and vegetables. Therefore, the regular consumption of this vegetable would supply adequate vitamins necessary to regulate numerous body functions, nervous system optimal maintenance of fat and also plays an important role as an antioxidant *in vivo*, both by preventing the adverse effect of reactive oxygen species (ROS), as well as by inhibiting lipid peroxidation [29-35].

The present investigation with *D. alata* showed potent antioxidant activities using DPPH and ABTS assay. The IC_{50} value of DPPH assay was found to be higher than that of ABTS assay which showed more antioxidant activities. The total phenolic component exhibited antioxidant activity through adsorption and neutralization of the free radicals, whereas flavonoid and flavonol showed antioxidant activity through scavenging or chelating process [36-37]. The antioxidant activities of the extractive solution represent an important parameter to evaluate the biological property of the plant. Therefore, it is necessary to characterize and quantify the important compounds like phenolic acids and flavonoids present in the plant and also to validate the method of separation and identification of active constituents. The occurrence of these phenolics in wild edible plant might be responsible for the strong antioxidant properties and thus help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases [38].

The HPLC analysis showed the presence of gallic acid content (Table 3) in the 80 % aq. ethanol extract of *D. alata* (0.20±0.0047 mg/100gm DPM**)** which is well compared with some common vegetables [39] whereas the consumption of the plant containing *p*-Hydroxybenzoic acid (0.192±0.0024 mg/100gm DPM) lowered risk of a variety of liver diseases, including liver cirrhosis and liver cancer [40].

Caffeic acid is one of the major hydroxycinnamic acid components found in wine and it is a well- known antioxidant which boosts immunity, controls lipid levels in blood and anti-mutagenic. The present study showed that the ethanol extract of the plant was found to contain a moderate amount of caffeic acid 0.215±0.0023 DPM and these were compatible with the same in cauliflower (0.058 mg/gm), carrot (0.09 mg/gm), lettuce (1.57 mg/gm) and potato (2.80 mg/gm) [41].

Syringic acid with hydroxy benzoic acid skeleton was found in the plant under investigation ((0.899 ± 0.0022 DPM was higher than that reported for common leafy vegetables, such as, cauliflower (0.0113 mg/gm) *Salvia officinalis* (0.0335 mg/gm), *Origanum vulgare* (0.0375 mg/gm) and this phenolic acid is well known for its anti-cancer, anti-proliferative, sedative, decongestant and hepato-protective actions [41].

One of the important phenolics, ferulic acid which is detected (0.089± 0.0005 DPM) in the 80 % aq.ethanol extract of *D. alata* in our study and regular intake of the vegetable leads to lower cholesterol level in serum and increases sperm viability [42]. A very significant amount of sinapic acid (0.202± 0.15 DPM) was noticed in the plant under investigation and consumption of this plant would be useful for health promotion because it showed antioxidant, anti-microbial, anti-inflammatory, anticancer, and anti- anxiety activity [43]. An appreciable amount of quercetin (0.687± 0.003 DPM), detected in *D. alata* were comparable to the same in apple (0.021 mg/gm), lettuce(0.011 mg/gm) and tomato (0.055 mg/gm) and this is reported to display anti-histamine, anti-cancer as also anti-inflammatory activities [44]. The HPLC analysis of the ethanol extract of *D. alata* showed the presence of good amount of myricetin (4.613±0.003 mg/100gm DPM), which is widely available in fruits, vegetables, tea, berries and red wine and reported to be useful for the prevention of diabetes mellitus and diabetic complications [45-46]. The plant contain a significant amount of kaempferol **(**9.219±0.0043 mg/100gm DPM**)** which showed potent pharmacological and nutraceutical activities. The regular intake of this plant containing kaempferol thereby producing numerous health benefits in the form of reducing the risk of cardio vascular diseases, cancer, arteriosclerosis etc. [47-48].

5. Conclusion

The present exploration showed that plant under investigation could provide essential nutrients like protein, fat, carbohydrate, fibre, and vitamins and these are required for maintaining normal body function. The dietary property of this plant was similar to and also sometimes better than the common vegetables. The tuber of the plant was also found to be a significantly useful source of various minerals in appreciable quantities. The toxic heavy metals Cd was not detected in the plant materials but Pb, As and Hg were detected within the permissible limit as prescribed by WHO and the presence of the heavy metals in the plant might not be harmful for mankind. The presence of various phenolic acids and flavonoids inferred that tuber of this plant could be used for the nutritional purpose of human being and adequate protection may be obtained against diseases arising from malnutrition.

Conflict of Interest: We have no conflict of interest

6. Acknowledgement

Authors of this paper are highly grateful to Dr. P. Singh, Director, Botanical Survey of India, Kolkata, for providing all facilities to carry out this work.

7. References

1. Seal Tapan, Pillai Basundhara, Chaudhuri Kausik. Nutritional potential of five unexplored wild edible plants consumed by the tribal people of Arunachal Pradesh state in India. International Journal of Food Science and Nutrition. 2017; 2(2):101-105.
2. Dutta B. Food and medicine values of certain species of *Dioscorea* with special reference of Assam. Journal of Pharmacology and Phytochemistry. 2015; 3(4):15-18.
3. Namasa ND, Mandal M, Tangjang S, Mandal SC. Ethnobotany of the Monpa ethnic group at Arunachal Pradesh, India. Journal of Ethnobiology and Ethnomedicine. 2011; 7:31.
4. Lalfakzuala R, Lalramnghinglova H, Kayang H. Ethnobotanical uses of plants in Western Mizoram. Indian Journal of Traditional Knowledge. 2007; 6(3):486-493.
5. Pradhan A, Chettri A, Gurung S. Diversity and distribution of genus *Dioscorea* in Sikkim Himalaya, India: an application of DIVA-GIS. NeBIO. 2015; 6(3):29-31.
6. Joy Jenit K, Siddhuraju P. Studies on nutritional profile and antioxidant potential of different *Dioscorea* sp with *Plectranthus rotundifolius*. Int J Curr Pharm Res. 2017; 9(4):65-74.
7. Seal Tapan, Pillai Basundhara, Chaudhuri Kausik. Evaluation of nutritional potential of five unexplored wild edible plants consumed by the tribal people of Arunachal Pradesh state in India. Journal of Food and Nutrition research. 2017; 5(1):01-05.
8. Hedge JE, Hofreiter BT. In: Carbohydrate Chemistry, 17 (*Eds.* Whistler RL, Be Miller JN), Academic Press, New York, 1962.
9. Guil-Guerrero JL, Gimenez-Gimenez A, Rodriguez-Garcia I, Torija-Isasa ME. Nutritional composition of *Sonchus* Species (*S. asper* L., *S. oleraceus* L. and *S. tenerrimus* L.). J Sci Food Agric. 1998; 76:628-632.
10. Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Current Science. 2005; 89:1252-1255.
11. Seal Tapan, Kausik Chaudhuri Kaushik. High performance liquid chromatography method for the estimation of water soluble vitamin in five wild edible fruits consumed by the tribal people of north-eastern region in India. International Journal of Current Microbiology and Applied Sciences. 2017; 6(10):2900-2913.
12. ICH-Q2A, Text on Validation of Analytical Procedures, 1995.
13. ICH-Q2B, Validation of Analytical Procedures: Methodology, 1996.
14. Seal Tapan, Chaudhuri Kausik. Antioxidant activities of five wild edible fruits of Meghalaya State in India and effect of solvent extraction system. International Journal of Pharmaceutical Sciences and Research. 2015; 6(12):5134-40.
15. Seal Tapan. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. Journal of Applied Pharmaceutical Science. 2016; 6(2):157-166.
16. Nielsen SS. Determination of moisture content, Food Analysis Laboratory Manual,Published Springer US, 2010, 17-27.
17. Gopalan C, Rama Sastri BV, Balasubramanian SC. Nutritive value of Indian foods, Printed by National Institute of Nutrition, Indian Council of Medical Research, Hyderabad-500 007, India, 2004, 2-58.
18. Satter MMA, Khan MMRL, Jabin SA, Abedin N, Islam MF, Shaha B. Nutritional quality and safety aspects of wild vegetables consume in Bangladesh. Asian Pacific Journal of Tropical Biomedicine. 2016; 6:125-131.
19. Sundriyal M, Sundriyal RC. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. Economic Botany 2004; 58:286-299.
20. Mohan VR, Kalidass C. Nutritional and antinutritional evaluation of some unconventional wild edible plants. Tropical and Subtropical Agroecosystems. 2010; 12:495-506.
21. Saupi N, Zakaria MH, Bujang JS. Analytic chemical composition and mineral content of yellow velvet leaf (*Limnocharis flava* L. Buchenau)'s edible parts. Journal of Applied Science. 2009; 9:2969-2974.
22. Ihedioha JN, Okoye COB. Nutritional evaluation of *Mucuna flagellipes* leaves: An underutilized legume in Eastern Nigeria. American Journal of Plant Nutrition and Fertilization Technology. 2011; 1:55-63.
23. Saikia P, Deka DC. Mineral content of some wild green leafy vegetables of North-East India. Journal of Chemical and Pharmaceutical Research. 2013; 5:117-121.
24. Sharma P, Dubey RS. Lead toxicity in plants. Braz. J Plant Physiol. 2005; 17:35-52.
25. Annan K, Dickson RA, Amponsah IK, Nooni IK. The heavy metal contents of some selected medicinal plants sampled from different geographical locations. Phcog Res. 2013; 5:103-8.
26. Olson RE. Water soluble vitamins. In Principles of Pharmacology. Edited by PL, Munson RA. Mueller & G. R. Bresse. (Chapter 59). NewYork: Chapman and Hall, 1999.
27. Rekha C, Poornim G, Manasa M, Abhipsa V, Devi J, Kumar H, *et al*. Ascorbic acid, total phenol content and

antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chem. Sci. Trans. 2012; 1:303-310.
28. Boyaci BB, Han J, Masatcioglu MT, Yalcin E, Celik S, Ryu G, *et al*. Effects of cold extrusion process on thiamine and riboflavin contents of fortified corn extrudates. Food Chemistry. 2012; 132:2165-2170.
29. Gregory SK. Pantothenic acid. Alternative Medicine Review. 2011; 16:263-274.
30. Groff JL, Gropper SS, Hunt SM. Advanced Nutrition and Human Metabolism. 2nd Edition. Minneapolis/St. Paul New York Los Angeles San Francisco: West Publishing Company, 1995.
31. Iyer R, Tomar SK. Folate: a functional food constituent. Journal of Food Science. 2009; 74:R114-R122.
32. McGuire JJ. Folic acid, folates and cancer. In AB Awad & PG Bradford (Eds.), Nutrition and Cancer Prevention, (DOI: 10.1201/9781420026399.ch8), Boca Raton, Forida, USA: CRC Press (Taylor & Francis Group, LLC), 2006.
33. Stover PJ. Folate biochemical pathways and their regulation. In L. B. Bailey (Ed.), Folate in Health and Disease. Boca Raton, Forida, USA: CRC Press (Taylor & Francis Group, LLC), 2010, 2:49-74.
34. Rivlin RS. Vitamin deficiencies. In CD, Berdanier EB, Feldman, J Dwyer (Eds.), Handbook of Nutrition and Food. Boca Raton, Forida, USA: CRC Press (Taylor & Francis Group, LLC). 2007: 2:177-292.
35. Merola N, Alonso FJG, Ros G, Cáston MJP. Antioxidant activity comparison between [6S]-5-methyltetrahydrofolic acid calcium salt and the related racemate form. Food Chemistry. 2013; 136:984-988.
36. Florence OJ, Adeolu AA, Anthony JA. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus* Rec Nat Prod. 2011; 5:29-42.
37. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5:1142-45.
38. Kakkar S, Bais S. A Review on Protocatechuic acid and its pharmacological potential. ISRN Pharmacology. 2014; 26:1-9.
39. Romaric GB, Fatoumata AL, Oumou HK, Mamounata D, Imael HNB, Mamoudou HD. Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. African Journal of Biotechnology. 2011; 10:13543-13547.
40. Tverdal A, Skurtveit S. Coffee intake and mortality from liver cirrhosis. Annals of Epidemiology. 2003; 13:419-423.
41. Seal Tapan, Pillai Basundhara, Chaudhuri Kausik. Identification and Quantification of phenolic acids by HPLC, in two wild edible plants viz. *Solanum gilo* and *Solanum kurzii* collected from North-Eastern region in India. Journal of Chemical, Biological and Physical Sciences. 2016; 6(4):1108-1121.
42. Ramadoss Karthikeyan SI, Devadasu, Chapala, Srinivasa Babu Puttagunta. Isolation, characterization and RP-HPLC estimation of p-coumaric acid from methanolic extract of Durva Grass (*Cynodon dactylon* Linn.) (Pers.). International Journal of Analytical Chemistry. 2015; 2015:1-7.
43. Mussatto G, Dragone I, Roberto C Ferulic. p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. Industrial Crops and Products. 2007; 25:231-237.
44. Sepulveda L, Ascacio A, Rodríguez-Herrera AR, Aguilera-Carbo A, Aguilar Cristóbal, N Ellagic. Acid: Biological properties and biotechnological development for production processes. African Journal of Biotechnology. 2011; 10:4518-4523.
45. Wach A, Pyrzynska K, Biesaga M. Quercetin content in some food and herbal samples. Food Chemistry. 2007; 100:699-704.
46. Ong KC, Khoo HE. Biological effects of myricetin. Gen.Pharmacol. 1997; 29:121-126.
47. Yong Li, Ye Ding. Minireview: Therapeutic potential of myricetin in diabetes mellitus, Food Science and Human Wellness. 2012; 1(1):19-25.
48. Moghaddasian B, Eradatmand AD, Alaghemand A. Simultaneous determination of rutin and quercetin in different parts of *Capparis spinosa*. Bull. Env. Pharmacol. Life Sci. 2013; 2:35-38.