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HPLC Based Identification of Water Soluble Vitamins and Nutraceutical Value of Three Common Grasses of West Bengal

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Abstract: The purpose of the present work was to evaluate the proximate composition, minerals content (Na, K, Ca, Fe, Mg, Cu, Zn, Mn), simultaneous quantification of water soluble vitamins (like ascorbic acid, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine & folic acid) by HPLC of three different grasses viz. *Cynodon dactylon, Apluda mutica* and *Eleusin indica.* The result showed the highest calorific value of *C. dactylon* (124.031 ± 1.154 kcal/100g) which was also found to contain highest amount of protein and carbohydrate. An appreciable quantity of carbohydrate was estimated in the aerial parts of *E. indica* (17.762 ± 0.122%). *E. indica* had the highest potassium content (2.207 ± 0.006 mg/g) and calcium content (6.023 ± 0.002 mg/g). The sodium content ranged between 0.049 – 0.289 mg/g. The aerial parts of *E. indica* (274.945 ± 0.085 mg/100g) contained a very good amount of vitamin C. The water soluble B vitamin content in these plants under investigation ranged between 0.009 to 12.133 mg/100gm. In conclusion, the results indicate that these IAS can be utilized as food supplement.

Keywords: Nutritional composition, Mineral content, Water soluble vitamins, *Cynodon dactylon, Apluda mutica, Eleusine indica*.

INTRODUCTION

The values of grasses have been recognized by mankind in various stages as food, medicine and as fooder. Their remarkable ability of persistence and re-growth has been attributed to several factors. Utilization of storage food is one such factor that contributes to their re-growth potential.

These storage foods contribute largely as food and source of vitamins by grazing animals. It can also be utilized by humans as a natural source of vitamins. The present study was conducted to evaluate the concentration of water soluble vitamins and nutritional potential of three common grasses viz. *Cynodon dactylon, Apluda mutica* and *Eleusine indica* of West Bengal, India.

C. dactylon paste is applied on bleeding piles [1], wounds [2], inflammation [3] and also used as fodder [1]. *A. mutica* is used to cure mouth sores in cattle [1], in dysentery [4] and as fodder [5]. Decoction of *E. indica* is given in fever [2], fruits are used as food supplement [6] and as fodder [7].

MATERIALS AND METHODS

Plant Materials

The fresh plants (aerial parts) of *C. dactylon, A. mutica* and *E. indica* were collected from various locations of Kolkata, India and were authenticated from Botanical Survey of India, Howrah. The voucher specimens were preserved in our office. The plant materials were taken in our laboratory at refrigerated temperature using cold packs. The refrigerated plant samples were stored at 15^0C and one part processed for vitamin estimation. The other parts were shed-dried, pulverized and stored in an airtight container to evaluate proximate composition, minerals content and antioxidant properties.

Chemicals

The standards chemicals like ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), sodium hydroxide, anthrone sodium carbonate, were purchased from Sigma Chemical Co. (St. Louis , MO, USA), Petrolium ether, nitric acid, sulphuric acid, hydrochloric acid and the HPLC-grade solvents such as acetonitrile, methanol, water and trifluoroacetic acid were purchased from Merck (Germany). All the chemicals used including the solvents, were of analytical grade.

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.

Proximate Composition

Estimation of ash

5 g of each sample was weighed in a silica crucible and heated in muffle furnace for about 5-6 h at 500 °C. It was cooled in a desiccator and weighed. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or grayish white). Weight of ash gave the ash content [8].

Estimation of moisture

2 g of each sample was taken in a flat-bottom dish and kept overnight in an air oven at 100–110°C and weighed. The loss in weight was regarded as a measure of moisture content [8].

Estimation of crude fat

2 g moisture free of each sample was extracted with petroleum ether (60-80°C) in a Soxhlet apparatus for about 6-8 h. After boiling with petrol, the residual petrol was filtered using Whatman no. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave crude fat [8].

Estimation of crude fibre

2 g of moisture and fat-free material of each sample was treated with 200 mL of 1.25 % H_2SO_4. After filtration and washing, the residue was treated with 1.25 % NaOH. It was the filtered, washed with hot water and then 1 % HNO_3 and again with hot water. The washed residue was dried in an oven at 130 °C to constant weight and cooled in a dessicator. The residue was scraped into a pre-weighed porcelain crucible, weighed, ashed at 550 °C for two hours, cooled in a dessicator and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition [8].

Estimation of crude protein

The crude protein was determined using micro Kjeldahl method. 2 g of each sample compound was decomposed by digestion with concentrated sulphuric acid in the presence of a catalyst, ammonium sulphate is produced. An excess of sodium hydroxide solution was added to the diluted reaction mixture, the liberated ammonia was distilled in steam and absorbed in a measured excess of standard sulphuric acid. Titration of the residual mineral acid with standard sodium hydroxide gives the equivalent of ammonia obtained from the weight of the sample taken. From this the percentage of nitrogen in the compound can was calculated. On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N × 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content [8].

Estimation of carbohydrate

The total carbohydrate content was estimated by the method of Hedge and Hofreiter [9]. 100mg of the sample was hydrolysed by keeping it in a boiling water bath for 3h.with 5.0 ml of 2.5N hydrochloric acid. Following hydrolysis it was cooled to room temperature and then neutralized with solid sodium carbonate until the effervescence ceased. After filtration, the volume of resulting mixture was made upto100 ml. To 0.2 ml of this mixture, 0.8 ml distilled waterand2.0 ml of 0.2 % anthrone (200 mg anthrone dissolved in 100 ml of ice cold 95% Sulphuric acid) was added, heated for 8 mins in a boiling water bath, cooled rapidly and absorbance was measured at 630 nm. Glucose was taken as standard. .Carbohydrate content in microgram per ml (µg/ml) of dry material was calculated using the following equation based on the calibration curve: $y = 0.0081x + 0.2475$, $R^2 = 0.9955$, where y was the absorbance and x was the carbohydrate content (µg/ml). % carbohydrate is given as amount of carbohydrate (µg/ml) dived by volume of sample taken for analysis.

Estimation of energy content

The three components of foods which provide energy are protein, carbohydrate and fat. One gram carbohydrate and protein yield 4 kcal energy whereas one gram fat yields 9 kcal energy. Therefore 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 [8].

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 % 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 [10].

Estimation of Water Soluble Vitamins

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 and volume made 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 volume 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 standard stock solutions by mixing 100 µl mixed vitamins standard (vitamin B9, B5 and B2), 800 µl phosphate buffer (1M, 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 80µg/ml were prepared accordingly.

Preparation of sample solution

Plant materials were cleaned, 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. One gm each of freeze-dried sample was soaked in 10 ml water. Then 1 ml 0.1M 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 [11].

Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by Ciulua *et al.*, [11] 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 22^0 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 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. Total analysis time 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 and the retention times were noted and used to identify the vitamins in the sample. 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. Each compound in the plant extracts were identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done 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 of means of three independent analyses.

RESULTS

Proximate composition

The proximate analyses of the nutritive contents of the grasses are depicted in Table 1. The grasses are mineral rich which is evident from their relatively high ash content. The proximate analysis expressed in percentages, showed the moisture, ash, crude fibre, crude protein, crude lipid, carbohydrate contents and metabolic energy in the aerial parts of the grasses.

Mineral Content

Mean values for mineral content of the selected grasses are presented in Table 2 and expressed as mg/g dry material and in µg/g for copper. Four macrominerals (calcium, magnesium, potassium and sodium) and four microminerals (iron, manganese, copper, zinc) were analyzed. The grasses were richer source of Calcium and Copper as compared to the other minerals evaluated.

Table-1: Proximate Composition of the Selected Grass Species

Plants	Proximate composition						
	Moisture %	Ash %	Crude fibre %	Crude fat %	Crude protein %	Carbohydrate %	Nutritive value kcal/100g
C. dactylon	53.815 ± 0.182	12.556 ± 0.101	11.058 ± 0.043	2.574 ± 0.114	6.956 ± 0.115	18.260 ± 0.673	124.031 ± 1.154
A. mutica	58.556 ± 0.309	7.013 ± 0.107	24.498 ± 0.317	1.185 ± 0.058	3.382 ± 0.461	12.066 ± 0.358	72.460 ± 0.577
E. indica	57.782 ± 0.288	19.657 ± 0.057	6.359 ± 0.378	2.092 ± 0.115	5.198 ± 0.011	17.762 ± 0.122	110.548 ± 1.378

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

Table-2: Mineral Content of the Selected Grass Species

Plants	Minerals present mg /g and copper µg/g							
	Sodium (Na)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Iron (Fe)	Copper (Cu)	Zinc (Zn)	Manganese (Mn)
C. dactylon	0.293 ± 0.002	1.294 ± 0.014	3.869 ± 0.035	0.044 ± 0.019	0.079 ± 0.006	3.696 ± 0.115	0.043 ± 0.011	0.007 ± 0.001
A. mutica	0.045 ± 0.002	1.233 ± 0.012	2.912 ± 0.010	0.049 ± 0.017	0.061 ± 0.011	0.907 ± 0.051	0.034 ± 0.003	ND
E. indica	0.183 ± 0.002	2.207 ± 0.006	6.023 ± 0.002	0.088 ± 0.005	0.187 ± 0.011	4.289 ± 0.346	0.061 ± 0.005	0.013 ± 0.001

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

Chromatographic estimation of water soluble vitamins

A typical HPLC chromatogram of the all standard vitamin mixture recorded at 275 nm is presented in fig. 1. As shown in the chromatogram, all investigated compounds had responses at 275 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 substances.

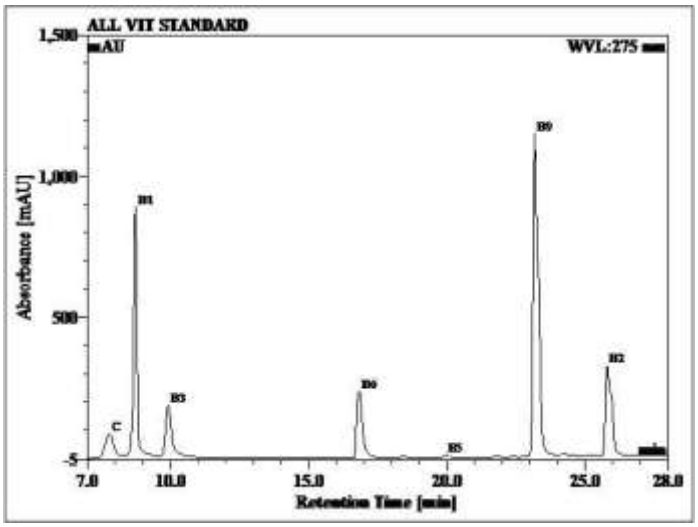

Fig-1: HPLC Chromatogram of mixture of Standard vitamin (C) Ascorbic acid; (B1) Thiamine; (B3) Niacin; (B6) Pyridoxine; (B5) Pantothenic acid; (B9) Folic acid; (B2) Riboflavin.

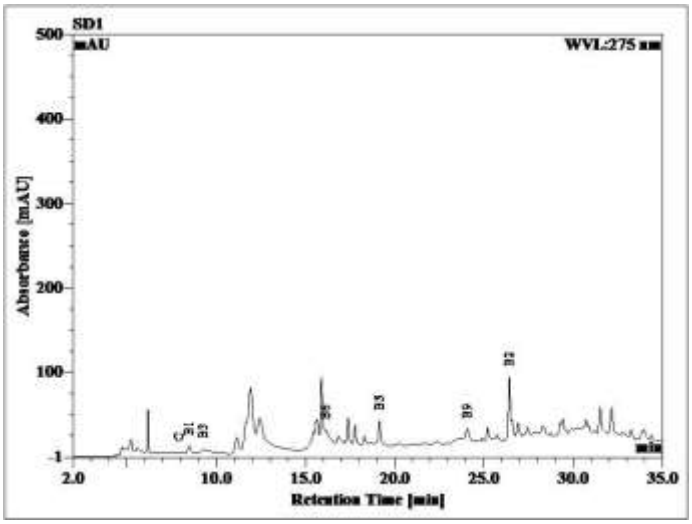

Fig-2: HPLC Chromatogram of *C. dactylon* (C) Ascorbic acid; (B1) Thiamine; (B3) Niacin; (B6) Pyridoxine; (B5) Pantothenic acid; (B9) Folic acid; (B2) Riboflavin.

The chromatogram of *C. dactylon* (Figure-2) showed the presence of vitamin C, vitamin B1, Vitamin B2, vitamin B3, vitamin B5, vitamin B6 and vitamin B9. *A. mutica* (Figure-3) showed the presence of only Vitamin B2, vitamin B6 and vitamin B9. From the HPLC chromatogram of *E. indica* (Figure-4) it is evident that it is a richer source of vitamins than the other two grass species analyzed and showed the presence of vitamin C, vitamin B1, Vitamin B2, vitamin B3, vitamin B6 and vitamin B9. The quantities of all the vitamins of the three grasses are expressed as mg/100g dry plant material and the data is represented in Table-3.

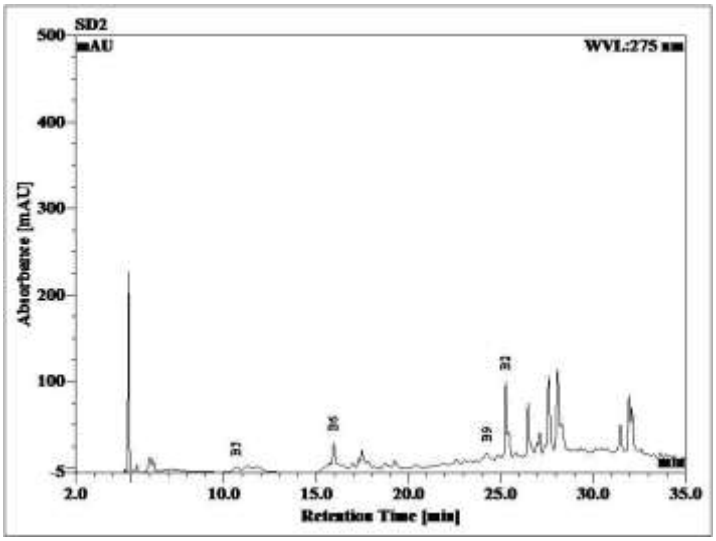

Fig-3: HPLC Chromatogram of *A. mutica* (B6) Pyridoxine; (B9) Folic acid; (B2) Riboflavin.

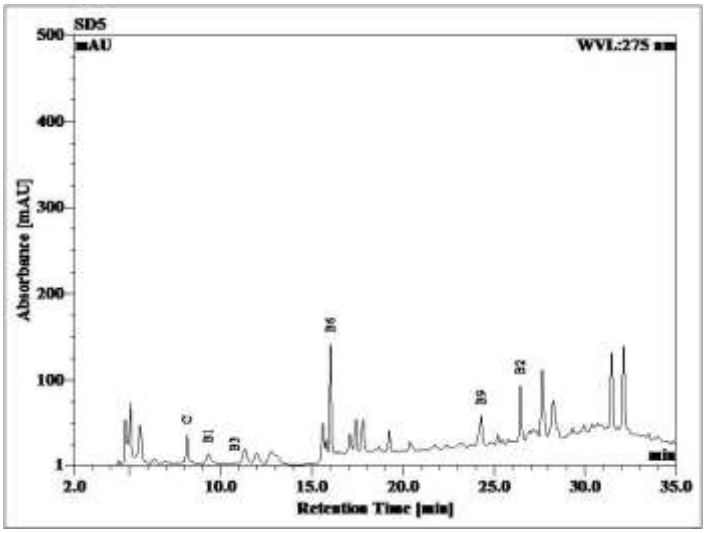

Fig-4: HPLC Chromatogram of *E. indica* (C) Ascorbic acid; (B1) Thiamine; (B3) Niacin; (B6) Pyridoxine; (B9) Folic acid; (B2) Riboflavin.

Table 3: Quantification of Water Soluble Vitamins in the Selected Grass Species

Plants	Vitamins present mg /100g						
	C	B1	B2	B3	B5	B6	B9
C. dactylon	18.279 ± 0.133	0.022 ± 0.001	1.261 ± 0.003	0.218 ± 0.005	0.962 ± 0.026	0.009 ± 0.001	0.043 ± 0.002
A. mutica	ND	ND	1.535 ± 0.005	ND	ND	0.923 ± 0.011	0.033 ± 0.003
E. indica	274.945 ± 0.085	0.885 ± 0.015	9.775 ± 0.113	0.211 ± 0.007	ND	12.933 ± 0.041	1.001 ± 0.017

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

DISCUSSION

Proximate Analysis

All the plant had a moderate moisture content ranging from 53 – 58%. The lowest moisture content was in *C. dactylon* (53.815 ± 0.182 %). Lesser moisture content indicates that storage of these species would be easier and less liable to deterioration. Ash content was relatively high with values ranging from 7.013 ± 0.107 % for *A. mutica* to 19.657 ± 0.057 % for *E. indica.* Ash content analyzed in *C. dactylon* and *A. mutica* in a different study by Sultan *et al*., [12] was 7 – 8 % and 8 – 9 % respectively. The ash content for *C. dactylon* is much lower to that obtained in this study 12.556 ± 0.101%. This variation may be due to ecological factors or age of the plant samples under study. These values indicate that these plant species may be considered as good sources of minerals and can be considered as fodder supplement.

The nutritive value of *C. dactylon* (124.031 ± 1.154 kcal/100g) was the maximum followed by that of *E. indica* (110.548 ± 0.548 kcal/100g). The outcome of investigation revealed that these plants had greater nutritive potential than the common leafy vegetables like cabbage (27 kcal/100g), spinach (26 kcal/100g) and lettuce (21 kcal/100g) [13].

The maximum carbohydrate content was in *C. dactylon* (18.260 % ± 0.673). Lowest carbohydrate content was observed in *A. mutica* (12.066 % ± 0.358). The carbohydrate content was lower than those reported from other wild leafy vegetables like *Brassica nigra* (76.14 %), *Eurya acuminata* (76.14 %) [14]. The recommended carbohydrate values for children and adults are 130 g. It implied that 9.28 to 14.04 % of the daily requirement could be reached when 100 g of these dried grasses were consumed.

The total fat content ranged between 1.396 ± 0.001– 3.79 ±0.002 % in these grasses, which was in congruence to the findings of many works which showed that leafy vegetables are poor sources of lipids [15]. However, it was important to note that diet providing 1 – 2 % of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yields to cardiovascular disorders such as atherosclerosis, cancer and aging [16]. Therefore, the consumption of these grasses in large amount may be recommended to individuals suffering from obesity. The crude fibre content in *A. mutica* was maximum (24.498 % ± 0.317) and would be advantageous for their active role in the regulation of intestinal transit, increasing dietary bulk due to their ability to absorb water [28]. The protein content ranged from 3.382 ± 0.461 % (in aerial parts of *A. mutica*) to 6.956 ± 0.115 % (in aerial parts of *C. dactylon*). Foods which provide more than 12 % of their calorific value from proteins have been shown to be good source of proteins [17]. This suggests that all the plants investigated are good sources of proteins and could play a significant role in providing cheap and available proteins for rural communities. Nutritional evaluation of the above common grasses suggests that these can be considered to be utilized as alternate food source.

Minerals Content

Mean values for mineral content of the selected leafy vegetables are presented in Table 2. Sodium (Na) concentration ranged from 0.045 ± 0.002 mg/g (*A. mutica*) to 0.293 ± 0.002 mg/g (*C. dactylon*). The sodium levels of some cultivated vegetables and fruits vary between 30-1249 mg/kg [13]. The potassium (K) ranged between 1.233 ± 0.012 (*A. mutica*) – 2.207 ± 0.006 (*E. indica*) mg/g. Na and K take part in 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 in prevention of hypertension and arteriosclerosis, with K depresses and Na enhances blood pressure [18].The ratio of K/Na in *A. mutica* (25.979) and *E. indica* (11.925) werer comparable to some common vegetables (Spinach 3.52, Celery leaves 5.91, cabbage 17.5, tomato 11.31) [19]. The calcium (Ca) content was highest in the aerial parts of *E. indica* (6.023 ± 0.002 mg/g). Ca levels varied within 2 – 6 mg/g whereas that in of some cultivated vegetables (lettuce, cabbage and spinach) varies between 0.39 –0.73 mg/g [13]. It is also very much required for the normal functioning of the cardiac muscles, blood coagulation and the regulation of cell permeability [10]. The iron content of these plants ranged between 0.061 – 0.187 mg/g. Iron is essential in oxygen binding to hemoglobin and also acts as catalyst for many enzymes like cytochrome oxidase [20]. Thus, the selected leaves of this study could be recommended in diets for reducing anemia. The magnesium content ranged between 0.044 ± 0.019 mg/g in *C. dactylon* to 0.88 ± 0.005 mg/g in *E. indica*. Magnesium helps to prevent cardiomyopathy, muscle degeneration, growth retardation, immunologic dysfunction, impaired spermatogenesis, congenital malformations and bleeding disorders [21]. The recommended dietary allowance (RDA) for minerals: calcium (1000 mg/day); magnesium (400 mg/day) and iron (8 mg/day) [22], the results suggest that these plants contribute substantially in improving the diet in terms of mineral requirement.

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 [29]. The maximum amount of Cu was observed in *E. indica* (4.289 ± 0.346) and the least amount in *A. mutica* (0.907 ± 0.051). 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 [23]. Mn is also essential for haemoglobin formation [10]. The Mn concentration in *C. dactylon* is 0.007 ± 0.001 mg/g and

that in *E. indica* is 0.013 ± 0.001 mg/g. Mn was below detection level in *A. mutica.* 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 [23]. All the grasses under study had a moderate Zn concentration ranging between 0.034–0.061 mg/g.

Quantification of water soluble vitamins

The vitamin content in the three grasses is depicted in Table-3 expressed as mg/100 dry plant material. 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. Highest amount of ascorbic acid was found in *E. indica* (274.945 ± 0.085 mg/100gm) which is high when compared to that found in common edible vegetable like tomato (23 mg/100g), spinach (51 mg/100g) and onion (190mg/100g) [24]. The recommended daily requirement for Vitamin C according to FAO/ WHO [22] is between 45.83 mg/day to 68.50 mg/day for both male and female adults between the ages of 19 to 65 years. Furthermore, the availability of reasonable amounts of vitamin C in *C. dactylon* and *E. indica* in this study provides a new source of antioxidants required for the maintenance of health. Vitamin C content was below detection level in *A. mutica.*

Thiamine (B1) is essential for energy production, carbohydrate metabolism and nerve cell function [22]. Amount of B1 in *E. indica* and *C. dactylon* is 0.885 ± 0.015 mg/100g and 0.022 ± 0.001 mg/100g. In *A. mutica* it remained undetected. Thiamine has been shown to occur in some common vegetables like beans (0.132mg/100gm), cauliflower (0.073 mg/100gm), spinach (0.076mg/100gm) [25]. As compared to these cultivated vegetables the above grasses are potentially richer source of vitamin B1.

Maximum amount of Riboflavin (B2) was found in *E. indica* (9.775 ± 0.113 mg/100g) and the least amount in C. dactylon (1.261 ± 0.003). The data obtained from this study is much higher than in some common vegetables like spinach (0.24 mg/100g), green beans (0.12±2 mg/100g, potato (0.023 ±1 mg/100g) [25]. The B2 content in these grasses is much higher than found in wild edible fruits like *D. indica* (0.525±0.004) and *Elaeagnus latifolia* (0.05±0.003) [26].

Niacin (vitamin B3) content did not show much variation in these grasses and ranged between 0.211 – 0.218 mg/100g. It was absent in *A. mutica.* Vitamin B3 plays an important role in DNA repair and fat metabolism [22].

Pantothenic acid (vitamin B5) is a component of CoA required in fatty acid metabolism [22]. Vitamin B5 was only detected in *C. dactylon.* Pyridoxine (Vitamin B6) is responsible for proper maintenance of nervous and immune system [27]. Vitamin B6 content showed a wide range of variation. It was found in maximum in *E. indica* (12.993 ± 0.041 mg/100g) and minimum in *C. dactylon* (0.009 ± 0.001 mg/100g).

Folic acid (B9) plays an important role in DNA synthesis and repair [22]. It also showed a wide range of variation and was found in maximum amount in *E. indica* (1.001 ± 0.017 mg/100g) and *A. mutica* contained the minimum amount (0.033 ± 0.003 mg/100g). Consuming these invasive species as vegetables would partly satisfy the vitamin requirement of human and therefore can be considered as alternative food and vitamin sources. The respective HPLC chromatograms are also provided (Figure 1-4).

CONCLUSION

These selected grasses were rich in protein, available carbohydrate, total dietary fibre and minerals, and could be used as an alternate nutritional and vitamin sources and might provide adequate protection against diseases with their antioxidant activity. *C. dactylon* was found to be more nutritious as per its calorific value is concerned whereas *E. indica* came out as a rich source of water soluble vitamins.

CONFLICT OF INTEREST

All authors have no conflict of interest to declare.

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