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ORIGINAL ARTICLE

A Rapid High-performance Liquid Chromatography Method for the Simultaneous Estimation of Water-soluble Vitamin in Ten Wild Edible Plants Consumed by the Tribal People of North-eastern Region in India

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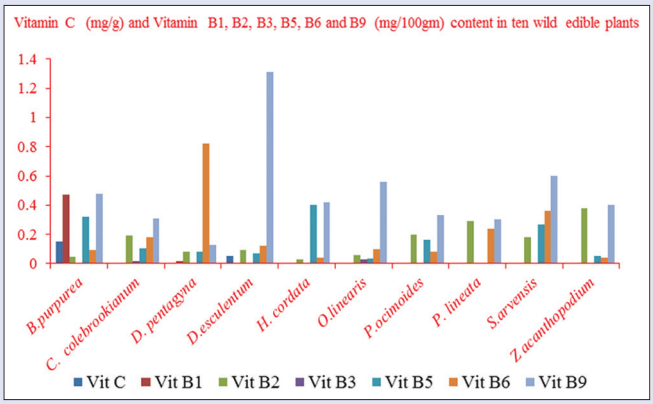
ABSTRACT

Background: *Bauhinia purpurea*, *Clerodendrum colebrookianum*, *Dillenia pentagyna*, *Diplazium esculentum*, *Houttuynia cordata*, *Oenanthe linearis*, *Potentilla lineata*, *Perilla ocimoides*, *Sonchus arvensis*, and *Zanthoxylum acanthopodium* are potent wild edible plants and consumed by the tribal people of North-eastern region in India. **Objective:** A reversed-phase high-performance liquid chromatographic method using photodiode array detector with gradient elution has been developed and validated for the simultaneous quantitation of several water-soluble vitamins such as ascorbic acid (Vitamin C), thiamine (Vitamin B_1), riboflavin (Vitamin B_2), niacin (Vitamin B_3), pantothenic acid (Vitamin B_5), pyridoxine (Vitamin B_6), and folic acid (Vitamin B_9) in these ten wild edible plants. **Materials and Methods:** The chromatographic separation of vitamins was carried out on Acclaim C 18 column (5 μm particle size, 250 mm × 4.6 mm), Dionex Ultimate 3000 liquid chromatograph and detection was carried out at three different wave lengths (210, 245, and 254 nm) using a mobile phase of acetonitrile and aqueous trifluoroacetic acid (0.01% v/v) solution with gradient elution. **Conclusion:** The results of investigation showed that these plants are rich sources of vitamins, especially the B group of vitamins that can contribute immensely to nutrition, food security, and health and therapeutic benefits. The high percentage of recovery (98%–99%), low coefficient of variation (R^2 > 0.99), low limit of detection, and limit of quantitation confirm the suitability of the method for simultaneous quantification of vitamins in these ten plants under investigation.

Key words: B group vitamins, High-performance liquid chromatography analysis, Vitamin C, water-soluble vitamins, wild edible plants

SUMMARY

- A reversed-phase high-performance liquid chromatographic method using photodiode array detector with gradient elution has been developed and validated for the simultaneous quantitation of several water-soluble vitamins such as ascorbic acid (Vitamin C), thiamine (Vitamin B_1), riboflavin (Vitamin B_2), niacin (Vitamin B_3), pantothenic acid (Vitamin B_5), pyridoxine (Vitamin B_6), and folic acid (Vitamin B_9) in ten wild edible plants consumed by the tribal people of North-eastern region in India
- The results of investigation showed that these plants are rich sources of vitamins, especially the B group of vitamins that can contribute immensely to nutrition, food security, and health and therapeutic benefits. The high percentage of recovery (98%–99%), low coefficient of variation (R^2 > 0.99), low limit of detection, and limit of quantitation confirm the suitability of the method for simultaneous quantification of vitamins in these ten plants under investigation.



Abbreviations used: RSD: Relative standard deviation; LOD: Limit of detection; LOQ: limit of quantification; SEM: Standard error of mean; ND: Not detected; DPM: Dried plant material; TFA: Trifluoroacetic acid; v/v: Volume by volume; HPLC: High-performance liquid chromatography; nm: Nanometer; mg: Milligram; μg: Microgram; R^2: Regression coefficient.

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INTRODUCTION

Vitamins are essential ingredients of food which are required in small amounts in the body on a regular basis to lead normal health and various physiological functions in the human body. They are widely distributed in natural food sources and can be easily introduced into the diets to satisfy daily needs. Vitamins are a group of organic compounds and can be categorized into two groups based on their solubility: fat-soluble vitamins and water-soluble vitamins. The former includes lipid-soluble Vitamins A, D, E, and K and other carotenoids, the latter is composed of water-soluble Vitamin C and eight B vitamins, namely thiamine (Vitamin B_1),

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riboflavin (Vitamin B_2), niacin (Vitamin B_3), pyridoxine (Vitamin B_6), pantothenic acid (Vitamin B_5), biotin (Vitamin B_7), folate (Vitamin B_9), and cyanocobalamin (Vitamin B_{12}).[1]

Measurement of vitamins in foods is complicated by many factors. It is very difficult to develop a single universal method for the simultaneous quantification of vitamin due to their diverse chemical structures and properties. Moreover, each vitamin can occur in different forms called vitamers that possess the same biological activity on ingestion. Vitamins often occur in food at relatively low levels and susceptible to degradation by exposure to light, air, heat, and high pH. Different methods including spectrophotometry, titration, high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), high-performance thin layer chromatography (HPTLC) and microbiological assays have been used for the determination of water-soluble vitamins in various conditions. The most widely used methods for the determination of ascorbic acid together with B group vitamins are reversed-phase HPLC coupled with diode array detector, using a C18 column and aqueous-organic mobile phases, in acidic media.[2]

Plants rich in fruits, vegetables, whole grains, and legumes provide an abundance of vitamins and minerals to meet one's nutritional needs. The therapeutic potential of the vegetables is largely dependent on the presence of vital vitamins as well as micronutrients. Even though vitamin is required a small amount per day in health, it plays a vital role in our health. The consumption of leafy vegetables and fruits rich in vitamins is reported to reduce the risk of attack of various acute and chronic diseases.[3]

The wild plants have been a main source of food and medicine for tribal people. In most developing nations, numerous types of edible wild plants are exploited as sources of food to provide supplementary nutrition to the inhabitants.[4]

These plants have rich nutrition and medicinal values. Regular consumption of vegetables is also recommended for better health and management of chronic diseases. The nutritive value, antioxidant properties of the fruits of wild edible plants such as *Bauhinia purpurea*, *Clerodendrum colebrookianum*, *Dillenia pentagyna*, *Diplazium esculentum*, *Houttuynia cordata*, *Oenanthe linearis*, *Potentilla lineata*, *Perilla ocimoides*, *Sonchus arvensis*, and *Zanthoxylum acanthopodium* consumed by the tribal people of North-eastern region in India has already been studied in our laboratory. The quantification of phenolic acids and flavonoids by HPLC has also been carried out with these plants.

Therefore, these wild edible plants have nutritional potential and are worthy of exploitation as a dietary resource due to the presence of sufficient amount of protein, carbohydrate, fat, and minerals. The antioxidant properties and the presence of phenolic acids and flavonoids in these wild edible plants in varying amounts have enriched the nutraceutical properties of these plants.[5-11]

This paper accounts a simple, gradient, and stability-indicating HPLC method for the rapid determination of water-soluble vitamins such as thiamine (Vitamin B_1), niacin (Vitamin B_3), pyridoxine (Vitamin B_6), ascorbic acid (Vitamin C), pantothenic acid (Vitamin B_5), riboflavin (Vitamin B_2), and folic acid (Vitamin B_9) in ten wild edible plants named as *B. purpurea*, *C. colebrookianum*, *D. pentagyna*, *D. esculentum*, *H. cordata*, *O. linearis*, *P. lineata*, *P. ocimoides*, *S. arvensis*, and *Z. acanthopodium* from North-eastern region in India, and all the vitamins were simultaneously analyzed in a single chromatographic run.

MATERIALS AND METHODS

Plant material

The wild edible plants named *B. purpurea*, *C. colebrookianum*, *D. pentagyna*, *D. esculentum*, *H. cordata*, *O. linearis*, *P. lineata*, *P. ocimoides*, *S. arvensis*, and *Z. acanthopodium* were collected from North-eastern region in India. It was duly authenticated, and a voucher specimen was kept at the Department of Plant Chemistry of Botanical Survey of India under the Registry No. BSITS 15, BSITS 7, BSITS 16, BSITS 17, BSITS 3, BSITS 5, BSITS 10, BSITS 6, BSITS 4, and BSITS 1 for future reference. The plant parts were taken in our laboratory at refrigerated temperature using cold packs. The refrigerated plant samples were stored at −15°C and then processed within 4 days of collection.

Chemicals

The standards chemicals such as ascorbic acid (Vitamin C), thiamine (Vitamin B_1), riboflavin (Vitamin B_2), niacin (Vitamin B_3), pantothenic acid (Vitamin B_5), pyridoxine (Vitamin B_6), and folic acid (Vitamin B_9) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and the HPLC-grade solvents such as acetonitrile, methanol, water sodium dihydrogen phosphate, and trifluoroacetic acid were purchased from Merck (Germany).

High-performance liquid chromatography equipment

HPLC analyses were performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 µl loop, and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim™ 120 C18 column (5 µm particle size, i. d. 4.6 mm × 250 mm).

Preparation of standard solutions

The stock standard solutions of Vitamin C, B_1, B_3, B_5, and B_6 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 B_9 and B_2, 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°C. The working standards were prepared from the stock standard solutions by mixing 100 µl mixed vitamins standard (Vitamin B_9, B_5, and B_2), 800 µl phosphate buffer (1 M, pH 5.5), and 100 µl mixed vitamins standard (Vitamin C, B_1, B_6, and B_3) which represent 100 µg/ml mixed working standards. The working standard solutions of concentrations 20, 40, 60, and 80 µg/ml were prepared accordingly.

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°C until analysis.

One g of each freeze-dried plant materials were soaked in 10 ml water. Then, 1 ml 0.1 M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in dark for 24 h. 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 *Marco Ciulua*[12] with minor modification. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoroacetic acid (TFA, 0.01% v/v) (Solvent B), the column was thermostatically controlled at 22°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% solvent A and 99% solvent B with flow rate 0.5 ml/min in 5 min, from 1% to 25% solvent A with flow rate 0.5 ml/min for 16 min, from 25% to 45 % solvent A, with flow rate 0.5 ml/min for 8 min. from 45 to 1% solvent 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 photodiode array UV detector at four different wavelengths (210, 245, 275, and 290 nm) according to absorption maxima of analyzed compounds. Each compound in the plant extracts was 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 means of three independent analyses, and the method was validated according to the United States Pharmacopeia (USP) and International Conference on Harmonisation guidelines.[13,14] Various parameters were studied to validate the reproducibility of the method, namely the effectiveness, the linearity, the limit of detection (LOD), the limit of quantitation (LOQ), the precision, and the accuracy.

Statistical analysis

Statistical analyses were performed according to the Statistical Package for Social Science (SPSS, version 22). The significant and nonsignificant variations within water-soluble vitamin contents and the ten wild edible plants were analyzed using one-way analysis of variance. The data were expressed as the mean ± standard error mean. P <0.05 was considered statistically significant.

RESULTS

Chromatographic method

A typical HPLC chromatogram of the all standard vitamin mixture recorded at 210 nm is presented in Figure 1. As shown in the chromatogram, all investigated compounds had responses at 245 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. The regression coefficient together with LOD and LOQ values is shown in Table 1. The high value of R^2 > 0.9906 in the range of analyzed concentrations at 210, 245, and 275 nm is indicative of responsive linearity.

Identification and quantification of water-soluble vitamins in the wild edible plants

The HPLC method was successfully performed for the estimation of water-soluble vitamin, for example, ascorbic acid (Vitamin C), thiamine (Vitamin B_1), riboflavin (Vitamin B_2), niacin (Vitamin B_3), pantothenic acid (Vitamin B_5), pyridoxine (Vitamin B_6), and folic acid (Vitamin B_9). The quantity of all vitamins of all plant materials has been expressed as mg/100 g dry plant material and data are presented in Table 2.

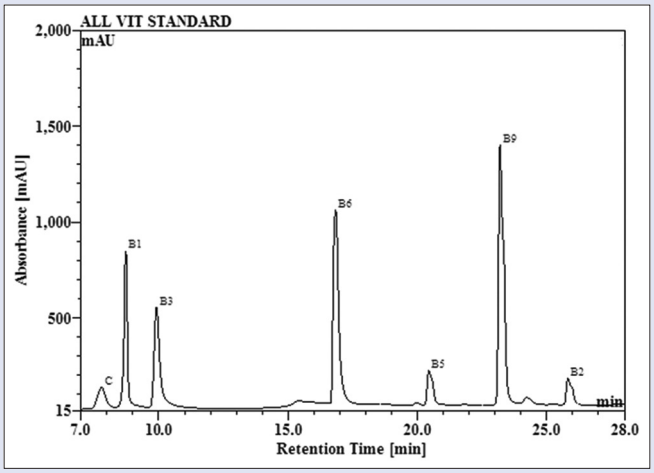

Figure 1: High-performance liquid chromatography chromatogram of mixture of standard vitamin. C: Ascorbic acid; B_1: Thiamine; B_3: Niacin; B_6: Pyridoxine; B_5: Pantothenic acid; B_9: Folic acid; B_2: Riboflavin

The HPLC analysis of the plants *B. purpurea* showed the presence of Vitamin C (15.35 ± 0.11 mg/100 g), Vitamin B_1 (0.47 ± 0.01 mg/100 g), Vitamin B_2 (0.047 ± 0.0001 mg/100 g), Vitamin B_5 (0.32 ± 0.014 mg/100 g), Vitamin B_6 (0.09 ± 0.003 mg/100 g), and Vitamin B_9 (0.48 ± 0.0001 mg/100 g).

The HPLC study of the plant *C. colebrookianum* revealed the presence of Vitamin B_2 (0.19 ± 0.002 mg/100 g), Vitamin B_3 (0.02 ± 0.001 mg/100 g), Vitamin B_5 (0.102 ± 0.002 mg/100 g), Vitamin B_6 (0.18 ± 0.002 mg/100 g), and Vitamin B_9 (0.31 ± 0.002 mg/100 g).

The presence of Vitamin B_1 (0.02 ± 0.0005 mg/100 g), Vitamin B_2 (0.08 ± 0.001 mg/100 g), Vitamin B_3 (0.37 ± 0.001 mg/100 g), Vitamin B_5 (0.08 ± 0.005 mg/100 g), Vitamin B_9 (0.13 ± 0.002 mg/100 g), and remarkable amount of Vitamin B_6 (0.82 ± 0.004 mg/100 g) were detected in *D. pentagyna*.

The leaves of *D. esculentum* were found to contain Vitamin C (5.41 ± 0.03 mg/100 g) along with Vitamin B_1 (0.006 ± 0.0005 mg/100 g), Vitamin B_2 (0.09 ± 0.001 mg/100 g), Vitamin B_5 (0.07 ± 0.001 mg/100 g), Vitamin B_6 (0.12 ± 0.001 mg/100 g), and significant amount of Vitamin B_9 (1.31 ± 0.001 mg/100 g).

The HPLC analysis showed the presence of Vitamin B_1 (0.0004 ± 0.00003 mg/100 g), Vitamin B_2 (0.03 ± 0.001 mg/100 g), Vitamin B_3 (0.006 ± 0.0002 mg/100 g), Vitamin B_5 (0.40 ± 0.003 mg/100 g), Vitamin B_6 (0.04 ± 0.0002 mg/100 g), and good amount of Vitamin B_9 (0.42 ± 0.002 mg/100 g) in the roots of *H. cordata*.

Our investigation disclosed the occurrence of Vitamin B_1 (0.0061 ± 0.002 mg/100 g), Vitamin B_2 (0.057 ± 0.003 mg/100 g), Vitamin B_3 (0.027 ± 0.001 mg/100 g), Vitamin B_5 (0.037 ± 0.001 mg/100 g), Vitamin B_6 (0.10 ± 0.002 mg/100 g), and Vitamin B_9 (0.56 ± 0.002 mg/100 g) in the leaves of *O. linearis*.

The HPLC study of the seeds of *P. ocimoides* showed the presence of Vitamin B_2 (0.20 ± 0.003 mg/100 g), Vitamin B_3 (0.012 ± 0.001 mg/100 g), Vitamin B_5 (0.16 ± 0.007 mg/100 g), and Vitamin B_6 (0.08 ± 0.001 mg/100 g) along with very sufficient amount of Vitamin B_9 (0.33 ± 0.003 mg/100 g).

The tuberous roots of *P. lineata* were found to contain Vitamin B_2, Vitamin B_6, and Vitamin B_9 amounting 0.29 ± 0.002, 0.24 ± 0.005, and

Table 1: Retention time and parameters of calibration curve, precision and repeatability, limit of detection, limit of quantification, and percentage recovery study of standard water-soluble vitamins for high-performance liquid chromatography method validation

Name of the standard vitamin	Detected at wavelength λ (nm)	Retention time	RSD (%) of the retention time	RSD (%) of the peak area at concentration 40 µg/ml	RSD (%) of the peak area at concentration 60 µg/ml	Regression coefficient R^2	LOD (µg/ml)	LOQ (µg/ml)	Percentage of recovery (%)
Vitamin C	245	7.79	0.956	0.138	0.149	99.88	0.186	0.565	98.76
Vitamin B_1	245	8.73	0.462	0.025	0.032	99.73	0.034	0.103	98.24
Vitamin B_3	245	9.92	0.706	0.206	0.171	99.83	0.277	0.839	98.50
Vitamin B_6	275	16.84	0.712	0.799	0.382	99.91	1.062	3.219	98.15
Vitamin B_5	210	20.44	0.830	0.173	0.103	99.89	0.233	0.705	98.33
Vitamin B_9	275	23.19	0.475	0.220	0.227	99.10	0.309	0.935	99.20
Vitamin B_2	275	25.82	0.453	0.114	0.144	99.68	0.156	0.472	98.25

RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification

Table 2: Quantification of Vitamin C and B_1, B_2, B_3, B_5, B_6, and B_9 in ten wild edible plants

	Vitamin content in mg/100 g dry plant material						
	Vitamin C	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_5	Vitamin B_6	Vitamin B_9
Bauhinia purpurea	15.35±0.11	0.47±0.01	0.047±0.0001	ND	0.32±0.014	0.09±0.003	0.48±0.0001
Clerodendrum colebrookianum	ND	ND	0.19±0.002	0.02±0.001	0.102±0.002	0.18±0.002	0.31±0.002
Dillenia pentagyna	ND	0.02±0.0005	0.08±0.001	0.37±0.001	0.08±0.005	0.82±0.004	0.13±0.002
Diplazium esculentum	5.41±0.03	0.006±0.0005	0.09±0.001	ND	0.07±0.001	0.12±0.001	1.31±0.001
Houttuynia cordata	ND	0.0004±0.00003	0.03±0.001	0.006±0.0002	0.40±0.003	0.04±0.0002	0.42±0.002
Oenanthe linearis	ND	0.0061±0.002	0.057±0.003	0.027±0.001	0.037±0.001	0.10±0.002	0.56±0.002
Perilla ocimoides	ND	ND	0.20±0.003	0.012±0.001	0.16±0.007	0.08±0.001	0.33±0.003
Potentilla lineata	ND	ND	0.29±0.002	ND	ND	0.24±0.003	0.30±0.003
Sonchus arvensis	ND	ND	0.18±0.002	ND	0.27±0.004	0.36±0.004	0.60±0.004
Zanthoxylum acanthopodium	0.76±0.03	0.001±0.0002	0.38±0.001	ND	0.05±0.001	0.04±0.001	0.40±0.001

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean±SEM. ND: Not detected; SEM: Standard error of mean

0.30 ± 0.004 mg/100 g, respectively, whereas Vitamin C, B_1, B_3, and B_5 were not detected.

The HPLC study of the leaves of *S. arvensis* showed the presence of Vitamin B_2 (0.18 ± 0.002 mg/100 g), Vitamin B_5 (0.27 ± 0.004 mg/100 g), Vitamin B_6 (0.36 ± 0.004 mg/100 g), and Vitamin B_9 (0.10 ± 0.004 mg/100 g) whereas the leaves of *Z. acanthopodium* found to contain vitamins (C, B_1, B_2, B_5, B_6, and B_9) amounting 0.76 ± 0.03,0.001 ± 0.00002, 0.38 ± 0.001, 0.05 ± 0.001, 0.04 ± 0.001, and 0.40 ± 0.001 mg/100 g, respectively.

DISCUSSION

Chromatographic method

The quantitative analysis of water-soluble vitamins was carried out using a photodiode array UV detector at four different wavelengths (210, 245, 275, and 290 nm). The detection of Vitamin C, Vitamin B_1, and Vitamin B_3 were carried out at wavelength 245 nm, Vitamin B_2, Vitamin B_6, and Vitamin B_9 were carried out at 275 nm. The detection wavelength was set at 210 nm for Vitamin B5 as it showed absorption at 209 nm. The chromatographic separation was performed at a flow rate of 0.5 ml/min. The method proposed was rapid, and all analytes were completely eluted within 30 min, and the whole chromatographic run was completed in 35 min. The solvent system (acetonitrile and aqueous TFA, 0.01% v/v) was used for the analysis and produced a sharp peak of the studied vitamins.

The repeatability of the retention time for all the standard samples and the relative standard deviation for the peak areas of two standards, namely 40 and 60 µg/ml was found to be below 1%. In this chromatographic method the percentage of recovery of standard vitamins is significantly high (98.15–99.20%). It follows that the method under consideration is characterized by precision, accuracy, and meticulousness and can be used for the qualitative as also quantitative estimation of water-soluble vitamins in the ten wild edible plants under investigation.

The aim of this study was to develop simple, gradient, and stability-indicating HPLC method for the determination of Vitamin C, B_1, B_2, B_3, B_5, B_6, and B_9 in ten wild edible plants. Vitamin C is extremely unstable in basic and neutral solutions, but relatively stable in acidic solutions; therefore, phosphate buffer (pH 5.5) was used as a diluting solution for Vitamin C, B_1, B_3, B_5, and B_6. Both the Vitamins (B_2 and B_9) were found slightly soluble in water and acidic aqueous solutions, but soluble in basic aqueous solutions. Hence, the stock solutions of Vitamin B_2 and B_9 were dissolved in 0.1M NaOH solution, and all working standard vitamins were diluted with phosphate buffer (pH 5.5) solution.

Identification and quantification of water-soluble vitamins in the wild edible plants

Vitamin C is the most important vitamin in fruits and vegetables. Plants, rich in Vitamin C, are well known for its antioxidant properties, and they help the body in inhibiting from viral infection, bacterial infections, and toxicity. It is required for the prevention of scurvy and maintenance of healthy skin, gums, and blood vessels, and the deficiency of this vitamin causes bruising, bleeding, dry skin, and depression.[15,16]

The experimental result showed that the amount of Vitamin C in ten wild edible plants ranged from 0.76 ± 0.03–15.35 ± 0.11 mg/100 g. The quantity of Vitamin C was found highest in the leaves of *B. purpurea* (95.54 ± 3.33 mg/100 g) followed by in the leaves of *D. esculentum* (5.41 ± 0.03 mg/100 g) [Figure 2]. The Vitamin C content in these wild edible plants is very much comparable with some common fruits and vegetables such as *Solanum tuberosum* (17.04 ± 1.18 mg/100 g), *Allium sativum* (13.06 ± 1.10 mg/100 g), and *Daucus carota sativus* (2.55 ± 0.72 mg/100 g).[15] Vitamin C was not detected in other plants under investigation.

Hence, the wild edible plants under investigation might be considered good sources of Vitamin C and therefore could be able to satisfy the

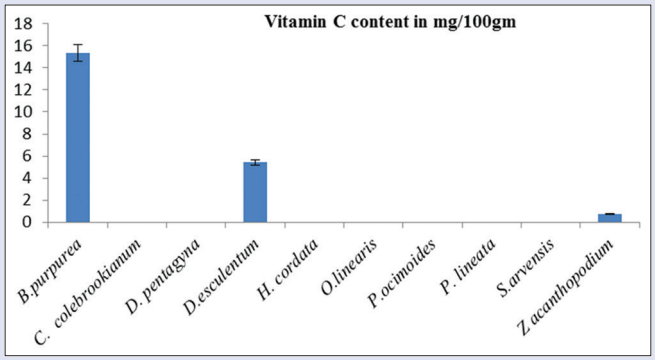

Figure 2: Vitamin C content in ten wild edible plants

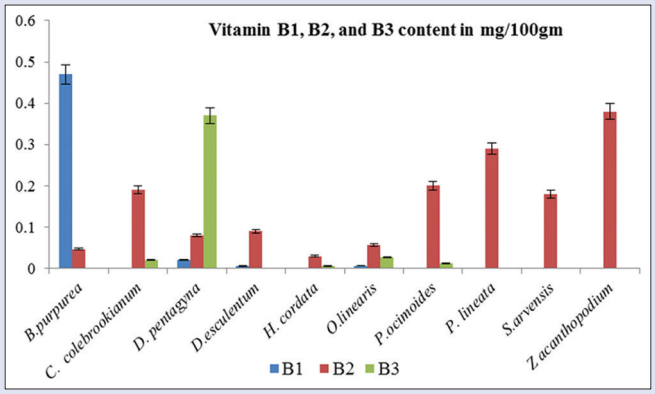

Figure 3: Vitamin B_1, B_2, and B_3 content in ten wild edible plants

recommended daily allowances of 75 mg/day and 90 mg/day for adult women and men, respectively, and 45 mg/day for children of 9–12 years old. Due to having antioxidant properties, Vitamin C-rich plant might be beneficial to reduce the risk of atherosclerosis and some forms of cancer.[17]

Thiamine (Vitamin B_1) is an essential nutrient required by the body for maintaining cellular function and consequently a wide array of organ functions. It is indispensable for energy production, carbohydrate metabolism, and nerve cell function. The deficiency of this vitamin is responsible for high blood pressure, cardiac diseases and deterioration of the nervous systems.[18,19]

The thiamine content in these wild edible fruits ranged from 0.0004 ± 0.00001 to 0.47 ± 0.01 mg/100 g. The highest amount of Vitamin B_1 was obtained from the leaves of *B. purpurea* followed by *D. pentagyna* and *D. esculentum*. The sufficient amount of this vitamin was also noted in *H. cordata*, *O. linearis*, and *Z. acanthopodium* and was not detected in other four plants under study [Figure 3].

Thiamine has been shown to occur in some common vegetables and fruits such as apple (0.016 mg/100 g), beans (0.132 mg/100 g), cauliflower (0.073 mg/100 g), and spinach (0.076 mg/100 g), and these amounts are very much similar to the thiamine content detected in the wild edible plants under investigation.

Riboflavin (Vitamin B_2) is a vital vitamin required for proper energy metabolism and a wide variety of cellular processes. It is the counterpart to thiamine used in the strengthening of food products.[20] A significant variation of riboflavin content was noticed among the tested wild edible plants. The highest amount of Vitamin B_2 was detected in the leaves of *Z. acanthopodium* (0.38 ± 0.001 mg/100 g) and the least amount was detected in *H. cordata* (0.03 ± 0.001 mg/100 g). The leaves of *C. colebrookianum*, *S. arvensis,* seeds of *P. ocimoides*, and tuberous roots of *P. lineata* were also found to contain a very good quantity of Vitamin B_2 [Figure 3] which are comparable with some common fruits and vegetables such as almonds (1.10 mg/100 g), spinach (0.24 mg/100 g), beet greens (0.41 mg/100 g), green beans (0.12 ± 2 mg/100 g), and potato (0.023 ± 1 mg/100 g).[21]

The niacin (Vitamin B_3) content in the wild edible plants under analysis ranged between 0.006 ± 0.0002 mg/100 g and 0.37 ± 0.001 mg/100 g. The highest amount of Vitamin B_3 was detected in *D. pentagyna* followed by in the leaves of *O. linearis* (0.027 ± 0.001 mg/100 g) [Figure 3]. Therefore, these plants are the important sources of Vitamin B_3. The other plants, namely *C. colebrookianum*, *H. cordata*, and *P. ocimoides* also found to have necessary amount of Vitamin B_3 which were comparable with cabbage, cauliflowers, cucumber, spinach, and tomatoes ranged between 0.19 and 0.97 mg/100 g.[22]

Vitamin B_3 is an important vitamin required for processing fat in the body, lowering cholesterol levels, and regulating blood sugar levels. It is important in DNA repair, Ca metabolism, intracellular respiration, and biosynthesis of fatty acid and steroids.[23] Hence, the regular consumption of these plants would supply adequate Vitamin B_3 necessary to maintain healthy body functions.

Vitamin B_5, or Pantothenic acid, is an essential vitamin required by the body for cellular processes and optimal maintenance of fat. The deficiency of Vitamin B5 leads to irritability, fatigue, apathy, numbness, paresthesia, and muscle cramps in human being.[24]

Pantothenic acid was detected highest in the roots of *H. cordata* (0.40 ± 0.003 mg/100 g). The leaves of *B. purpurea*, *C. colebrookianum*, and *S. arvensis* were also found to contain a very good amount of Vitamin B_5 and was not detected in *P. lineata* [Figure 4].

Pyridoxine (B_6) is another water-soluble vitamin necessary for the proper maintenance of red blood cell metabolism, the nervous system, the immune system, and many other bodily functions. It also plays a role in homocysteine synthetic and degradative reactions.[25] This vitamin is found in most food products and also, due to its stability, is often used for fortifying food products.[26] It was quantified in all the wild edible plants under our investigation. The highest Vitamin B_6 was observed in *D. pentagyna* (0.82 ± 0.004 mg/100 g) whereas the minimum was detected in both *H. cordata* and *Z. acanthopodium* (0.04 mg/100 g). The Vitamin B_6 content ranged between 0.08 ± 0.001 and 0.36 ± 0.004 mg/100 g in other seven wild edible plants under investigation [Figure 4].

The amount of Vitamin B_6 obtained in these wild edible plants was comparable with some common vegetable and fruits such as banana (0.37 mg/100 g), avocados (0.29 mg/100 g), spinach (0.24 mg/100 g), broccoli (0.134 mg/100 g), cauliflower (0.115 mg/100 g), and cucumber (0.2 mg/100 g). Hence, the regular intake of these plants would supply sufficient Vitamin B_6 necessary to maintain healthy body functions.

Vitamin B_9 (folic acid) is a water-soluble B vitamin with many rich natural sources. It is required for numerous body functions including DNA synthesis and repair, cell division, and cell growth. The shortage of folate can lead to anemia in adults and slower development in children.[27-30] It plays an important role as an antioxidant *in vivo*, both by preventing the adverse effect of reactive oxygen species), as well as by inhibiting lipid peroxidation.[30]

The extent of Vitamin B_9 in ten wild edible plants ranged from 0.13 ± 0.001 to 1.31 ± 0.002 mg/100 g. The content of Vitamin B_9 was found highest in *D. esculentum* and the leaves of *O. linearis* contained second highest amount of Vitamin B_9 (0.56 ± 0.002 mg/100 g). Among ten wild edible plants, Vitamin B_9 was lowest in *D. pentagyna*. A good amount of Vitamin B_9 was also detected in the leaves of *B. purpurea* (0.48 ± 0.0001 mg/100 g), *S. arvensis* (0.60 ± 0.004 mg/100 g), and *Z. acanthopodium* (0.40 ± 0.001 mg/100 g) [Figure 4].

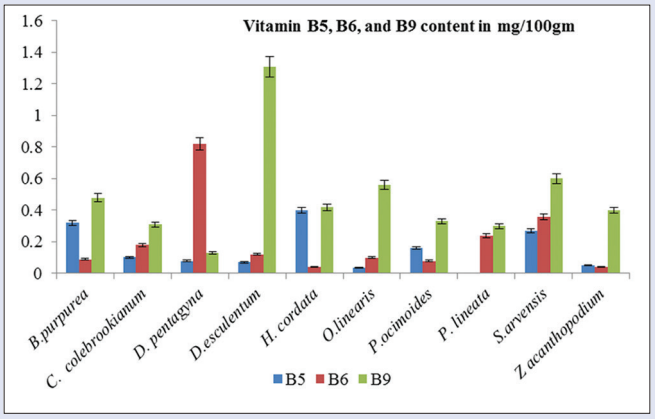

Figure 4: Vitamin B_5, B_6, and B_9 content in ten wild edible plants

CONCLUSION

The reversed-phase HPLC method with diode array detection was developed for the quantitative estimation of water-soluble B vitamins (B_1, B_2, B_3, B_5, B_6, and B_9) and Vitamin C in ten wild edible plants such as *B. purpurea*, *C. colebrookianum*, *D. pentagyna*, *D. esculentum*, *H. cordata*, *O. linearis*, *P. lineata*, *P. ocimoides*, *S. arvensis*, and *Z. acanthopodium* collected from North-eastern region in India. The established HPLC assay showed a well separation of the compounds, and also, the developed method was linear, sensitive, accurate, meticulous, and reproducible. Therefore, the method can be used for the simultaneous determination of water-soluble B vitamins and Vitamin C in different formulations with "shorter run time" and "high efficiency." RP-HPLC results showed that the plants contained several water-soluble B and C vitamins in varying amounts. The result of analysis of vitamin content in the wild edible plants under investigation will serve as a useful means to calculate dietary intake of C and B vitamins in the general population. These data will also be helpful in the preparation of a complete food composition table for nutritional survey and also for other research purposes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Harris DC. Quantitative Chemical Analysis. 6th ed. New York: Published by W.H. Freeman and Company; 2003.
2. Pengfei J, Lufeng X, Zheng L, Ning C, Ding Z, Xin H. Rapid determination of thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine, folic acid and ascorbic acid in vitamins with minerals tablets by high-performance liquid chromatography with diode array detector. J Pharm Biomed Anal 2012;70:151-7.
3. Hasan N, Akhtaruzzaman M, Sultan Z. Estimation of Vitamins B-complex (B2, B3, B5 and B6) of some leafy vegetables indigenous to Bangladesh by HPLC method. J Anal Sci Methods Instrum 2013;3:24-9.
4. Sudhakaran A, Nair GA. Nutritional evaluation of fruits of *Gynochthodes umbellata* (L.) Razafim. and B. Bremer – An underutilized edible fruit plant. Pharmacogn J 2016;8:72-6.
5. Seal T. Determination of nutritive value, mineral contents and antioxidant activity of some wild edible plants from Meghalaya state, India. Asian J Appl Sci 2011;4:238-46.
6. Seal T. Nutritional composition of wild edible fruits in Meghalaya state of India and their ethno-botanical importance. Res J Botany 2011;6:58-67.
7. Seal T. Antioxidant activity of some wild edible fruits of Meghalaya state in India. Adv Biol Res 2011;5:155-60.
8. Seal T, Pillai B, Chaudhuri K. 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. J Chem Biol Phys Sci 2016;6:1108-21.
9. Seal T, Pillai B, Chaudhuri K. Identification and quantification of phenolic acids by HPLC in three wild edible plants viz. *Viburnum foetidum*, *Houttuynia cordata* and *Perilla ocimoides* collected from North-Eastern region in India. Int J Curr Pharm Rev Res 2016;7:267-74.
10. Seal T, Chaudhuri K. Identification and quantification flavonoids in two wild edible plants *Viburnum foetidum* and *Perilla ocimoides* of North-Eastern region in India using high performance liquid chromatography with diode array detection. J Med Plant Stud 2016;4:79-85.
11. Seal T, Chaudhuri K, Pillai B. Identification and quantification flavonoids in three wild edible plants, *Houttuynia cordata, Solanum gilo* and *Solanum kurzii* of North-Eastern region in India, using high performance liquid chromatography with diode array detection. J Chem Pharm Res 2016;8:859-67.
12. Ciulu M, Solinas S, Floris I, Panzanelli A, Pilo MI, Piu PC, *et al.* RP-HPLC determination of water-soluble vitamins in honey. Talanta 2011;83:924-9.
13. ICH-Q2A, Text on Validation of Analytical Procedures; March, 1995.
14. ICH-Q2B, Validation of Analytical Procedures: Methodology; November, 1996.
15. Deekshika B, Lakshmi BP, Singuluri H, Sukumaran MK. Estimation of ascorbic acid content in fruits & vegetables from Hyderabad, India – A theoretical assessment of Vitamin C activity. Int J Curr Microbiol Appl Sci 2015;4:96-9.
16. Asghari G, Palizban A, Bakhshaei B. Quantitative analysis of the nutritional components in leaves and seeds of the Persian *Moringa peregrina* (Forssk.) fiori. Pharmacognosy Res 2015;7:242-8.
17. 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;1:303-10.
18. Akah NP, Onweluzo JC. Evaluation of water-soluble vitamins and optimum cooking time of fresh edible portions of elephant grass (*Pennisetum purpureum* L. Schumach) shoot. Niger Food J 2014;32:120-7.
19. Akyilmaz E, Yaşa I, Dinçkaya E. Whole cell immobilized amperometric biosensor based on *Saccharomyces cerevisiae* for selective determination of Vitamin B1 (thiamine). Anal Biochem 2006;354:78-84.
20. 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 Chem 2012;132:2165-70.
21. Watada AE. Vitamins C, Bl9 and B2 contents of stored fruits and vegetables as determined by high performance liquid chromatography. J Am Soc Hort Sci 1987;112:794-7.
22. Institute of Medicine Standing Committee on the Scientific Evaluation. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline, National Academies Press (US), Washington (DC); 1998. p. 1-591.
23. Gregory SK. Pantothenic acid. Altern Med Rev 2011;16:263-74.
24. Rivlin RS. Vitamin deficiencies. In: Berdanier CD, Feldman EB, Dwyer J, editors. Handbook of Nutrition and Food. 2nd ed. Boca Raton, Forida, USA: CRC Press, Taylor and Francis Group, LLC; 2007. p. 177-92.
25. Russell LF. Water soluble vitamins. In: Nollet LM, Toldrá F, editors. Food Analysis by HPLC. 3rd ed. Boca Raton, Forida, USA: CRC Press; 2012. p. 325-442.
26. Groff JL, Gropper SS, Hunt SM. Advanced Nutrition and Human Metabolism. 2nd ed. Minneapolis, New York, San Francisco: St. Paul, Los Angeles, West Publishing Company; 1995.
27. Iyer R, Tomar SK. Folate: A functional food constituent. J Food Sci 2009;74:R114-22.
28. McGuire JJ. Folic acid, folates and cancer. In: Awad AB, Bradford PG, editors. Nutrition and Cancer Prevention. Ch. 8. Boca Raton, Forida, USA: CRC Press, Taylor and Francis Group, LLC; 2006.
29. Stover PJ. Folate biochemical pathways and their regulation. In: Bailey LB, editor. Folate in Health and Disease. 2nd ed. Boca Raton, Forida, USA: CRC Press, Taylor and Francis Group, LLC; 2010. p. 49-74.
30. Merola N, Alonso FJ, Ros G, Castón MJ. Antioxidant activity comparison between [6S]-5-methyltetrahydrofolic acid calcium salt and the related racemate form. Food Chem 2013;136:984-8.