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Research Article

Simultaneous Estimation of Water Soluble Vitamin in Five Wild Edible Plants Consumed By the Tribal People of North-Eastern Region in India by High Performance Liquid Chromatography (HPLC) Method

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method has been developed for the simultaneous quantitation of water-soluble vitamins like ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9) in five potent wild edible plants named *Castanopsisindica, Fagopyrum cymosum, Ficus clavata, Ficus geniculata* and *Ficus pomifera consumed* by the tribal people of North-eastern region in India.

The chromatographic separation of vitamins were carried out on Acclaim C 18 column (5 µm particle size, 250 x 4.6 mm) , Dionex Ultimate 3000 liquid chromatography and detection was carried out at three different wave lengths (210, 245 and 254 nm) using a mobile phase of acetonitrile and aqueous trifluoro acetic acid (TFA, 0.01% v/v) solution with gradient elution. The experimental results showed that for different plants, the vitamin C content ranged between0.18± 0.003to 21.29± 1.00mg/100g dry plant material (DPM). The B2 content was determined high in C.*Indica* (1.32± 0.33mg/100gDPM) and significant amount of B9 (8.66± 0.30 mg/100g) was detected in this plant. The results of investigation showed that these plants are rich sources of vitamins, which can contribute immensely to nutrition and food security. The high percentage of recovery and low limit of detection confirm the suitability of the method for simultaneous quantification of vitamins in these five wild edible fruits.

INTRODUCTION

Vitamins are fundamental components of sustenance which are required in unobtrusive amounts in the body constantly to lead regular prosperity and diverse physiological limits in the human body. They are extensively circled in ordinary sustenance sources and can be successfully familiar into the weight control plans with satisfy each day needs. Vitamins are a gathering of organic compounds and can be sorted into two gatherings dependent on their solvency: fat-dissolvable vitamin and water-dissolvable vitamin. The previous incorporates lipid dissolvable nutrients A, D, E, and K and various carotenoids, the latter is made out of water dissolvable vitamins C and eight B-vitamins, specifically thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic destructive (B5), biotin (B7), folate (B9) and cyanocobalamin (B12) [1].

Estimation of vitamins in nourishments is perplexed by various parts. It is extraordinarily difficult to develop a singular across the board methodology for the

simultaneous assessment of supplement in light of their various substance structures and properties. Moreover, every vitamin can occur in different structures considered vitamins that have the equal natural action upon ingestion. Vitamins as often as possible occur in nourishment at decently low levels and feeble to corruption by introduction to light, air, warmth and high pH. Particular instrumental methods have been used for the affirmation of Vitamin C and B-pack supplements, including spectrophotometry, titration, High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), High Performance Thin Layer Chromatography (HPTLC) and microbiological looks at have been represented the confirmation of water-dissolvable supplements in various conditions. The most extensively used strategies for the confirmation of ascorbic acid together with B-bunch supplements are pivoted organize HPLC joined with diode array indicator, using a C18 column and aqueous–organic mobile phases, in acidic media [2].

Plants well off in regular items, vegetables, whole grains, and give an abundance of supplements and minerals to meet one's supporting needs. The supportive capacities of the vegetables are, all things considered, dependent on the proximity of vital supplements similarly as micronutrients. In spite of the way that vitamin is required an unassuming amount for consistently in prosperity, it accept a crucial activity in our prosperity. The use of verdant vegetables and fruits abundant in supplements, are represented to decrease the peril of attack of various serious and ceaseless contaminations [3].

The wild plants have been a key wellspring of sustenance and medicine for inherent people. These plants have rich sustenance and therapeutic characteristics. Standard usage of vegetables is furthermore recommended for better prosperity and the leading body of relentless ailments. The nutritive worth, cell reinforcement properties of the results of wild agreeable plants like *Castanopsisindica, Fagopyrum cymosum, Ficus clavata, Ficus geniculataand Ficuspomifera* ate up by the innate people of North-eastern territory in India has quite recently been packed in our research facility [4-5].

Thus, these wild consumable plants has supportive potential and are meriting maltreatment as a dietary resource in light of the closeness of sufficient proportion of protein, starch, fat and minerals. The cell fortification properties and the proximity of phenolic acids and flavonoids in these wild attractive plants in fluctuating wholes have been propelled the nutraceutical properties of these plants.

This paper accounts a simple, gradient and stability-indicating HPLC method for the rapid determination of water soluble vitamins like, thiamine (B1), niacin (B3), pyridoxine (B6), ascorbic acid (C), pantothenic acid (B5), riboflavin (B2) and folic acid (B9) in five wild edible plants named C. *indica, F. cymosum, F. clavata, F. geniculata and F. Pomiferafrom* 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 *Castanopsis indica* Roxb (ex Lindl.) A.DC (Family: Fagaceae), *Fagopyrum cymosum* (Trev) Meisn (Family: Polygonaceae), *Ficus clavata* Wall ex. Miq (Family: Moraceae), *Ficus geniculate* Kurz. (Family: Moraceae) and *FicuspomiferaWall*. ex King (Family: Moraceae) 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 12, BSITS20, BSITS 21, BSITS 22 and BSITS 23 for future reference. The plant parts were taken in our laboratory at refrigerated temperature using cold packs. The refrigerated plant samples were stored at -150C and then processed within four days of collection.

Chemicals

The standards chemicals like ascorbic acid ($C_6H_8O_6$,vitamin C), thiamine ($C_{12}H_{17}N_4OS$,vitamin B1), riboflavin ($C_{17}H_{20}N_4O_6$,vitaminB2), niacin ($C_6H_5NO_2$,vitamin B3), pantothenic acid ($C_9H_{17}NO_5$,vitamin B5), pyridoxine ($C_8H_{11}NO_3$, vitaminB6) and folic acid ($C_{19}H_{19}N_7O_6$,vitamin B9) were purchased from Sigma Chemical Co. (St. Louis , MO , USA) and the HPLC-grade solvents such as acetonitrile, methanol, water sodium di hydrogen phosphate and trifluoroacetic acid were purchased from Merck (Germany).

HPLC equipment

HPLC analyses were completed with Dionex Ultimate 3000 liquid chromatography (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD)with a Diode Array

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Detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20μl 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 x 250 mm).

Preparation of standard 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 and 10 ml double distilled water in a25 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 mixing100 µl mixed vitamins standard (vitaminB9, 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 80µg/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 gm of each freeze-dried plant materials were soaked in 10 ml double distilled water with stirring for 30 minutes. 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 hours. The solution was first filtered through a What man 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.

Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by Seal et al., [6] 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 220 C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. 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 means of three independent analyses and the method was validated according to the USP and ICH guidelines [7-8].Various parameters were studied to validate the reproducibility of the method *viz* .the effectiveness, the linearity, the Limit Of Detection (LOD), the Limit Of Quantitation (LOQ), the precision and the accuracy.

STATISTICAL ANALYSIS

The significant and non-significant variations within water soluble vitamin contents and the five wild edible plants were Analyzed Using One-Way Analysis Of Variance (ANOVAs). All

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the investigation was completed utilizing triplicate tests. Test Results Were Exposed to Univariate Analysis Of Variance (ANOVA), trailed by Tukey test ($p \leq 0.05$) utilizing the statistical package for the social sciences (SPSS variant 7.5).

RESULTS

Chromatographic method

A typical HPLC chromatogram of the all standard vitamin mixture is presented in (Figure 1). 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, are shown in (Table 1). The high value of R2 > 0.9906 in the range of analyzed concentrations at 210, 245 and 275 nm is indicative of responsive linearity.

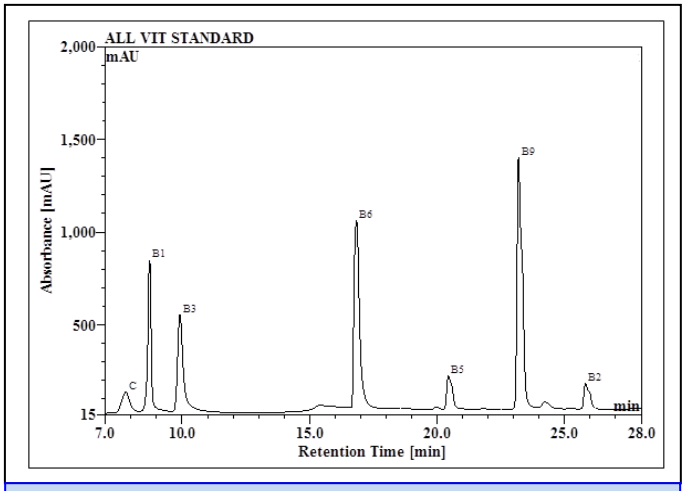

Figure 1: HPLC Chromatogram of mixture of Standard vitamin.

C: Ascorbic acid; B_1: Thiamine; B_3: Niacin; B_6: Pyridoxine; B_5: Pantothenic acid; B_9: Folic acid; B_2: Riboflavin

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 e.gascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9). The HPLC chromatogram of all five wild edible plants has been presented in (Figure 2-6) and the quantity of all vitamins of all plant materials has been expressed as mg/100gm dry plant material (DPM) and data presented in table 2.

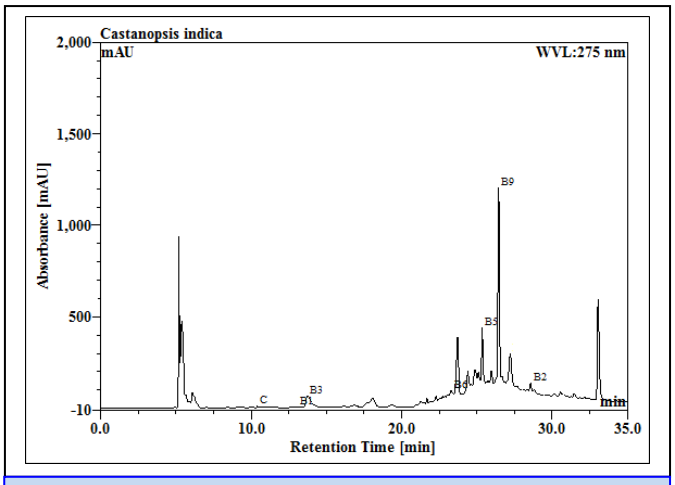

Figure 2: HPLC Chromatogram of *Castanopsis indica*.

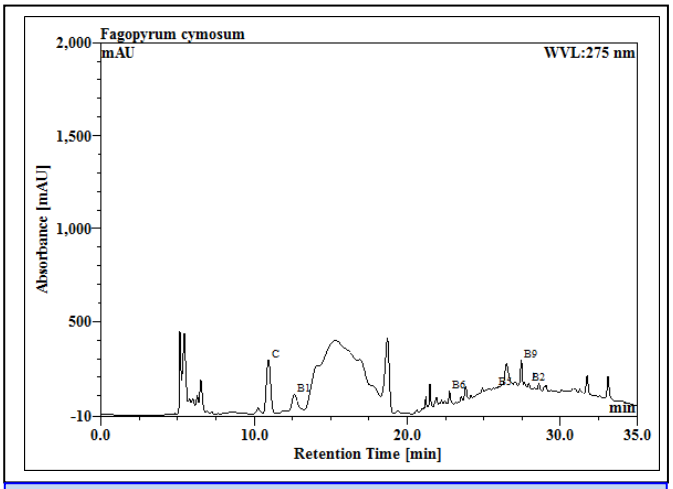

Figure 3: HPLC Chromatogram of *Fagopyrum cymosum*.

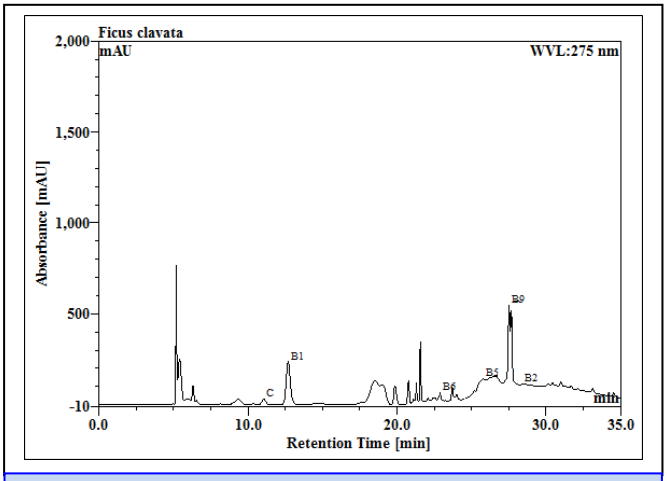

Figure 4: HPLC Chromatogram of *Ficus clavata*.

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Table 1: Retention time and parameters of calibration curve, precision and repeatability, LOD, LOQ and percent recovery study of standard water soluble vitamins for HPLC 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 conc 40 µg/ml	RSD (%) of the peak area at conc 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 B1	245	8.73	0.462	0.025	0.032	99.73	0.034	0.103	98.24
Vitamin B3	245	9.92	0.706	0.206	0.171	99.83	0.277	0.839	98.50
Vitamin B6	275	16.84	0.712	0.799	0.382	99.91	1.062	3.219	98.15
Vitamin B5	210	20.44	0.830	0.173	0.103	99.89	0.233	0.705	98.33
Vitamin B9	275	23.19	0.475	0.220	0.227	99.10	0.309	0.935	99.20
Vitamin B2	275	25.82	0.453	0.114	0.144	99.68	0.156	0.472	98.25

Note: RSD Relative standard deviation, LOD Limit of detection, LOQ limit of quantification.

Table 2: Quantification of Vitamin C and B1, B2, B3, B5, B6 and B9 in five wild edible plants.

Vitamin content in mg/ 100 gm dry plant material							
Plant	Vitamin C	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B5	Vitamin B6	Vitamin B9
C. indica	0.18± 0.003^{e}	0.12± 0.003^{b}	1.32± 0.33^{a}	0.43± 0.006^{a}	0.26± 0.006^{a}	0.40± 0.006^{c}	8.66± 0.30^{a}
F. cymosum	21.29± 1.00^{a}	0.23± 0.013^{a}	0.62± 0.013^{b}	ND	0.096± 0.002^{c}	0.73± 0.02^{a}	1.51± 0.03^{b}
F. clavata	2.08± 0.03^{b}	0.13± 0.016^{b}	0.61± 0.02^{b}	ND	0.13± 0.003^{b}	0.64± 0.02^{b}	1.31± 0.02^{d}
F. geniculata	0.51± 0.01^{c}	0.084± 0.003^{c}	0.51± 0.013^{c}	0.007± 0.0003^{b}	0.078± 0.003^{e}	0.39± 0.01^{c}	0.64± 0.016^{e}
F. pomifera	0.21± 0.003^{d}	0.079± 0.003^{d}	0.26± 0.023^{d}	ND	0.084± 0.002^{d}	0.14± 0.003^{d}	1.40 ±0.033^{c}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c, d and e denotes the significant differences within same parameters of individual plant ND: Not detected.

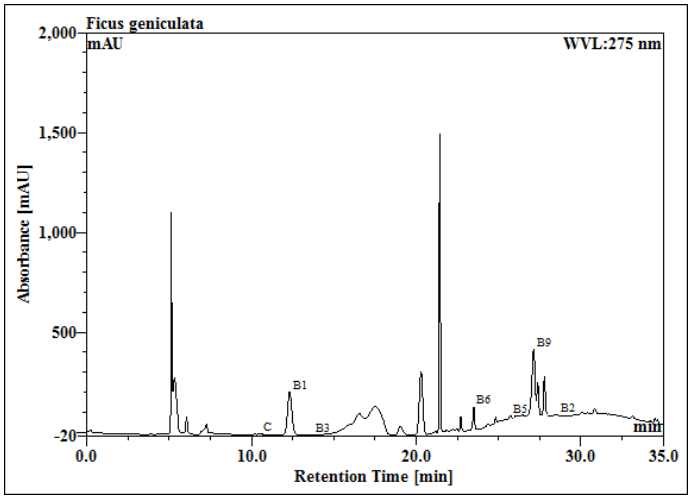

Figure 5: HPLC Chromatogram of *Ficus geniculata*.

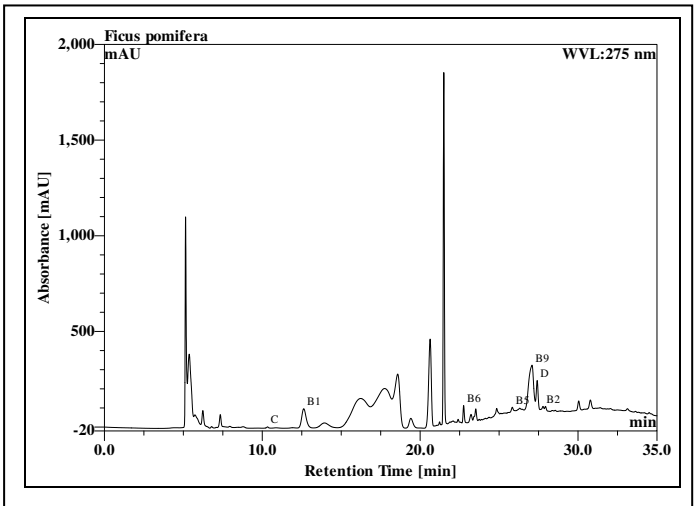

Figure 6: HPLC Chromatogram of *Ficus pomifera*.

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The HPLC analysis of the plants C.*indica* showed the presence of vitamin C (0.18± 0.003mg/100gm DPM), B1 (0.12±0.003 mg/100gm DPM), B2 (1.32±0.33 mg/100gm DPM), B3 (0.43±0.006), B5 (0.26±0.006 mg/100gm DPM), B6 (0.40±0.006 mg/100gm DPM) and B9 (8.66±0.30 mg/100gm DPM).

The HPLC study of the plant *F. cymosum* revealed the presence of significant amount vitamin C (21.29±1.00 mg/100gm DPM) along withB1 (0.23±0.013 mg/100gm DPM), B2 (0.62±0.013 mg/100gm DPM), B5 (0.096±0.002 mg/100gm DPM), B6 (0.73±0.02 mg/100gm DPM) and B9 (1.51±0.03 mg/100gm DPM).

The presence of vitamin B1 (0.13± 0.016mg/100gm DPM), B2 (0.61±0.02 mg/100gm DPM), B5 (0.13±0.003 mg/100gm DPM), B6 (0.64±0.02 mg/100gm DPM), B9 (1.31±0.02 mg/100gm DPM) and appreciable amount of Vitamin C (2.08±0.03 mg/100gm DPM) were detected in *F. clavata*.

The leaves of *F. Geniculata* were found to contain vitamin C (0.51± 0.01 mg/100gm DPM), B1 (0.084±0.003 mg/100gm DPM), B2 (0.51±0.013 mg/100gm DPM), B3 (0.007±0.0003 mg/100gm DPM), B5 (0.078±0.003 mg/100gm DPM), B6 (0.39±0.01 mg/100gm DPM) and good amount of B9 (0.64±0.016 mg/100gm DPM).

Our investigation disclosed the occurrence of vitamin C (0.21± 0.003 mg/100gm DPM), B1 (0.079±0.003 mg/100gm DPM), B2 (0.26±0.023 mg/100gm DPM), B5 (0.084±0.002 mg/100gm DPM), B6 (0.14±0.003 mg/100gm DPM) and substantial amount of B9 (1.40±0.033 mg/100gm DPM) in the leaves of *F. pomifera*.

DISCUSSION

Chromatographic method

The quantitative analysis of water soluble vitamins was completed using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm). The detection of vitamin C, B1 and B3 were carried out at wavelength 245 nm, vitamin B2, B6 and B9 were carried out at 275nm. The detection wavelength was set at 210 nm for vitamin B5 as it exhibited absorption at 210 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 trifluoro acetic acid (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 *viz*., 40µg/ml and 60µg/ml was found to be below one percent. The significantly high rate of recovery (98.15 – 99.20%) of the standard vitamins worth's mention.

As shown in Table 1, LOD values varied from 0.034 µg/ml (vitamin B1) to 1.062 µg/ml (vitamin B6), while LOQ values ranged from 0.103 µg/ml (vitamin B1) to 3.219 µg/ml (vitamin B6).As shown in Table 1, very good correlation coefficients (R^2) were also observed for all vitamins, ranging from 99.10 (vitamin B9) to 99.91 (vitamin B6). These observations also exclude any deviation from linearity for analytes amounts that largely exceed the concentrations usually found in wild edible plants under investigation.

It follows that the method under consideration is characterized by precision, accuracy, meticulousness and can be used for the qualitative as also quantitative estimation of water soluble vitamins in the five 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, B1, B2, B3, B5, B6 and B9 in five 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, B1, B3, B5 and B6. Both the vitamins (B2 and B9) were found slightly soluble in water and acidic aqueous solutions, but soluble in basic aqueous solutions. So the stock solutions of vitamin B2 and B9 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 significant nutrient in foods grown from the ground. It is notable for its cell reinforcement properties and it helps the body in repressing from viral disease, bacterial contaminations and poisonous quality. It is required

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for the avoidance of scurvy and upkeep of solid skin, gums and veins and the inadequacy of this nutrient causes wounding, bleeding, dry skin and sadness [9].

The experimental result showed that, the amount of vitamin C was found highest in the leaves of *F. Cymosum* (21.29± 1.00 mg/100gm DPM) followed by in F. clavata(2.08±0.03 mg/100gm DPM) (Table2). The vitamin C content in these wild edible plants are very much comparable with some common fruits and vegetables like Solanum tuberosum (17.04± 1.18 mg/100gm), Allium sativum (13.06 ± 1.10 mg/100gm), Daucus carota sativus (2.55± 0.72 mg/100gm) etc., [9]. An appreciable amount of Vitamin C was also detected in other plants under investigation.

So the wild consumable plants under investigation might be seen as extraordinary wellsprings of vitamin C, likewise, along these lines, may satisfy the proposed Regular Dietary Allowance (RDA) of 75 mg/day and 90 mg/day for grown-up women and men independently, and 45 mg/day for posterity of 9–12 years old. Due to having cell support properties, vitamin C rich plant might be useful to diminish the risk of atherosclerosis and a couple of sorts of dangerous development [10].

Thiamine (B1), is a fundamental enhancement required by the body for keeping up cell work and in this way a wide display of organ limits. It is essential for imperativeness age, starch processing and nerve cell work. The absence of this supplement prompts markdown degeneration of the body, particularly the on edge and circulatory systems, hypertension and heart infirmities [11-12].

The thiamine content in these wild edible plants ranged from 0.079±0.003to 0.23±0.013mg/100gmDPM. The highest amount of B1 was obtained from the leaves of F. cymosum followed by in C. *indicaand F. clavata* (Table 2).

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

Riboflavin (B2) is a principal supplement required for suitable essentialness processing and a wide combination of cell structures. It is the accomplice to thiamine used in the sustaining of sustenance things [13]. A basic assortment of riboflavin content was seen among the attempted wild consumable characteristic items.

The maximum sum of B2 was detected in the nuts of C. *indica* (1.32±0.03mg/100gm DPM) and the least amount was detected in F. pomifera (0.26±0.023mg/100gm DPM). The leaves of *F.cymosum, F. clavata* and *F. Geniculata* were also found to contain a very good quantity of vitamin B2 (Table 2) which are comparable with some common fruits and vegetables like almonds (1.10 mg/100g), spinach (0.24 mg/100g), beet greens (0.41 mg/100g), green beans (0.12±2 mg/100g, potato (0.023 ±1 mg/100g) etc., [14].

The niacin (B3) was detected in the nuts of C. *indica* (0.43± 0.006mg/100gm DPM) and a small amount was noticed in the leaves of *F. geniculata* (0.007± 0.0003mg/100gm). The edible parts of these plants are the important sources of vitamin B3, which were comparable with cabbage, cauliflowers, cucumber, spinach, tomatoes ranged between 0.19 -0.97 mg/100gm [14]. Vitamin B3, is a critical supplement required for dealing with fat in the body, cutting down cholesterol levels, and overseeing glucose levels. It is huge in DNA fix, Ca assimilation, intracellular breath, and biosynthesis of unsaturated fat and steroids [15]. So the ordinary usage of these edible plants would supply agreeable B3 critical to keep up strong body limits. Vitamin B5, or Pantothenic acid, is an essential supplement required by the body for cell structures and perfect upkeep of fat. The insufficiency of supplement B5 prompts irritability, exhaustion, reserved quality, deadness, paresthesia, and muscle issues in person [16].

Pantothenic acid was detected highest in the leaves of C. *indica* (0.26±0.006mg/100gm DPM). The edible parts of *F. Cymosum F. clavata, F. geniculata* and *F. pomifera* were also found to contain a very good amount of B5 (Table 2).

Pyridoxine (B6) is another water dissolvable vitamin key for the most ideal help of red platelet absorption, the tangible framework, the sheltered system, and various other genuine limits. It in like manner expects a vocation in homocysteine made and degradative reactions [17]. This supplement is found in most sustenance things and besides, as a result of its stabilty, is routinely used for reinforcing sustenance things [18]. It was

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measured in all the wild eatable plants under our examination. The highest B6 was observed in the leaves of F. cymosum (0.73±0.02mg/100gm DPM) whereas the minimum was detected in *F. Pomifera* (0.14±0.003mg/100gm DPM). An appreciable amount of B6 was detected in other plants under investigation (Table 2). The amount of B6 obtained in these wild edible fruits were comparable with some common vegetable and fruits like banana (0.37 mg/100g), avocados (0.29 mg/100g), spinach (0.24 mg/100g), broccoli (0.134 mg/100g), cauliflower (0.115mg/100g), cucumber (0.2 mg/100g) etc. So the regular intake of these plants would supply sufficient B6 necessary to maintain healthy body functions.

Vitamin B9 (folic acid) is a water-solvent B vitamin with numerous rich characteristic sources. It is required for various body capacities including DNA combination and fix, cell division, and cell development. The lack of folate can prompt pallor in grown-ups, and more slow improvement in kids [19-22]. It assumes a significant job as cell reinforcement in vivo, both by averting the unfriendly impact of Receptive Oxygen Species (ROS), just as by repressing lipid per oxidation [23]. The extent of B9 in five wild edible plants ranged from 0.64±0.016 to 8.66±0.30mg/100gm DPM. The content of B9 was found highest in C. *indica* and a good amount of B9 was also detected in other plants under investigation (Table 2).

CONCLUSION

The reversed-phase stage HPLC system with diode array detection was made for the quantitative estimation of water dissolvable B vitamins (B1, B2, B3, B5, B6 and B9) and vitamin C in five wild edible plants like C. *indica, F. cymosum, F. clavata, F. geniculata* and *F. Pomifera* assembled from North-eastern territory in India. The established HPLC test showed a well partition of the mixes and moreover the made strategy was straight, delicate, precise, fastidious and reproducible. As such, the technique can be used for the simultaneous estimation of water dissolvable B vitamin and vitamin C in different plans with 'shorter run time' and 'high viability'. RP-HPLC results showed the plants contained a couple of water dissolvable B and C supplements in varying amounts. The eventual outcome of assessment of supplement substance in the wild satisfactory plants under investigation will fill in as a significant method to register dietary affirmation of C and B supplements in the comprehensive network. These data will similarly be valuable in the preparation of an all out sustenance creation table for empowering outline and moreover for other research purposes.

CONFLICT OF INTEREST

We have no conflict of interest.

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