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Chromatographic Estimation of Water Soluble Vitamins in Wild Edible Plants

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A reversed-phase high performance liquid chromatographic technique has been developed for the simultaneous quantitation of water soluble vitamins in 10 potent wild edible plants consumed by the tribal people of North-eastern region in India. The chromatographic separations of vitamins were assessed on Acclaim C18 column using a mobile phase of acetonitrile and aqueous trifluoro acetic acid solution with gradient elution. The experimental results exhibited that for different plants, the vitamin C content ranged between 0.15 ± 0.003 to 8.10 ± 0.03 mg/100g dry plant material (DPM). The vitamin B_2 content was determined high in *C. album* (2.64 ± 0.03 mg/100g DPM) and significant amount of vitamin B_9 (1.44 ± 0.03 mg/100g) was detected in *E. acuminata*. The results 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 estimation of vitamins in these 10 wild edible plants.

Keywords: Wild edible leaves, Water soluble vitamins, Vitamin B, Vitamin C, HPLC analysis.

INTRODUCTION

Vitamins are potent organic compounds, which are needed in modest amounts in the body continually to lead customary success and varied physiological activities in the human body. Apart from that they can't be produced or then again combined by organisms and their absence brings about explicit lack illness. The reasons for these vitamin insufficiencies incorporate poor dietary patterns, liquor addiction, enthusiastic pressure, the inappropriate ingestion of nutrients generally because of liver or intestinal issues and the admission of medications that meddle with the ingestion of nutrients and of presentation to daylight [1].

Vitamins differ from each other in physiological function, in chemical structure and in their distribution in food. They are broadly divided into two categories: fat soluble vitamin and water soluble vitamin. The previous incorporates lipid soluble nutrients A, D, E and K and various carotenoids, the latter is made out of water soluble vitamins C and eight B-vitamins, specifically thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), pantothenic acid (B_5), biotin (B_7), folate (B_9) and cyanocobalamin (B_{12}) [2].

Vitamins, utilized remedially, can be of tremendous assistance in battling sickness and speeding recuperation. They can be utilized in two different ways, to be specific, adjusting efficiencies and treating sickness instead of medication. Vitamins treatment has a particular bit of leeway over medication treatment. While drugs are consistently poisonous and have man unwanted results, vitamins, when in doubt are non-harmful and safe [3].

Estimation of vitamins in nourishments is bewildered by various methods. 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 vitamers 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 estimation 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 methods

have been accounted for the estimation of water-soluble nutrients in different conditions. The most broadly utilized techniques for the quantification of ascorbic acid together with B-complex vitamins are turned around stage HPLC combined with diode array detector, utilizing a C_{18} column and aqueous-organic mobile phases, in acidic media [4].

Plants well off in fruits, vegetables, whole grains and provide an abundance of supplements and minerals to meet one's supporting needs. The therapeutic potentials of the vegetables are, all things considered, dependent on the proximity of vital supplements similarly as micronutrients. The consumption of vegetables and fruits abundant in vitamin, are responsible to decrease the exposure of attack of various acute and chronic diseases [5]. The wild plants have been a key wells pring of sustenance and medicine for inherent people. These plants have rich sustenance and therapeutic characteristics. The nutritive potential, antioxidant properties of these wild edible leaves like *Allium hookeri*, *Brassica nigra*, *Chenopodium album*, *Eurya acuminata*, *Gentiana pedicellata*, *Hypochaeris radicata*, *Leea sambucina*, *Neptunia olearacea*, *Spilanthes acmella* and *Tricyrtis pillosa* consumed by the innate people of North-eastern territory in India has recently been studied [6-9]. 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 antioxidant properties and the proximity of phenolic acids and flavonoids in these wild edible plants in variable quantities have been enhanced 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 (B_1), niacin (B_3), pyridoxine (B_6), ascorbic acid (C), pantothenic acid (B_5), riboflavin (B_2) and folic acid (B_9) in ten wild edible leaves named *A. hookeri*, *B. nigra*, *C. album*, *E. acuminata*, *G. pedicellata*, *H. radicata*, *L. sambucina*, *N. olearacea*, *S. acmella* and *T. pillosa* collected from North-eastern region in India and all the vitamins were simultaneously analyzed in a single chromatographic run.

EXPERIMENTAL

Standards chemicals like ascorbic acid ($C_6H_8O_6$, vitamin C), thiamine ($C_{12}H_{17}N_4OS$, vitamin B_1), riboflavin ($C_{17}H_{20}N_4O_6$, vitamin B_2), niacin ($C_6H_5NO_2$, vitamin B_3), pantothenic acid ($C_9H_{17}NO_5$, vitamin B_5), pyridoxine ($C_8H_{11}NO_3$, vitamin B_6) and folic acid ($C_{19}H_{19}N_7O_6$, vitamin B_9) were purchased from Sigma Chemical Co. (St. Louis, USA) and the HPLC-grade solvents such as acetonitrile, methanol, water sodium dihydrogen phosphate and trifluoroacetic acid were purchased from Merck (Germany).

Plant materials: The wild edible plants named *A. hookeri*, *B. nigra*, *C. album*, *E. acuminata*, *G. pedicellata*, *H. radicata*, *L. sambucina*, *N. olearacea*, *S. acmella* and *T. pillosa* 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 36, BSITS 46, BSITS 49, BSITS 42, BSITS 24, BSITS 37, BSITS 40, BSITS 41, BSITS 39 and BSITS 38, respectively 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 ºC and then processed within two days of collection.

HPLC equipment: HPLC analyses were completed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) with a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 5l loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim™ 120 C_{18} column (55 mm particle size, i.d. 4.6 × 250 mm).

Preparation of standard solutions: The stock standard solutions of vitamin C, B_1, B_3, B_5 and B_6 were prepared by dissolving 25 mg of each standard in one mL 0.1 M HCl and 10 mL double distilled water in a 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 1 mL 0.1 M NaOH in 25 mL standard volumetric flask and made up to mark with double distilled water. The standard solution was stored in Amber-glass bottles at 4 ºC . 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 ºC until analysis. Each freeze-dried plant materials (1 g) were soaked in 10 mL double distilled water with stirring for 30 min. Then, 1 mL 0.1 M NaOH and 10 mL phosphate buffer (1 M, 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.

Chromatographic analysis of water soluble vitamins: The chromatographic analysis was carried out as described by Seal *et al*. [10] with minor modification. The mobile phase contains acetonitrile (solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (solvent B), the column was thermostatically controlled at 22 ºC 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 compo-

sition 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 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 [11,12]. Various parameters were studied to validate the reproducibility of the method *viz.* the effectiveness, linearity, the limit of detection (LOD), the limit of quantitation (LOQ), precision and accuracy.

Statistical analysis: All the analysis was done using triplicate samples values were presented as mean ± standard error mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey test ($p \leq 0.05$) and Principal Component Analysis (PCA) were employed to evaluate the differences and identify the plants with similar characteristics in relation to their nutritional composition, minerals and vitamin content. The data analysis was performed in Minitab version 18.0. (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

In this study, a 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 10 wild edible plants is developed. 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. So the stock solutions of vitamin B_2 and B_9 were dissolved in 0.1 M NaOH solution and all working standard vitamins were diluted with phosphate buffer (pH 5.5) solution.

The quantitative analysis of water soluble vitamins were analyzed using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm). The detection of vitmain C, B_1 and B_3 were carried out at wavelength 245 nm, while for vitamin B_2, B_6 and B_9 were carried out at 275 nm. The detection wavelength was set at 210 nm for vitamin B_5 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. A typical HPLC chromatogram of the all standard vitamin mixture is presented in Fig. 1.

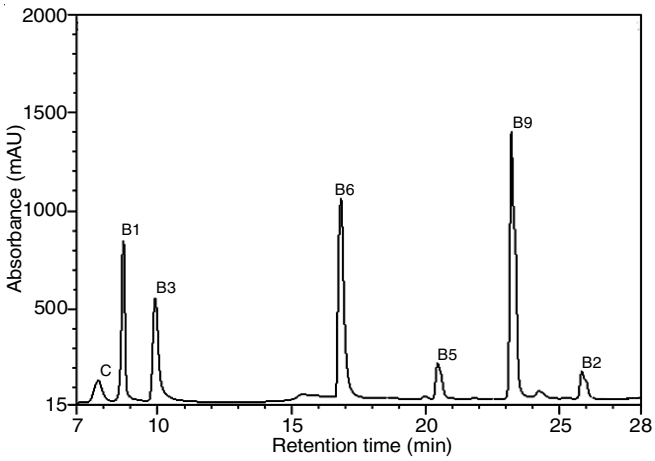

Fig. 1. HPLC chromatogram of mixture of standard vitamin

The repeatability of the retention time for all the standard samples and the relative standard deviation for the peak areas of two standards *viz.* 40 µg/mL and 60 µg/mL was found to be below 1%. 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 B_1) to 1.062 µg/mL (vitamin B_6), while LOQ values ranged from 0.103 µg/mL (vitamin B_1) to 3.219 µg/mL (vitamin B_6). A good correlation coefficients (R^2) were also observed for all

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 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

vitamins, ranging from 99.10 (vitamin B_9) to 99.91 (vitamin B_6). These observations also exclude any deviation from linearity for analyte amounts that largely exceed the concentrations usually found in wild edible plants under investigation. Thus, 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.

Thus, 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.

Identification and quantification of water soluble vitamins in wild edible plants: The HPLC method was successfully performed for the estimation of water soluble vitamin *e.g.* ascorbic acid (C), thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6) and folic acid (B_9). The HPLC chromatograms of all ten wild edible leaves have been presented in Fig. 2 and the quantity of all vitamins of all plant materials has been expressed as mg/100 g dry plant material (DPM) and data are presented in Table-2.

The experimental results showed that the amount of vitamin C was found highest in the leaves of *B. nigra* (8.10 ± 0.03 mg/100 g DPM) followed by *S. acmella* (5.59± 0.067 mg/100 g DPM) (Fig. 3). The vitamin C content in these wild edible plants are very much comparable with some wild edible plants like *Diplazium esculentum* (5.41± 0.03 mg/100 g), *Begonia hatacoa* (3.41 ± 0.01 mg/100 g), *Cardamine hirsuta* (6.23± 0.02 mg/100 g), *etc*. [2,10]. An appreciable amount of vitamin C was also detected in other plants under investigation.

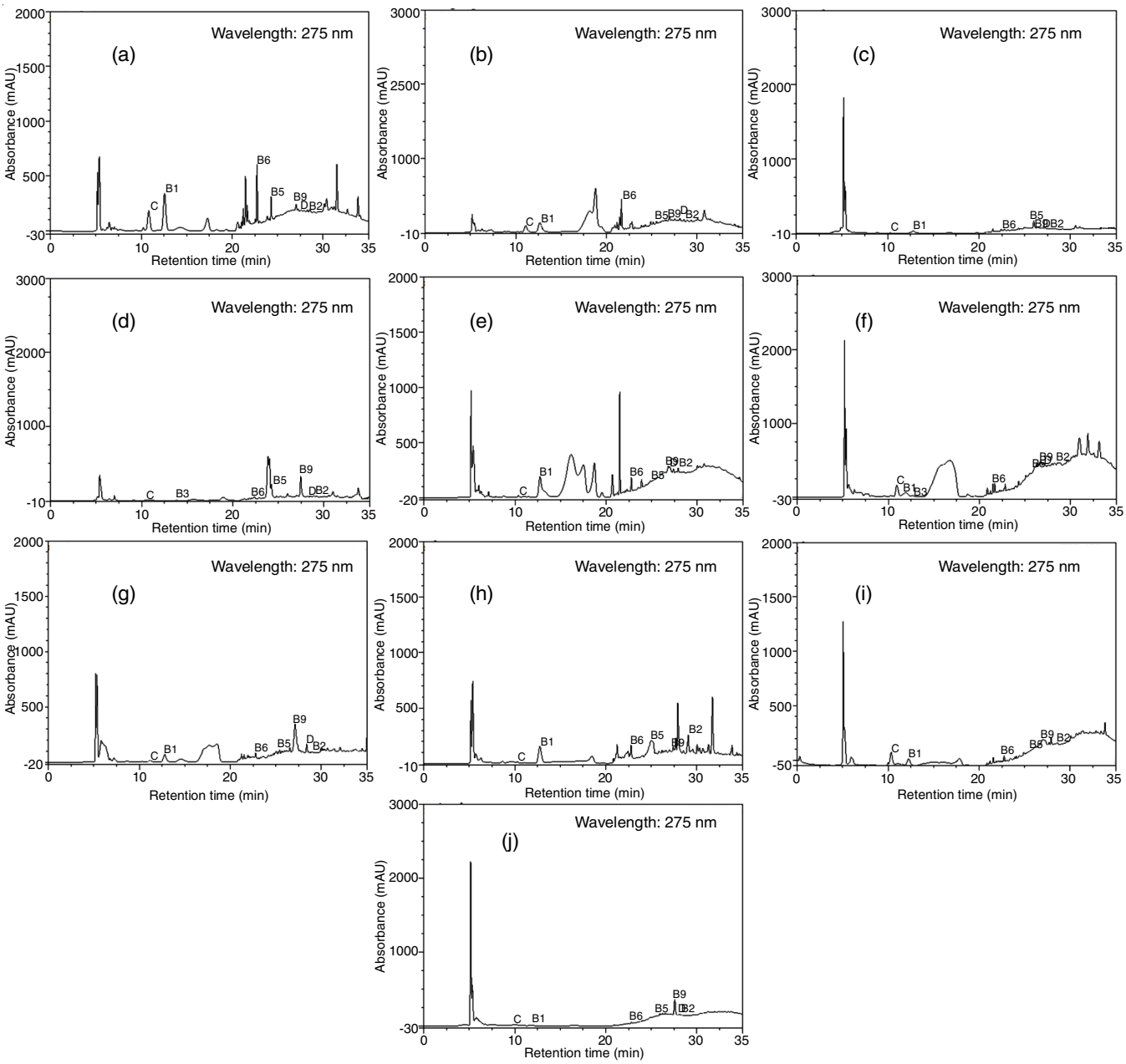

Fig. 2. HPLC chromatograms of (a) *A. hookeri*; (b) *B. nigra*; (c) *C. album*; (d) *E. acuminata*; (e) *G. pedicellata*; (f) *H. radicata*; (g) *L. sambucina*; (h) *N. oleracea*; (i) *S. acmella*; (j) *T. pillosa*

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
A. hookeri	3.65 ± 0.06^{c}	0.54 ± 0.003^{b}	0.12 ± 0.006^{e}	ND	0.43 ± 0.003^{b}	0.68 ± 0.003^{a}	1.34 ± 0.023^{b}
B. nigra	8.10 ± 0.03^{a}	0.62 ± 0.016^{a}	0.043 ± 0.001^{f}	ND	0.051 ± 0.003^{f}	0.30 ± 0.016^{d}	0.16 ± 0.003^{f}
C. album	0.19 ± 0.003^{h}	0.52 ± 0.006^{b}	2.64 ± 0.03^{a}	ND	0.09 ± 0.002^{c}	0.37 ± 0.006^{c}	0.11 ± 0.003^{h}
E. acuminata	0.45 ± 0.01^{g}	ND	0.83 ± 0.013^{b}	0.036 ± 0.002^{a}	0.46 ± 0.026^{a}	0.16 ± 0.014^{e}	1.44 ± 0.03^{a}
G. pedicellata	0.51 ± 0.003^{f}	0.015 ± 0.002^{h}	0.15 ± 0.003^{d}	ND	0.067 ± 0.002^{e}	0.17 ± 0.006^{e}	0.18 ± 0.003^{ef}
H. radicata	1.62 ± 0.02^{d}	0.044 ± 0.001^{f}	0.12 ± 0.003^{e}	0.026 ± 0.002^{b}	0.059 ± 0.002^{f}	0.43 ± 0.003^{b}	0.16 ± 0.003^{f}
L. sambucina	1.01 ± 0.03^{e}	0.24 ± 0.003^{d}	0.36 ± 0.02^{c}	ND	0.074 ± 0.002^{d}	0.08 ± 0.001^{g}	0.62 ± 0.013^{d}
N. oleracea	0.63 ± 0.02^{f}	0.31 ± 0.016^{c}	0.14 ± 0.003^{d}	ND	0.094 ± 0.003^{c}	0.11 ± 0.003^{f}	0.14 ± 0.002^{g}
S. acmella	5.59 ± 0.067^{b}	0.066 ± 0.003^{e}	0.034 ± 0.001^{g}	ND	0.072 ± 0.003^{d}	0.013 ± 0.001^{i}	0.05 ± 0.001^{i}
T. pillosa	0.15 ± 0.003^{i}	0.024 ± 0.002^{g}	0.05 ± 0.002^{ef}	ND	0.029 ± 0.001^{g}	0.04 ± 0.002^{h}	0.84 ± 0.026^{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,e,f,g,h,i and j denotes the significant differences within studied parameters among different plants ND: Not detected.

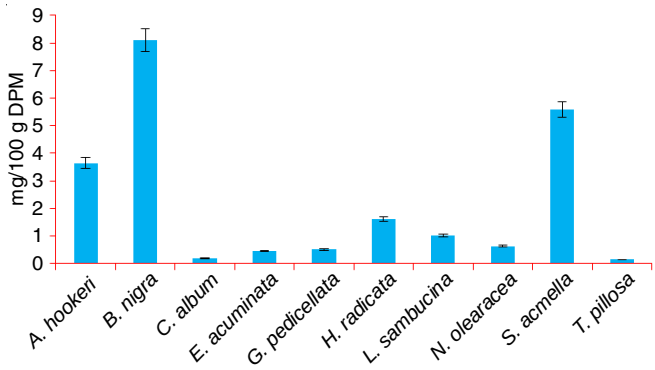

Fig. 3. Vitamin C contents in 10 wild edible leaves

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 children 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 different ailments [13].

The thiamine content in these wild edible plants ranged from 0.015 ± 0.002 to 0.62 ± 0.016 mg/100 g DPM. The highest amount of B_1 was obtained from the leaves of *B. nigra* followed by in *A. hookeri* and *C. album* (Fig. 4). The maximum sum of B_2 was detected in the leaves of *C. album* (2.64 ± 0.03 mg/100 g DPM) and the least amount was detected in *B. nigra* (0.043 ± 0.001 mg/100 g DPM). The leaves of *E. acuminata*, *L. sambucina* and *G. pedicellata* were also found to contain a very good quantity of vitamin B_2 (Fig. 4), which are comparable with some common fruits and vegetables like almonds (1.10 mg/100 g), spinach (0.24 mg/100 g), beet greens (0.41 mg/100 g), green beans (0.12 ± 2.0 mg/100 g, potato (0.023 ±1 mg/100 g), *etc*. [14].

Niacin (B_3) was detected only in the leaves of *E. acuminata* (0.036± 0.002 mg/100 g DPM) and in *H. radicata* (0.026 ± 0.002 mg/100 g) (Fig. 4). The edible parts of these plants are the important sources of vitamin B_3, which were comparable with cabbage, cauliflowers, cucumber, spinach, tomatoes ranged between 0.19 -0.97 mg/100 g [14].

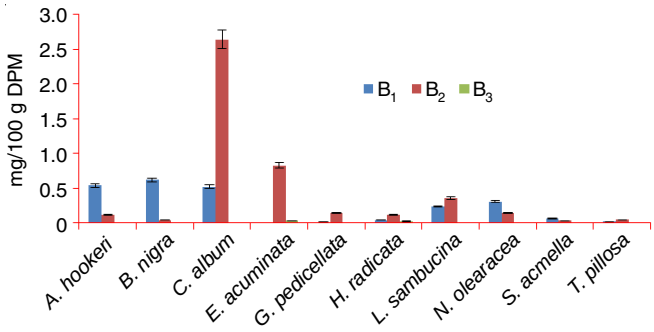

Fig. 4. Vitamin B_1, B_2 and B_3 contents in 10 wild edible leaves

Pantothenic acid (vitamin B_5) was detected highest in the leaves of *E. acuminata* (0.46 ± 0.026 mg/100 g DPM) followed by *A. hookeri* (0.43±0.003 mg/100 g DPM). The edible parts of all other plants were also found to contain a very good amount of vitamin B_5 (Fig. 5). The highest pyridoxine (vitamin B_6) was observed in the leaves of *A. hookeri* (0.68 ± 0.003 mg/100 g DPM) followed by *H. radicata* and *C. album*, whereas the minimum was detected in *S. acmella* (0.013± 0.001 mg/100 g DPM). An appreciable amount of vitamin B_6 was also detected in other plants too (Fig. 5).

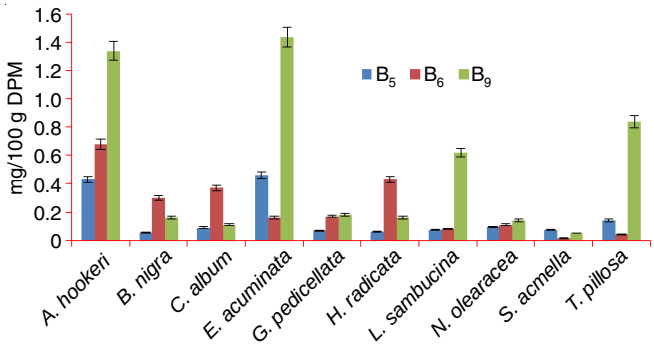

Fig. 5. Vitamin B_5, B_6 and B_9 contents in 10 wild edible leaves

The extent of vitamin B_9 (folic acid) in ten wild edible leaves ranged from 0.05 ± 0.001 to 1.44 ± 0.03 mg/100 g DPM. The content of vitamin B_9 was found highest in *E. acuminata* and a good amount of vitamin B_9 was also detected in other plants (Fig. 5).

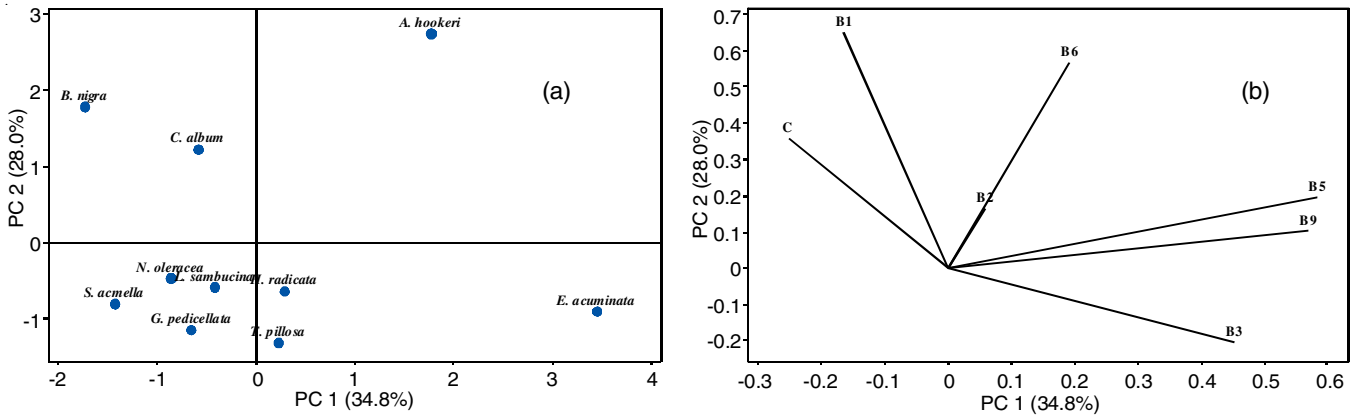

Fig. 6. Principal component analysis using variables listed in Table-2. Score plot (a) and loading plot (b) of first two principal components for clustering of plant samples. Variables: 7 (Vitamin C, B_1, B_2, B_3, B_5, B_6 and B_9)

Principal component analysis (PCA): To better discriminate among the samples under investigation, principal component analysis (PCA) was performed separately on the combined proximate composition, vitamin C, vitamin B_6, vitamin B_9, vitamin B_2 and mineral data. The PCA score plots of all of the plant samples are shown in Fig. 6a (based on all proximate, vitamin C, B_1, B_2, B_3, B_5, B_6 and B_9 variables) and their corresponding loading plots are presented in Fig. 6a. Though the PCA results yielded four principal components (PC) with eigen values >1, only first two principal components (PC2) were kept to simplify the analysis of results. The first two principal components accounted for 62.8% (Fig. 6a-b) of the total variance based on the water soluble vitamins with PC1 (34.8%) explaining ~ 1.24 times as much as PC2 (28.0%). In Fig. 6b, PC1 was negatively associated with vitamin C and vitamin B_1 and positively associated with the vitamin B_2, B_3, B_5, B_6 and B_9 variables. PC2 was negatively correlated with vitamin B_2 while positively correlated with the others. The leaves of *E. acuminata*, *A. hookeri* and *B. nigra* were clearly separated and distant from all other samples on the right side and left side (Fig. 6a) as a result of the high contents of water soluble vitamins in variable amounts.

Conclusion

A reversed-phase stage HPLC system with diode array detection was analyzed for the quantitative estimation of water dissolvable B vitamins (B_1, B_2, B_3, B_5, B_6 and B_9) and vitamin C in ten wild edible leaves collected from North-eastern territory in India. An established HPLC method displayed a well partition of the components and moreover the made strategy was linear, sensitive, precise, meticulous and reproducible. As such, the technique can be used for the simultaneous estimation of water dissolvable B vitamin and vitamin C in different formulations with shorter run time and high viability. The results showed the plants contained a couple of water soluble B and C supplements in varying amounts. The eventual outcome of assessment of supplement substance in the wild edible 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 food composition table for nutritional survey and moreover for other research purposes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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