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WATER-SOLUBLE VITAMIN IN TEN WILD EDIBLE FRUITS CONSUMED BY THE TRIBAL PEOPLE OF NORTH-EASTERN REGION IN INDIA: SIMULTANEOUS ESTIMATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD

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ABSTRACT: The present study aimed to develop a reversed-phase high-performance liquid chromatographic method 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 ten potent wild edible fruits named *Artocarpus gomezianus*, *Baccurea sapida*, *Cyclanthera pedata*, *Flacourtia jangomas*, *Gymnopetalum cochinchinense*, *Hodgsonia heteroclite*, *Prunus nepaulensis*, *Spondias axillaris*, *Terminalia bellirica* and *Zhanthoxylum armatum* consumed by the tribal people of North-eastern region in India. The chromatographic separation of vitamins was 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 wavelengths (210, 245 and 254 nm) using a mobile phase of acetonitrile and aqueous trifluoroacetic acid (TFA, 0.01% v/v) solution with gradient elution. The experimental results showed that for different plants, the vitamin C content ranged between0.05 ± 0.001to 4.99 ± 0.03 mg/100g dry plant material (DPM). The B2 content was determined high in *F. jangomas* (0.62 ± 0.013 mg/100g DPM), and a significant amount of B9 (1.87± 0.03mg/100g) was detected in this plant. The investigation 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 detection limit confirm the suitability of the method for simultaneous quantification of vitamins in these ten wild edible fruits.

INTRODUCTION: Vitamins are potent organic compounds that are needed in unassuming amounts in the body continues to lead to customary success and assorted physiological activities in the human body. Aside from a couple, 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 presentation to daylight [1].

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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-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 acid (B5), biotin (B7), folate (B9) and cyanocobalamin (B12) [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 an unwanted result, 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 supplements in light of their various substance structures and properties. Moreover, every vitamin can occur in different structures considered vitamers that have 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-dissolvable nutrients in different conditions. The most broadly utilized techniques for quantifying ascorbic acid together with B-bunch 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 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 to micronutrients.

In spite of the way that vitamin is required an unassuming amount for consistently in prosperity, it accepts a crucial activity in our prosperity. The use of verdant vegetables and fruits abundant in supplements is represented to decrease the exposure of various serious and ceaseless contaminations [5]. 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, antioxidant properties of these wild edible fruits like *Artocarpus gomezianus, Baccurea sapida, Cyclanthera pedata, Flacourtia jangomas, Gymnopetalum cochin-chinense, Hodgsonia heteroclite, Prunus nepaulensis, Spondias axillaris,Terminalia bellirica* and *Zhanthoxylum armatum* ate up by the innate people of North-eastern territory in India has recently been studied in our research facility [6-9]. Thus, these wild consumable plants have supportive potential and are meriting maltreatment as a dietary resource in light of the closeness of a 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 for 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 ten wild edible fruits named *Artocarpus gomezianus, Baccurea sapida, Cyclanthera pedata, Flacourtia jangomas, Gymnopetalum cochinchinense, Hodgsonia heteroclite, Prunus nepaulensis, Spondias axillaris,Terminalia bellirica* and *Zhanthoxylum armatum* 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 *Artocarpus gomezianus, Baccurea sapida, Cyclanthera pedata, Flacourtia jangomas, Gymnopetalum cochinchinense, Hodgsonia heteroclite, Prunus nepaulensis, Spondias axillaris, Terminalia bellirica*, and *Zhanthoxylum armatum* 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 Registry No. BSITS 45, BSITS 48, BSITS 33, BSITS 47, BSITS 43, BSITS 44, BSITS 30, BSITS 52, BSITS 31 and BSITS 32 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 °C 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 ($C_8H_{11}NO_3$, vitamin B6) 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 dihydrogen 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 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 a data processor. The separation was achieved by a reversed-phase Acclaim ^{TM} 120 C_{18} column (5 μm particle size, i.d. 4.6 x 250 mm).

Preparation of Standard Solutions: The standard stock solutions of vitamin C, B1, B3, B5, and B6 and were prepared by dissolving 25 mg of each standard in one ml 0.1 M hydrochloric acid and 10 ml double distilled water in a 25 ml standard volumetric flask and topped up to mark with double distilled water. For the preparation of standard stock solutions of vitamin B9 and B2, 25 mg of 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 mixing100 µl mixed vitamins standard (vitamin B9, B5 and B2), 800 µl phosphate buffer (1 M, pH 5.5) and 100 µl mixed vitamins standard (vitamin C, B1, B6 and B3) which represent 100 µg/ml mixed working standards. The working standard solutions of concentrations 20, 40, 60 and 80 µg/ml were prepared accordingly.

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 a 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 material was soaked in 10 ml double distilled water with stirring for 30 min. Then 1 ml 0.1 M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in the 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 the 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 the 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 Segal *et al.* 2018 with minor modification [10]. 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. Gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elution 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 the initial condition (solvent A: solvent B: 1: 99) in 34 min and allowed to run for another 1 min, before the injection of another sample. The total 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 the 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 measuring the integrated peak area. The content was calculated using the calibration curve by plotting the peak area against the 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, 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 ten wild edible fruits were analyzed using one-way analysis of variance (ANOVAs). All the investigation was completed utilizing triplicate tests. Test results were exposed to the 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 **Fig. 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 R^2> 0.9906 in the range of analyzed.

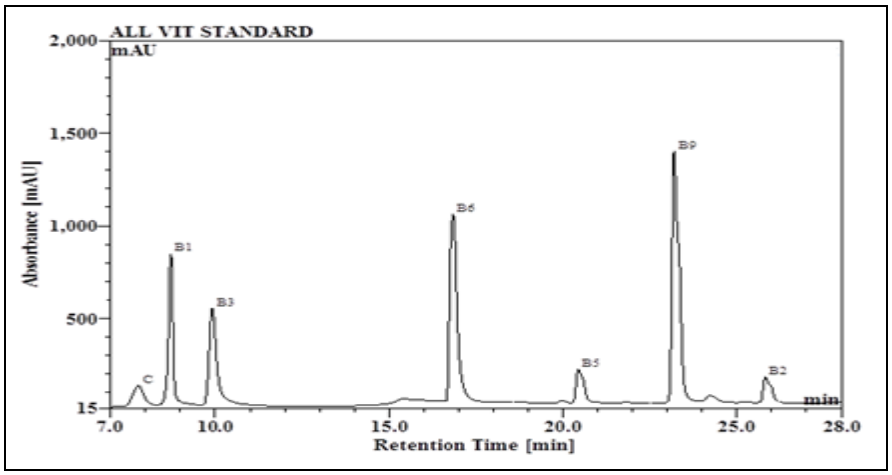

FIG. 1: HPLC CHROMATOGRAM OF MIXTURE OF STANDARD VITAMIN. C: Ascorbic acid; B1: Thiamine; B3: Niacin; B6: Pyridoxine; B5: Pantothenic acid; B9: Folic acid; B2: Riboflavin..

TABLE 1: RETENTION TIME AND PARAMETERS OF CALIBRATION CURVE, PRECISION, AND RE-PEATABILITY, LOD, LO 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	Percent Age of Recover y (%)
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. Absorbance 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 *e.g.* ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9). The HPLC results of all ten wild edible fruits have been presented in **Fig. 2-3,** 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.**

TABLE 2: QUANTIFICATION OF VITAMIN C AND B1, B2, B3, B5, B6 AND B9 IN TEN WILD EDIBLE FRUITS

	Vitamin Content in mg/ 100 gm Dry plant Material						
	Vitamin C	**Vitamin B1**	**Vitamin B2**	**Vitamin B3**	**Vitamin B5**	**Vitamin B6**	**Vitamin B9**
A. gomezianus	0.22±0.003^{h}	0.15±0.002^{b}	0.57±0.007^{b}	0.18±0.001^{b}	0.11±0.003^{d}	0.15± 0.003^{g}	0.73± 0.001^{f}
B. sapida	0.12± 0.001^{i}	0.07± 0.001^{e}	0.07 ±0.002^{gh}	0.41±0.006^{a}	0.05±0.002^{g}	0.60± 0.013^{d}	1.51±0.06^{d}
C. pedata	0.58± 0.003^{g}	0.08± 0.002^{d}	0.14± 0.003^{e}	ND	0.19± 0.016^{b}	0.86± 0.02^{a}	0.60± 0.016^{g}
F. jangomas	3.12±0.05^{b}	0.04±0.001^{f}	0.62±0.013^{a}	0.02± 0.001^{d}	0.48±0.02^{a}	0.69± 0.02^{c}	1.87± 0.03^{a}
G.cochinchinense	1.74± 0.006^{c}	0.20±0.01^{a}	0.08± 0.002^{g}	ND	0.09± 0.003^{e}	0.08± 0.001^{h}	0.15± 0.003^{j}
H. heteroclita	0.65± 0.05^{f}	ND	0.15± 0.016^{e}	ND	0.14± 0.003^{c}	0.33± 0.006^{e}	0.26± 0.026^{i}
P. nepaulensis	1.51± 0.033^{d}	0.08± 0.001^{d}	0.26 ±0.02^{d}	ND	0.04± 0.001^{h}	0.61± 0.015^{d}	0.83± 0.02^{e}
S. axillaris	0.05± 0.001^{j}	0.01± 0.0003^{g}	0.11± 0.003^{f}	0.02± 0.001^{d}	0.06± 0.002^{f}	0.28± 0.013^{f}	1.83± 0.02^{b}
T. bellirica	4.99± 0.03^{a}	0.04± 0.002^{f}	0.49± 0.016^{c}	0.004±0.0003^{e}	0.09± 0.002^{e}	0.14± 0.003^{g}	0.42± 0.003^{h}
Z. armatum	1.28± 0.016^{e}	0.12± 0.006^{c}	0.05± 0.002^{h}	0.04± 0.001^{c}	0.05± 0.003^{g}	0.76± 0.026^{b}	1.78± 0.013^{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 was carried out by Tukeys test at 95% confidence level and statistical significance was 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.

The HPLC analysis of the plants *A. gomezianus* showed the presence of vitamin C (0.22± 0.003 mg/100gm DPM), B1 (0.15±0.002 mg/100gm DPM), B2 (0.57±0.007 mg/100gm DPM), B3 (0.18±0.001), B5 (0.11±0.003 mg/100gm DPM), B6 (0.15±0.003 mg/100gm DPM) and B9 (0.73±0.001 mg/100gm DPM). The HPLC study of the fruits of B. sapida revealed the presence of vitamin C (0.12±0.001 mg/100gm DPM) along withB1 (0.07±0.001 mg/100gm DPM), B2 (0.07±0.002 mg/100gm DPM), B3 (0.41±0.006 mg/100gm DPM), B5 (0.05±0.002 mg/100gm DPM), B6 (0.60±0.013 mg/100gm DPM) and B9 (1.51±0.06 mg/100gm DPM).

The presence of vitamin B1 (0.08± 0.002 mg/100gm DPM), B2 (0.14±0.003 mg/100gm DPM), B5 (0.19±0.016 mg/100gm DPM), B6 (0.86±0.02 mg/100gm DPM), B9 (0.60±0.016 mg/100gm DPM) and appreciable amount of Vitamin C (0.58±0.03 mg/100gm DPM) were detected in *C. pedata*. The fruits of *F. Jangomas* were found to contain good amount of vitamin C (3.12± 0.05 mg/100gm DPM), along with B1 (0.04±0.001 mg/100gm DPM), B2 (0.62±0.013 mg/100gm DPM), B3 (0.02±0.001 mg/100gm DPM), B5 (0.48±0.02 mg/100gm DPM), B6 (0.69±0.01 mg/100gm DPM) and appreciable amount of B9 (1.87±0.03 mg/100gm DPM). Our investigation disclosed the occurrence of vitamin B1 (0.20±0.01 mg/100gm DPM), B2 (0.08±0.002 mg/100gm DPM), B5 (0.09±0.003 mg/100gm DPM), B6 (0.08±0.001 mg/100gm DPM), B9 (0.15±0.033 mg/100gm DPM) and substantial amount of vitamin C (1.74± 0.006 mg/100gm DPM) in the fruits of G. cochinchinense. The HPLC study of the fruits H. heteroclita revealed the presence of vitamin B2 (0.15±0.016 mg/100gm), B5 (0.14±0.003 mg/100 gm), B6 (0.33±0.006 mg/100 gm) and B9 (0.26±0.026 mg/100gm). The presence of vitamin B1 (0.08± 0.001 mg/100 gm), B2 (0.26±0.02 mg/100gm), B5 (0.04±0.001 mg/100 gm), B6 (0.61±0.015 mg/100gm), B9 (0.83±0.02 mg/100gm) and good amount of Vitamin C (1.51±0.033mg/100gm) were detectedin P. nepaulensis.

The fruits of *S. axillaris* were found to contain vitamin C (0.05±0.001 mg/100gm), B1 (0.01 ± 0.0003 mg/100gm), B2 (0.11±0.003 mg/100gm), B3 (0.02±0.001 mg/100 gm), B5 (0.06±0.002 mg/100 gm), B6 (0.28±0.003 mg/100gm) and significant amount of B9 (1.83±0.02 mg/100gm). Our investigation disclosed the occurrence of vitamin B1 (0.04±0.002 mg/100gm), B2 (0.49 ± 0.016 mg/100gm), B3 (0.004±0.0003 mg/100gm), B5 (0.09±0.002 mg/100 gm), B6 (0.14±0.003 mg/100 gm), B9 (0.42±0.003 mg/100 gm) and good amount of vitamin C (4.99±0.03 mg/100gm) in the fruits of *T. bellirica*. The HPLC investigation

of the fruits of *Z. armatum* showed the presence of vitamin C (1.28± 0.016 mg/100gm DPM), B1 (0.12±0.006 mg/100gm DPM), B2 (0.05±0.002 mg/100gm DPM), B3 (0.04±0.001), B5 (0.05± 0.003 mg/100gm DPM), B6 (0.76±0.026 mg/100 gm DPM) and B9 (1.78±0.013 mg/100gm DPM).

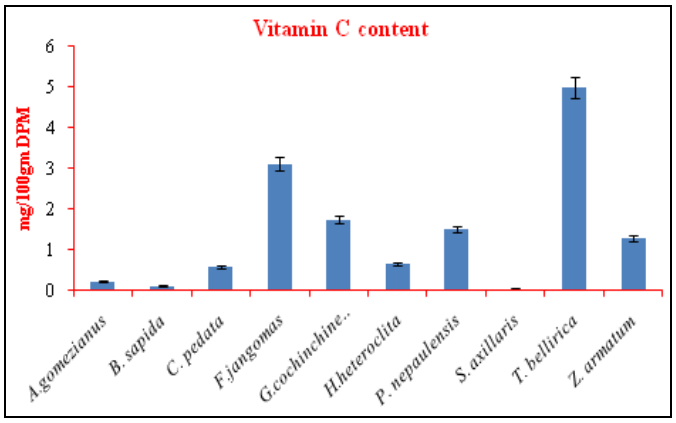

FIG. 2: VITAMIN C CONTENT IN TEN WILD EDIBLE FRUITS

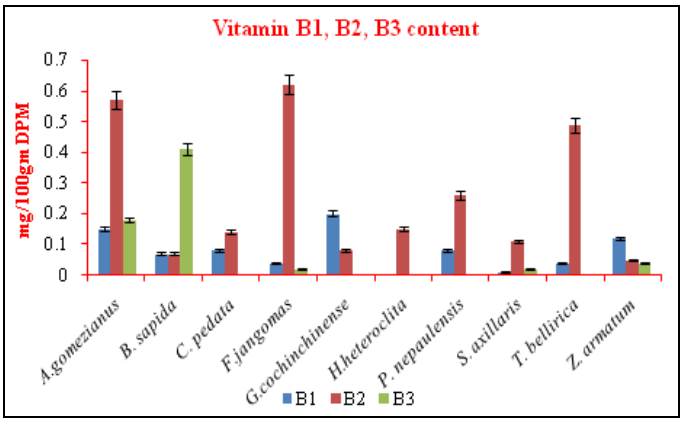

FIG. 3: VITAMIN B1, B2 AND B3CONTENT IN TEN WILD EDIBLE FRUITS

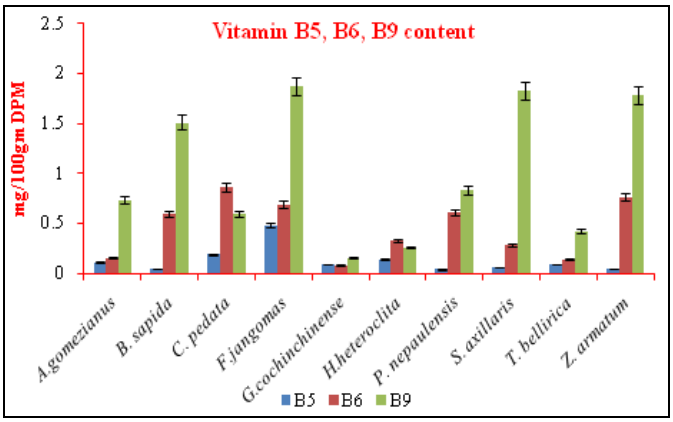

FIG. 4: VITAMIN B5, B6 AND B9 CONTENT IN TEN WILD EDIBLE PLANTS

DISCUSSION:

Chromatographic Method: The quantitative analysis of water-soluble vitamins was completed using a photodiode 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.*, 40 µg/ml and 60 µg/ml was found to be below one percent. The significantly high recovery rate (98.15 – 99.20%) of the standard vitamins is worth mentioning. 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 analyte 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, meticulous-ness 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 a simple, gradient, and stability-indicating HPLC method for the determination of Vitamin C, B1, B2, B3, B5, B6, and B9 in ten wild edible fruits. 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 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 [13-14]. The experimental result showed that the amount of vitamin C was found highest in the fruits of *T. bellirica* (4.99± 0.03mg/100gm DPM) followed by in *F. jangomas* (3.12±0.05mg/100gm DPM) **Table 2**.

The vitamin C content in these wild edible fruits is very much comparable with some wild edible plants like *Diplazium esculentum* (5.41± 0.03 mg/100gm), *Begonia hatacoa* (3.41± 0.01 mg/100gm), *Cardamine hirsute* (6.23± 0.02 mg/100gm) *etc.* [2, 10]. 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 plants might be useful to diminish the risk of atherosclerosis and a couple of sorts of dangerous development [15]. 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 the edge and circulatory systems, hypertension, and heart infirmities [16-17]. The thiamine content in these wild edible plants ranged from 0.01± 0.0003to 0.20±0.01mg/100gm DPM. The highest amount of B1 was obtained from the fruits of *G. cochinchinense* followed by in *A. gomezianus*a and *Z armatum* **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 [18]. A basic assortment of riboflavin content was seen among the attempted wild consumable characteristic items. The maximum sum of B2 was detected in the fruits of *F. jangomas* (0.62±0.013mg/100gm DPM), and the least amount was detected in *Z. armatum* (0.05± 0.002 mg/100gm DPM).

The fruits of *A. gomezianus*, *T. bellirica*, and *P. nepaulensis*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* [19]. The niacin (B3) was detected highest in the fruits of *B. sapida* (0.41±0.006mg/100gm DPM) and the least amount was noticed in the fruits of *T. bellirica* (0.004± 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 [19]. 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 [20]. 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 [21]. Pantothenic acid was detected highest in the fruits of *F. jangomas* (0.48±0.02mg/100gm DPM). The edible parts of *G. cochinchinense, C. pedata, H. heteroclita*and *A. gomezianus*were also found to contain a very good amount of B5 **Table 2.**

Pyridoxine (B6) is another water dissolvable vitamin key for the ideal help of red platelet absorption, the tangible framework, the sheltered system, and various other genuine limits. It is like manner expect a vocation in homocysteine made and degradative reactions [22]. This supplement is found in most sustenance things and, besides, as a result of its stability, is routinely used for reinforcing sustenance things [23]. It was measured in all the wild eatable plants under our examination.The highest B6 was observed in the fruits of *C. pedata* (0.86±0.02mg/100gm DPM) followed by in *F. jangomas* and in *P. nepaulensis*, whereas the minimum was detected in *G. Cochinchinense* (0.08±0.001mg/100gm DPM). An appreciable amount of B_6 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 (0.115 mg/100g), cucumber (0.2 mg/100g) *etc.* So the regular intake of these plants would supply sufficient B_6 necessary to maintain healthy body functions. Vitamin B_9 (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 [24]. 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 peroxidation [24]. The extent of B_9 in ten wild edible fruits ranged from 0.15± 0.003to 1.87± 0.03mg/100gm DPM. The content of B_9 was found highest in *F. Jangomas*, and a good amount of B_9 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 ten wild edible fruits like *A. gomezianus, B. sapida, C. pedata, F. jangomas, G. cochinchinense, H. heteroclita, P. nepaulensis, S. axillaris, T. bellirica*and *Z. Armatum* collected 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 the 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.

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