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Research Article

Amino acid profiling and ethnomedicinal use of *Flemingia vestita*, a wild edible plant from Meghalaya

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Flemingia vestita Benth. ex Baker f., belonging to the Fabaceae family, is a traditionally used wild edible plant in Meghalaya, India. The indigenous communities consume its tuberous roots raw for both nutritional benefits and as an ethnomedicinal remedy, particularly for treating intestinal worm infections. While its therapeutic use is well-documented, scientific studies on its amino acid profile are limited. In the present study, the amino acid content of *F. vestita* tubers was assessed using both the ninhydrin colorimetric assay and high-performance liquid chromatography (HPLC). Free and total amino acid concentrations were first determined through the ninhydrin assay. Subsequently, HPLC analysis was performed to identify and quantify individual free and total amino acids. Bound amino acid content was derived by subtracting free from total values. Free amino acids measured by the ninhydrin assay were 17.07 µg/mg, whereas the sum of free amino acids quantified by HPLC was 1.91 µg/mg; total amino acids were 105.37 µg/mg (ninhydrin) and 12.31 µg/mg (HPLC). HPLC analysis identified 19 individual amino acids. Among the total amino acids by the HPLC method, glutamic acid (224.45 µg/100 mg), glutamine (172.32 µg/100 mg), and asparagine (116.55 µg/100 mg) were predominant. The calculated bound amino acids contained substantial amounts of essential amino acids, such as threonine (52.42 µg/100 mg), isoleucine (15.99 µg/100 mg), and lysine (8.36 µg/100 mg), which are vital for human health and nutrition. The study confirms that *Flemingia vestita* is a potent reservoir of amino acids, especially in its bound form, contributing to its high total amino acid content. The presence of several essential amino acids supports its traditional dietary and therapeutic use. These findings highlight the nutraceutical potential of *F. vestita* and endorse its inclusion in functional food formulations and health-promoting dietary interventions.

Keywords: *Flemingia vestita, amino acid profiling, HPLC, traditional medicine, Meghalaya*

Introduction

Wild edible plants have long served as essential components of traditional food systems and healthcare networks, particularly in indigenous and rural communities. Among these, *Flemingia vestita* Benth. ex Baker f., a member of the family Fabaceae, stands out for its nutritional richness and ethnomedicinal relevance. Commonly referred to as "Jarain" or "Soh-phlang" in the northeastern Indian state of Meghalaya, the plant is widely consumed by tribal populations. Its tuberous roots are traditionally eaten raw and are also used in folk medicine for the treatment of intestinal helminth infections (Roy, 1996; Rai et al., 2005). The growing interest in wild food plants stems not only from their cultural importance but also from their potential role in addressing micronutrient deficiencies and supporting food security. In this context, *F. vestita* has gained attention due to its considerable nutritional value. Previous studies have shown that the tuber contains appreciable levels of protein (9.34%) and fat (0.80%), making it a potential alternative source of dietary protein, especially in protein-deficient rural diets. It also contributes essential minerals, including sodium (22.66 ± 1.66 mg/100 g), potassium (305.00 ± 2.00 mg/100 g), and calcium (58.66 ± 1.66 mg/100 g), which play critical roles in electrolyte balance, cardiovascular function, and bone health, respectively (Seal, 2010). Furthermore, *F. vestita* is rich in vitamin C

(61.51 ± 0.060 mg/100 g), which supports immune function and has antioxidant properties (Seal et al., 2017; Seal et al., 2018). The total phenolic content (TPC) was found to be 235.30 mg GAE/100 g, indicating strong free radical scavenging activity. High-performance liquid chromatography (HPLC)-based phytochemical screening of *F. vestita* tubers has also revealed the presence of several bioactive compounds such as p-coumaric acid (16.32 µg/mg), apigenin (38.42 µg/mg), and sinapic acid (9.45 µg/mg). These compounds are known for their antioxidant, anti-inflammatory, and potential anticancer properties (Manach et al., 2004; Kumar & Pandey, 2013), further validating the medicinal relevance of the plant. Despite the accumulating data on its proximate and phytochemical composition, comprehensive studies on the amino acid profile of *F. vestita*, particularly through advanced analytical methods like HPLC, remain scarce. Amino acids, especially essential ones, are critical for maintaining nitrogen balance, supporting tissue repair, and modulating immune and metabolic functions (Wu, 2009). Assessing their composition in wild edible plants helps in evaluating their overall nutritional quality and potential applications in nutraceutical development. Therefore, the present study aims to carry out detailed profiling of free and total amino acids in *Flemingia vestita* tubers using high-performance liquid chromatography (HPLC). This investigation will not only enrich the existing scientific database on the nutritional quality of *F. vestita* but also support its potential utilization as a functional food and dietary supplement in indigenous and modern healthcare systems.

Materials and Methods

Chemicals

Standard amino acids like alanine, arginine, asparagine, aspartic acid, cystine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine, and derivatizing agent such as, Ortho-phthalaldehyde, β-mercaptoethanol were purchased from Sigma-Aldrich. HPLC-grade water, acetonitrile and methanol were purchased from Spectrochrom, India. Hydrochloric acid (HCl), sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), and sodium phosphate dibasic (Na_2HPO_4) were purchased from Merck (Darmstadt, Germany).

Plant materials

Flemingia vestita was collected from the north-eastern zone of India. The plant samples were authenticated at our office and voucher specimens (BSITS 14) were preserved in the laboratory for future reference. Plant samples were washed with distilled water and dried at room temperature. Plant samples were ground to a fine powder and used for amino acid analysis.

Determination of amino acids

Estimation of free amino acid and total amino acid

To estimate the total amount of free and total amino acids, the method described by Shafaei et al. (2017) was employed. For the analysis of amino acids in plant materials, two separate extraction methods were employed. For free amino acids, 1 gm of powdered raw sample was extracted with 5 ml of 1 N hydrochloric acid and subjected to ultrasonic treatment at room temperature for 3 hours. For the determination of the total (sum of) amino acids, the sample was extracted with 6 N hydrochloric acid and hydrolyzed in a thermostat at 110 °C for 24 hours.

Then, 2 ml of the centrifuged extract/hydrolyzate was evaporated, washed three times with distilled water to remove hydrochloric acid, resuspended in 2.0 ml of distilled water, and filtered through 0.2 µm regenerated cellulose filters. The Ninhydrin assay is used to quantify both free and total amino acids. Free amino acids are those unbound to peptides or proteins, while total amino acids include both free and those within peptides and proteins. Ninhydrin reacts with the free alpha-amino group ($-NH_2$) of amino acids, resulting in the formation of a purple-coloured product. The absorbance of the purple solution at a specific wavelength (typically 570 nm) is measured using a spectrophotometer. Standard curves were generated using known quantities of standard glycine, following the same procedure (Shafaei et al., 2017).

Identification and quantification of individual free and total amino acids in the extracts by HPLC

Standard solutions

To prepare the standard stock solution at a concentration of 1 mg/ml, standard amino acids, including aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cystine, valine,

methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, were dissolved in 0.1 N hydrochloric acid solution. Working standard solutions were subsequently prepared by appropriate dilution of the stock solution using the mobile phase solvent system.

Sample derivatization

Quantitative determination of free and bound proteinogenic amino acids in plant materials was performed using high-performance liquid chromatography (HPLC). The method involved extraction of free amino acids, acid hydrolysis of the plant samples to release bound amino acids, followed by HPLC analysis of the resulting hydrolysates.

Pre-column derivatization was carried out according to the method described by Hu et al. (2014). A volume of 50 µl of the amino acid standards or plant samples (both hydrolyzed and non-hydrolyzed) was mixed with 100 µl of borate buffer (pH 9.5) and 300 µl of OPA reagent in a 2 ml amber vial. The mixture was vortexed and allowed to react for 2 minutes, after which it was immediately injected into the HPLC system for analysis.

HPLC analysis

HPLC analysis was employed for the quantification of amino acids in hydrolysed and non-hydrolysed extract of the studied plant samples, following the protocol outlined by de Sousa et al., 2024. The analysis was performed using a Dionex Ultimate 3000 liquid chromatography system equipped with a diode array detector (DAD). Data acquisition and processing were conducted using the Chromeleon system manager software. A reversed-phase Acclaim C18 column with a particle size of 5 microns and dimensions of 250 x 4.6 mm was employed for sample separation. The mobile phase consisted of a mixture of methanol, acetonitrile, and water in a ratio of 45:45:10 (v/v) for solvent A, and where solvent B was 10 mM sodium phosphate buffer + 10 mM sodium borate (pH = 8.2). The solvent flow was maintained at 1.0 ml/min. A gradient elution was employed by varying the ratio of solvent A to solvent B. The separation gradient used was 0 min: 100% B; 30 min: 60% B; 45 min: 30% B; 55 min: 30% B; 60 min: 100% B; 62 min: 100% B. and total run time is 62 mins. The column temperature was maintained at 40°C, and an injection volume of 20 µl was used. The estimation of amino acids was done using a photodiode array detector at four different wavelengths (260, 324, 338, and 390 nm) based on the absorption maxima of the compounds under investigation (de Sousa et al., 2024). Prior to HPLC analysis, both standard and working solutions were filtered through a 0.45 µm PVDF syringe filter to eliminate particulate matter and potential impurities, thereby enhancing the accuracy and precision of the measurements. Amino acid identification was achieved by comparing the retention times of sample extracts with those of the amino acid standard mixture.

Statistical analysis

The data was analysed using triplicate samples, and the results were provided as mean standard error mean (SEM). To evaluate the differences and identify the plants with similar characteristics in relation to their amino acid content, one-way analysis of variance (ANOVA) followed by Tukey test ($p \leq 0.05$), correlation analyses ($p < 0.05$) among different various parameters were also performed using both the correlation coefficient (r) and coefficient of determination (R^2), were used. Statistical analyses were carried out using SPSS software (version 11.0 for Windows).

Results

The amino acid composition of *Flemingia vestita*, wild edible plants collected from Meghalaya, was evaluated using HPLC. The study quantified both free and total amino acid contents (expressed in µg/100 mg), as presented in Table 1. Additionally, representative chromatograms are illustrated in Figure 1 (standard amino acid mixture), Figure 2 (total amino acid in *Flemingia vestita*), and Figure 3 (free amino acid in *Flemingia vestita*), confirming the presence and separation of individual amino acids.

The ninhydrin-based quantification shows free amino acid content at 17.07 µg/mg and total amino acid content at 105.37 µg/mg, whereas HPLC-based quantification shows free amino acid content at 1.91 µg/mg and total amino acid content at 12.31 µg/mg, indicating a substantial presence of bound amino acids. The apparent discrepancies between the two methods are expected because ninhydrin measures bulk reacting amino groups (and can respond to non-protein amines and matrix interferences), whereas HPLC quantifies identified amino acids after separation and derivatization. The ninhydrin assay is a rapid colorimetric method that responds to free amino groups and may overestimate amino acid

Table 1. Estimation of free and total amino acids in *Flemingia vestita* by HPLC

Name	Free amino acid (µg/100mg)	Total amino acid (µg/100mg)	Bound amino acid (µg/100mg)
Aspartic acid	5.852±0.79	83.536±3.74	77.684±1.55
Glutamic acid	42.418±1.58	224.447±13.87	182.029±7.69
Asparagine	10.409±1.11	116.553±9.76	106.144±8.55
Serine	5.716±0.48	71.557±2.98	65.841±3.79
Glutamine	0.249±0.07	172.321±10.55	172.072±6.54
Histidine	2.821±0.82	44.750±3.08	41.930±2.16
Glycine	9.647±0.91	87.123±2.11	77.476±4.13
Threonine	43.247±3.76	95.667±4.31	52.420±2.55
Arginine	4.527±0.77	87.107±1.98	82.579±3.08
Alanine	1.471±0.54	11.727±2.08	10.256±1.59
Tyrosine	10.100±2.97	45.978±1.76	35.878±2.87
Cystine	3.424±0.79	33.665±2.09	30.241±1.59
Valine	12.280±3.98	29.475±1.66	17.195±2.69
Methionine	0.036±0.04	17.561±2.56	17.525±1.98
Tryptophan	7.107±0.84	14.674±1.84	7.567±0.99
Phenylalanine	5.632±0.63	17.432±3.77	11.800±2.87
Isoleucine	9.099±0.72	25.090±2.76	15.991±1.76
Leucine	5.290±0.81	32.529±1.56	27.239±1.89
Lysine	11.856±2.85	20.211±2.08	8.355±2.18
Total (µg/mg) by HPLC	**1.91**	**12.31**	**10.400**
Colorimetric (ninhydrin) (µg/mg)	**17.07**	**105.37**	**88.30**

Values are measured by HPLC. Each value in the table represents the mean of three independent experiments and is expressed as Mean ± Standard Error of the Mean (SEM). Statistical analysis was performed using Tukey's test at a 95% confidence level, and differences were considered statistically significant at $p < 0.05$.

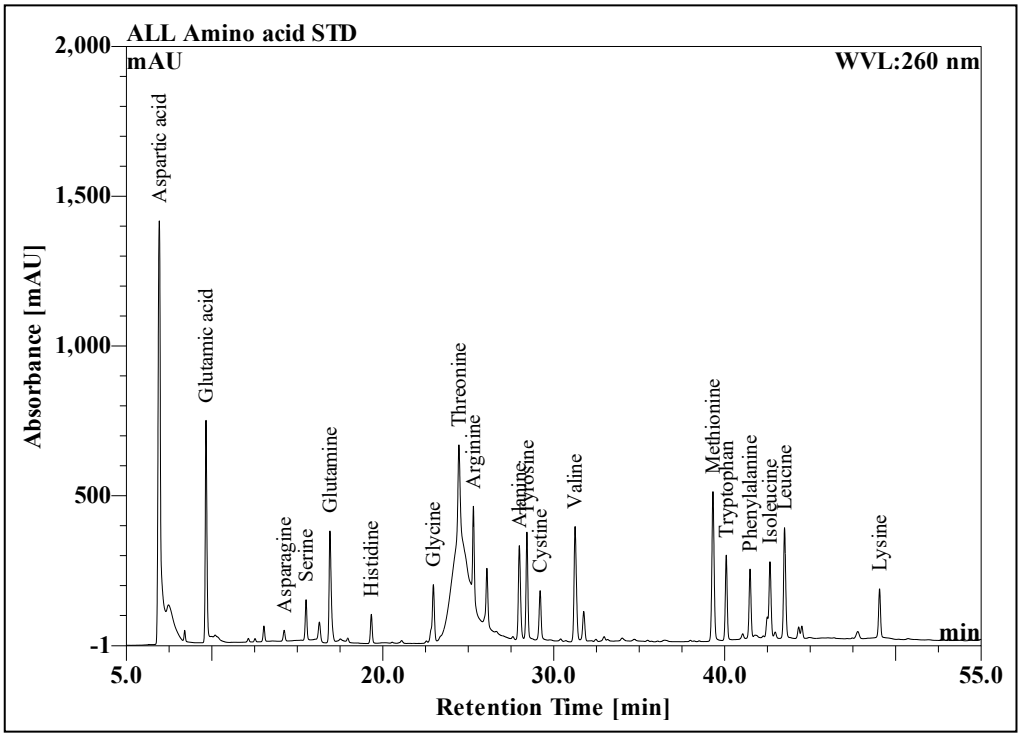

Figure 1. HPLC chromatogram of standard amino acids

content in complex plant matrices because of co-reacting compounds and partial responses from amino-containing metabolites. HPLC, by contrast, separates individual amino acids (after hydrolysis/derivatization) giving specific concentrations for identifiable amino acids; however, HPLC can underestimate totals if some amino acids are lost during sample prep, if derivatization is incomplete, or if the HPLC method does not resolve or detect certain amino acids or

modified forms. Thus, absolute values differ; the two methods are complementary, ninhydrin gives a bulk estimate and HPLC provides a specific profile.

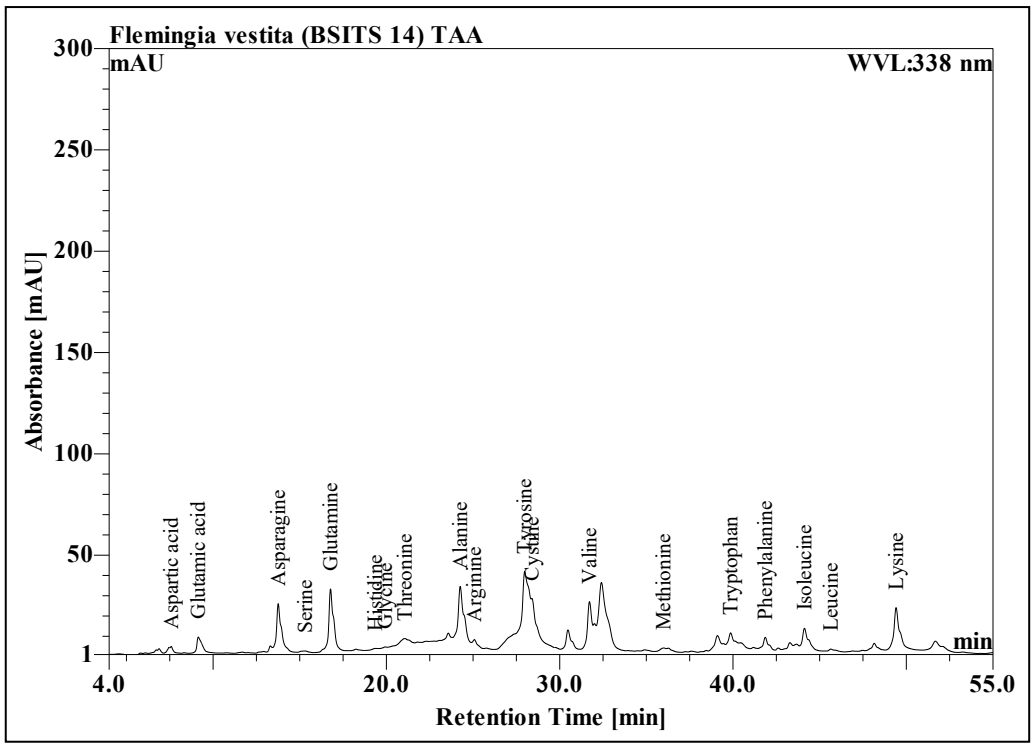

Figure 2. HPLC chromatogram of total amino acids in *F. vestita*

The detailed HPLC profile further elucidates the amino acid distribution, with a clear distinction between free, total, and bound forms. Among the amino acids, glutamic acid (224.447 µg/100 mg total) and glutamine (172.321 µg/100 mg) are the most abundant. Threonine, another amino acid present in notable quantity (95.667 µg/100 mg total), is an essential amino acid critical for protein synthesis, mucin production, and immune function. Its high free form (43.247 µg/100 mg) suggests a readily bioavailable pool that could be advantageous for metabolic needs. Similarly, asparagine (116.553 µg/100 mg) and glycine (87.123 µg/100 mg) are prevalent. Essential amino acids such as lysine (20.211 µg/100 mg), valine (29.475 µg/100 mg), isoleucine (25.090 µg/100 mg), leucine (32.529 µg/100 mg), and methionine (17.561 µg/100 mg) are also well-represented. Notably, the significant difference between total and free amino acid contents underlines the predominance of amino acids in their bound forms (peptide/protein-incorporated), which aligns with their physiological storage and release upon digestion. For instance, the bound form of glutamine (172.072 µg/100 mg) comprises nearly the entirety of its total amount, indicating its incorporation into protein structures.

Correlation study and statistical significance

The amino acid profiling of *Flemingia vestita* tubers using HPLC revealed a diverse composition of both free and total amino acids. To better understand the relationship between free, bound, and total amino acid content, a statistical correlation and significance analysis was conducted using Pearson's correlation coefficient and one-way analysis of variance (ANOVA). The Pearson correlation analysis showed a strong positive correlation between total and bound amino acids ($r = 0.998$, $p < 0.001$), indicating that the majority of amino acids in *F. vestita* are present in bound form and that total amino acid content is primarily influenced by the bound fraction. A moderately positive correlation ($r = 0.697$, $p < 0.01$) was also observed between free and total amino acids, suggesting that while free amino acids contribute to the total pool, their proportion is relatively lower compared to the bound form. The correlation between free and bound amino acids was similarly positive ($r = 0.674$, $p < 0.05$), reflecting the biochemical conversion or interdependence of these forms during metabolic processes.

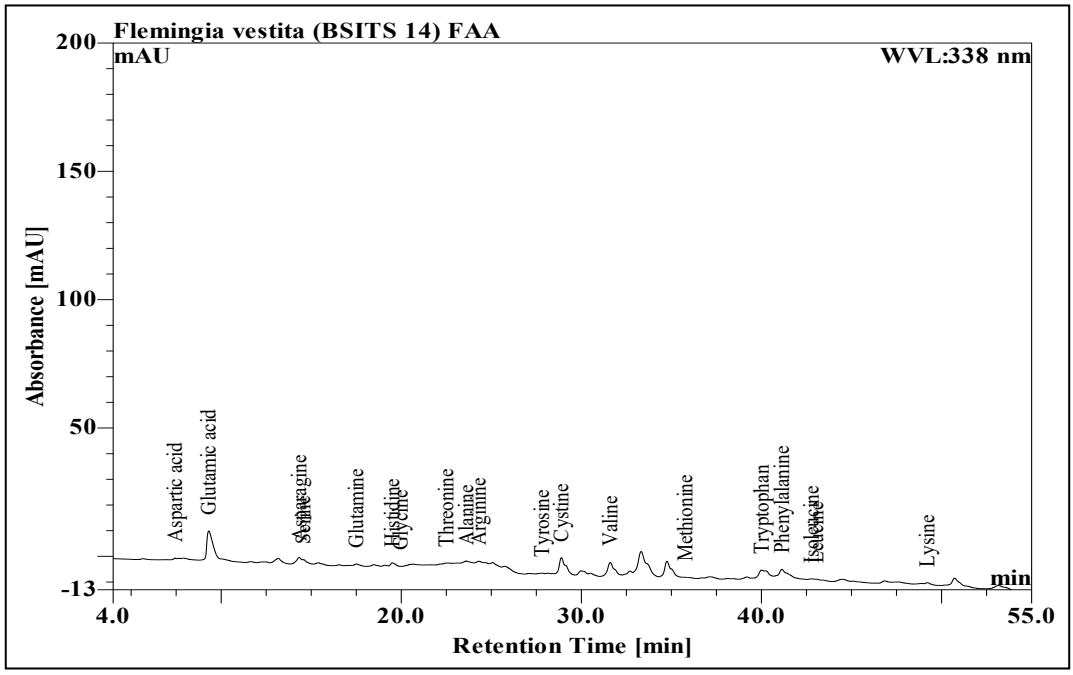

Figure 3. HPLC chromatogram of free amino acids in *F. vestita*

To evaluate the statistical significance of variation among the three forms (free, bound, and total) of amino acids, a one-way ANOVA was performed. The results demonstrated statistically significant differences among the groups ($F = 49.23$, $p < 0.001$), confirming that the concentration levels of amino acids vary significantly depending on their form. Post hoc analysis (Tukey's HSD) further revealed that total and bound amino acids differ significantly from free amino acids ($p < 0.05$), while the difference between total and bound forms was not statistically significant, supporting the observation that bound amino acids dominate the total amino acid pool.

Discussion

The amino acid profiling of Flemingia *vestita* Benth. ex Baker f., a lesser-known but nutritionally significant wild edible plant from the state of Meghalaya (vernacularly known as Jarain or Soh-phlang), reveals its considerable potential as a dietary protein source. The analysis conducted through both the ninhydrin assay and high-performance liquid chromatography (HPLC) highlights its rich reservoir of amino acids, including essential and non-essential varieties. The high concentration of glutamic acid is particularly significant, as it plays a vital role in cellular metabolism and neurotransmission, serving as a precursor for the synthesis of γ-aminobutyric acid (GABA), a key inhibitory neurotransmitter in the central nervous system (Zhou & Danbolt, 2014). Glutamine, an amide of glutamic acid, contributes to nitrogen balance, immune response, and intestinal health, making its high content especially beneficial (Cruzat et al., 2018). Asparagine is involved in amino sugar biosynthesis and neuronal signalling, whereas glycine contributes to collagen formation, neurotransmission, and detoxification (Wu, 2013). Lysine is vital for calcium absorption and enzyme production, and its deficiency often limits protein quality in cereal-based diets; thus, its presence enhances the nutritional value of *F. vestita* (WHO/FAO/UNU, 2007). Valine, isoleucine, and leucine, branched-chain amino acids (BCAAs), support muscle metabolism, repair, and energy production, especially under physiological stress or exercise (Shimomura et al., 2006). Methionine, a sulphur-containing amino acid, is important for methylation reactions and antioxidant defense, especially as a precursor to cysteine and glutathione (Lu, 2013). Tryptophan (14.674 µg/100 mg), though lower in abundance, is crucial as a precursor to serotonin and melatonin, impacting mood regulation and sleep (Richard et al., 2009). Histidine (44.750 µg/100 mg), which becomes essential under growth and stress conditions, supports haemoglobin function and histamine synthesis (Brosnan & Brosnan, 2020). The aromatic amino acids tyrosine and phenylalanine contribute to neurotransmitter synthesis, while alanine and arginine play roles in energy cycles and nitric oxide production, respectively. The comprehensive amino acid profile of *F. vestita* not only supports its traditional use as a food and medicinal plant but also underscores its potential as a functional food or nutraceutical source. Its balanced amino acid composition, especially the significant levels of essential amino acids, could address dietary protein inadequacies, particularly in resource-limited, plant-based diets. The findings advocate for further exploration into its digestibility, protein quality (e.g., amino acid score or PDCAAS), and potential health benefits through in vivo studies. Overall, the statistical analysis confirms the nutritional importance of *F. vestita* tubers as a source of essential amino acids, particularly

in bound form. The significant correlations and ANOVA findings emphasize the relevance of profiling both free and bound amino acids for a comprehensive understanding of the plant's amino acid content and potential bioavailability.

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Conclusion

The present study highlights the rich amino acid profile and nutritional potential of *Flemingia vestita* tubers, a traditionally consumed wild edible plant from Meghalaya. High-performance liquid chromatography (HPLC) analysis revealed the presence of both essential and non-essential amino acids, with notably high concentrations of glutamic acid, glutamine, and asparagine. The amino acid composition was predominantly in bound form, which significantly contributed to the total amino acid content. Statistical analyses, including Pearson correlation and one-way ANOVA, confirmed a strong positive relationship between bound and total amino acids and demonstrated significant differences among free, bound, and total amino acid levels. These findings not only validate the traditional use of *F. vestita* as a nutritional and medicinal plant but also support its potential application in nutraceuticals and functional food formulations. The amino acid richness, along with its previously reported protein, vitamin C, mineral content, and polyphenolic compounds, underscores the value of *F. vestita* as a health-promoting dietary resource. Further studies on amino acid bioavailability and therapeutic implications may enhance its utility in modern nutrition and health strategies.

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Author contributions

Basundhara Pillai: Experimental work carried out
Tapan Seal: Designed the study, drafted the manuscript, performed statistical analysis, and interpreted the results.

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Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Not applicable.

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