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Amino acid profiling of *Houttuynia cordata* and *Solanum kurzii*: wild edible plants from Northeast India using HPLC

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ABSTRACT

Background: Wild edible plants play a crucial role in traditional diets and healthcare systems of North-East India, yet many remain scientifically underexplored. *Houttuynia cordata* and *Solanum kurzii* are lesser-known edible plants traditionally consumed in Meghalaya for both nutritional and ethnomedicinal purposes. **Objective**: The present study aimed to evaluate the ethnomedicinal significance and amino acid composition of *H. cordata* and *S. kurzii* to assess their nutritional value and potential as nutraceutical resources. **Materials and Methods**: Ethnobotanical information was documented through field surveys among local communities of Meghalaya. Free and total amino acid contents were quantified using the ninhydrin assay, while individual amino acids were identified and quantified by high-performance liquid chromatography (HPLC). **Results**: Both plants are traditionally used as vegetables, condiments, and remedies for ailments such as jaundice, diarrhea, allergies, and gastrointestinal disorders. Significant variation was observed in amino acid profiles. *H. cordata* contained 15.54 µg/mg free and 73.31 µg/mg total amino acids, whereas *S. kurzii* showed 6.93 µg/mg free and 116.05 µg/mg total amino acids. HPLC analysis identified 19 amino acids in both species. *H. cordata* was rich in tyrosine, lysine, and asparagine, while *S. kurzii* exhibited notably high levels of threonine, glutamic acid, and methionine. **Conclusion:** The study demonstrates that *H. cordata* and *S. kurzii* are valuable sources of essential and non-essential amino acids, supporting their traditional dietary and medicinal uses. Their rich amino acid profiles highlight their potential for further development as functional foods and nutraceuticals.

Keywords: *Houttuynia cordata*, *Solanum kurzii*, Amino Acid Profile, HPLC, Ethnomedicine, Wild Edible Plants.

INTRODUCTION

Wild edible plants (WEPs) have long served as vital nutritional and medicinal resources for indigenous communities worldwide, particularly in biodiversity-rich regions like Northeast India. These plants not only provide essential dietary nutrients but also possess numerous bioactive compounds with potential health-promoting properties. Among the many biochemical constituents found in WEPs, amino acids play a crucial role in human health. They are the building blocks of proteins and are involved in various physiological processes including enzyme function, neurotransmission, immune response, and cellular repair [1,2].

The amino acid profile of a plant provides insight into its nutritional value and therapeutic potential. Both essential and non-essential amino acids contribute to a balanced diet, with essential ones being indispensable as the human body cannot synthesize them. Free amino acids are readily absorbed and participate in quick metabolic functions, while total amino acids, including those bound in protein structures, indicate the plant's capacity to contribute to long-term protein nutrition [3].

Houttuynia cordata Thunb. (family: Saururaceae), locally known as Jamyrdoh in Meghalaya, is one such wild plant consumed for both food and traditional medicine. The leaves and shoots are eaten raw, cooked, or used as a condiment. This plant is reputed in ethnomedicine for its effectiveness in treating jaundice, gastrointestinal disorders, and skin infections, and is traditionally believed to improve sleep and mental clarity. It has been scientifically reported to exhibit antibacterial, anti-inflammatory, antiviral, and diuretic activities [4,5].

Similarly, *Solanum kurzii* Brace ex Prain (family: Solanaceae), known as Soh-ngangrit in Meghalaya, is consumed for its fruits, which are eaten raw or processed into chutneys. It is traditionally used by the Mao Naga tribe for managing stomach disorders and allergic conditions,

wherein fruit juice is applied topically to treat allergic reactions [6]. Despite their ethnobotanical significance, the amino acid composition of these plants has not been thoroughly characterized using advanced techniques such as High-Performance Liquid Chromatography (HPLC). High-performance liquid chromatography (HPLC) is a widely accepted analytical method for the separation and quantification of amino acids due to its precision, sensitivity, and reliability [7,8].

Amino acid profiling of wild edible fruits has attracted considerable attention due to their potential nutritional and functional benefits. In a study conducted on five wild edible fruits native to Assam, North-East India, *Grewia sapida*, *Ottelia alismoides*, *Aporosa dioica*, *Antidesma bunius*, and *Eugenia operculata*, reverse-phase high-performance liquid chromatography (RP-HPLC) equipped with a C18 column was employed to characterize their amino acid composition. The analysis revealed the presence of 17 amino acids in varying concentrations, comprising eight essential and nine non-essential amino acids. Among these, six amino acids, namely aspartic acid (1.151-3.837%), glutamic acid (2.283-9.667%), arginine (0.904-7.187%), valine (0.142-1.029%), leucine (1.849-19.665%), and histidine (0.467-12.986%), were consistently detected across all species examined. These findings underscore the diversity and abundance of amino acids in underutilized wild fruits and highlight their potential as valuable dietary sources of essential and non-essential amino acids [9].

This study aimed to determine the free and total amino acid content of *H. cordata* and *S. kurzii* using both colorimetric and HPLC methods. By elucidating their amino acid profiles, this research contributes to understanding the nutritional potential of these lesser-known wild edible plants and supports their promotion as functional foods or nutraceuticals in local and broader contexts.

MATERIAL AND METHODS

Chemicals

Amino acids: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cystine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine and lysine, and o-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

Plant specimens viz. *H. cordata* and *S. kurzii* were collected from the northeastern region of India. The samples were taxonomically authenticated at our institute, and voucher specimens (BSITS 3, and BSITS 9) were deposited in the laboratory herbarium for future reference. The collected plant materials were thoroughly washed with distilled water and air-dried at room temperature. The dried samples were then finely ground into powder and used for subsequent amino acid analysis.

Determination of Amino Acids

Estimation of free amino acid and total amino acid

To estimate the total content of free and total amino acids, the method described by Shafaei et al. (2017) was followed [10].

Sample preparation and analysis of plant materials:

a) Free Amino Acids: One gram of the powdered plant material was extracted with 5 ml of 1 N hydrochloric acid and placed in an ultrasonic bath at room temperature for 3 hours.
b) Total Amino Acids: A separate portion of the sample was extracted with 6 N hydrochloric acid and subjected to hydrolysis in a thermostat at 110°C for 24 hours.

After extraction/hydrolysis, 2 ml of the solution was centrifuged, evaporated to dryness, and washed three times with distilled water to remove residual hydrochloric acid. The dried residue was then resuspended in 2 ml of distilled water and filtered through 0.2 µm regenerated cellulose filters.

The quantification of both free and total amino acids was carried out using the Ninhydrin assay. Free amino acids are those not bound to peptides or proteins, whereas total amino acids include both free and protein-bound amino acids released upon hydrolysis. Ninhydrin reacts with the free alpha-amino group ($-NH_2$) of amino acids to form a purple-colored complex. The absorbance of this complex was measured spectrophotometrically at 570 nm. Standard curves were prepared using known concentrations of glycine, processed using the same method, to ensure accurate quantification [10].

Identification and Quantification of Individual Free and Total Amino Acids in the Extracts by HPLC

Standard solutions

For the preparation of the stock solution at a concentration of 1 mg/ml, standard amino acids (aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cystine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine) were dissolved in 0.1N hydrochloric acid solution. The preparation of working solutions involved dilution of the standard solution with the mobile phase solvent system.

Sample derivatization

Quantitative determination of free and bound proteinogenic amino acids in plant materials was carried out by high performance liquid chromatography (HPLC). The method is based on the extraction of free amino acids from plant materials, acid hydrolysis, and subsequent analysis of hydrolysates by HPLC.

The pre-column derivatization reaction was performed following the methodology of Hu *et al.* (2014) [11]. 50 µl of the amino acid standard and samples (hydrolysed and non-hydrolysed) were mixed with 100 µl of borate buffer (pH = 9.5) and 300 µL of the OPA reagent for 2 minutes in a 2 mL amber vial and subjected to vortexing for subsequent injection into the HPLC. The reaction mixture was then immediately analyzed using HPLC.

HPLC analysis

HPLC analysis was harnessed for the quantification of amino acids in hydrolysed and non-hydrolysed extract of the investigated plants, following the methodology outlined by de Sousa *et al*., 2024 and modified by Datta *et al*., 2025 [12,13]. The analysis was carried out utilizing a Dionex Ultimate 3000 liquid chromatograph furnished with a diode array detector (DAD) incorporating a 5 cm flow cell. Data processing was facilitated by a Chromeleon system manager. 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 [12].

Before introducing the standard and working solutions into the HPLC apparatus, a filtration step was undertaken using a 0.45 µm PVDF-syringe filter. This process ensured the removal of particulate matter and other impurities, contributing to the accuracy and precision of the HPLC analyses.

Identification of amino acids was done by comparing the retention times of amino acid extracts with the retention times of a mixture of amino acid standards. HPLC analysis was carried out following established procedures reported in the literature [11-13].

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 parameters were also performed using both correlation coefficient (r) and coefficient of determination (R^2), and Principal Component Analysis (PCA) were used. SPSS software (version 11.0 for Windows) was used to conduct statistical analysis.

RESULTS AND DISCUSSION

The amino acids composition of two wild edible plants from Northeast India, *H. cordata* and *S. kurzii*, were 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 (*H. cordata*), and Figure 3 (*S. kurzii*), confirming the presence and separation of individual amino acids. Figure 4 and Figure 5 present a comparative analysis of non-essential and essential amino acids in *H. cordata* and *S. kurzii* respectively.

The HPLC-based profiling of individual amino acids in *H. cordata* and *S. kurzii* revealed a diverse array of both free and total amino acids, highlighting the nutritional and therapeutic potential of these wild edible plants. The total amino acid content was found to be 10.11µg/mg in *H. cordata* and 28.87 µg/mg in *S. kurzii*, while the free amino acid content was 3.14 µg/mg and 10.75 µg/mg, respectively. The higher concentration of free amino acids in *S. kurzii* suggests greater bioavailability and digestibility, which is crucial for absorption and utilization in human metabolism [14].

In *H. cordata*, tyrosine was the most abundant amino acid (76.548 µg/100 mg free; 283.490 µg/100 mg total). Tyrosine is a precursor to catecholamines such as dopamine, epinephrine, and norepinephrine, which play key roles in mood regulation, stress response, and cognitive function [15]. Lysine, another major component (51.248 µg/100 mg free; 87.780 µg/100 mg total), is an essential amino acid involved in calcium absorption, collagen formation, and immune function [16]. Threonine (31.905 µg/100 mg free; 43.994 µg/100 mg total), important for mucin production in the gastrointestinal tract, supports intestinal barrier function and immune modulation [17].

The high content of aspartic acid (39.671 µg/100 mg free; 84.072 µg/100 mg total) and glutamic acid (29.544 µg/100 mg free; 51.483 µg/100 mg total) in *H. cordata* suggests their importance in energy production, neurotransmission, and amino acid metabolism [18]. Methionine (14.577 µg/100 mg free; 30.743 µg/100 mg total) and cystine (3.088 µg/100 mg free; 11.200 µg/100 mg total), as sulfur-containing amino acids, are crucial for methylation reactions, detoxification, and the synthesis of glutathione, one of the body's most important antioxidants [19].

In contrast, *Solanum kurzii* demonstrated a strikingly high total glutamic acid concentration (1130.151 µg/100 mg), accompanied by a free form value of 183.263 µg/100 mg, which may contribute to umami taste and enhance palatability. Glutamate also serves as a key excitatory neurotransmitter and has been implicated in learning and memory processes [20]. Threonine was also remarkably elevated (707.011 µg/100 mg free; 992.435 µg/100 mg total), suggesting potential gut health and immunological benefits.

Additionally, the presence of asparagine (43.827 µg/100 mg free; 219.935 µg/100 mg total) and methionine (44.396 µg/100 mg free; 170.598 µg/100 mg total) in *S. kurzii* further emphasizes its nutritional value. Asparagine plays a role in protein synthesis and central nervous system development, while methionine supports liver function and acts as a precursor to S-adenosylmethionine, a universal methyl donor [21].

The essential branched-chain amino acids (BCAAs), valine, isoleucine, and leucine, were also detected in both plants. These are critical for muscle metabolism, protein synthesis, and the prevention of muscle wasting, particularly under conditions of stress or malnutrition [22]. Although present in moderate quantities (e.g., leucine: 62.631 µg/100 mg in *H. cordata* total; 11.119 µg/100 mg in *S. kurzii* total), their presence across both samples reflects the completeness of the amino acid profiles

The data confirm that both *H. cordata* and *S. kurzii* are valuable sources of essential and non-essential amino acids, many of which are linked to antioxidant, immunomodulatory, neuroprotective, and metabolic regulatory functions. These findings support their ethnobotanical use as traditional vegetables and underline their potential application as nutraceutical ingredients in functional food development, especially for communities in Northeast India that rely on wild plant resources.

Statistical Analysis and Correlation Study

The statistical evaluation of amino acid profiles in *H. cordata* and *S. kurzii* revealed significant differences in their free and total amino acid contents. A one-way ANOVA was performed to assess the overall variance among four amino acid groups: free and total amino acids from *H. cordata* and *S. kurzii*. The ANOVA test yielded a highly significant result ($F = 6.06$, $p = 0.0016$), indicating that at least one of the groups differed significantly in mean amino acid content.

To further explore group-wise differences, Tukey's Honest Significant Difference (HSD) post-hoc test was conducted. The test showed that the total amino acid content of *S. kurzii* was significantly higher than the free amino acid content of *H. cordata* ($p < 0.05$). Similarly, significant differences were also observed between *S. kurzii* total and *S. kurzii* free, as well as between *S. kurzii* total and *H. cordata* total contents. However, the difference between *H. cordata* free and *H. cordata* total was not statistically significant, suggesting relatively moderate variation within this species.

Pearson correlation analyses provided further insights into interrelationships between amino acid types. A very strong positive correlation was observed between free and total amino acid content in both *H. cordata* ($r = 0.997$, $p < 0.0001$) and *S. kurzii* ($r = 0.999$, $p < 0.0001$), suggesting that the free amino acid content strongly predicts the total content in each plant. A strong correlation was also found between the free amino acid contents of *H. cordata* and *S. kurzii* ($r = 0.87$, $p < 0.0001$), indicating a comparable pattern of amino acid distribution across species. Moreover, total amino acid contents between the two species also exhibited strong correlation ($r = 0.89$, $p < 0.0001$), further supporting the notion of similarity in biochemical composition.

These findings highlight significant quantitative and relational differences in amino acid content between the studied wild edible plants, suggesting species-specific biochemical profiles that may relate to their differing nutritional and medicinal potentials.

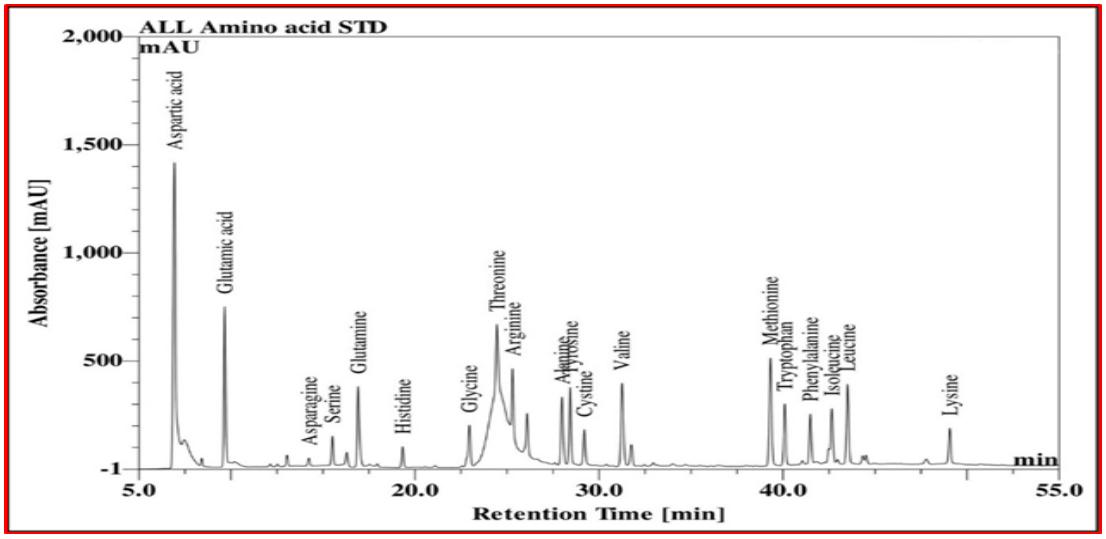

Figure 1: HPLC chromatogram of standard amino acids

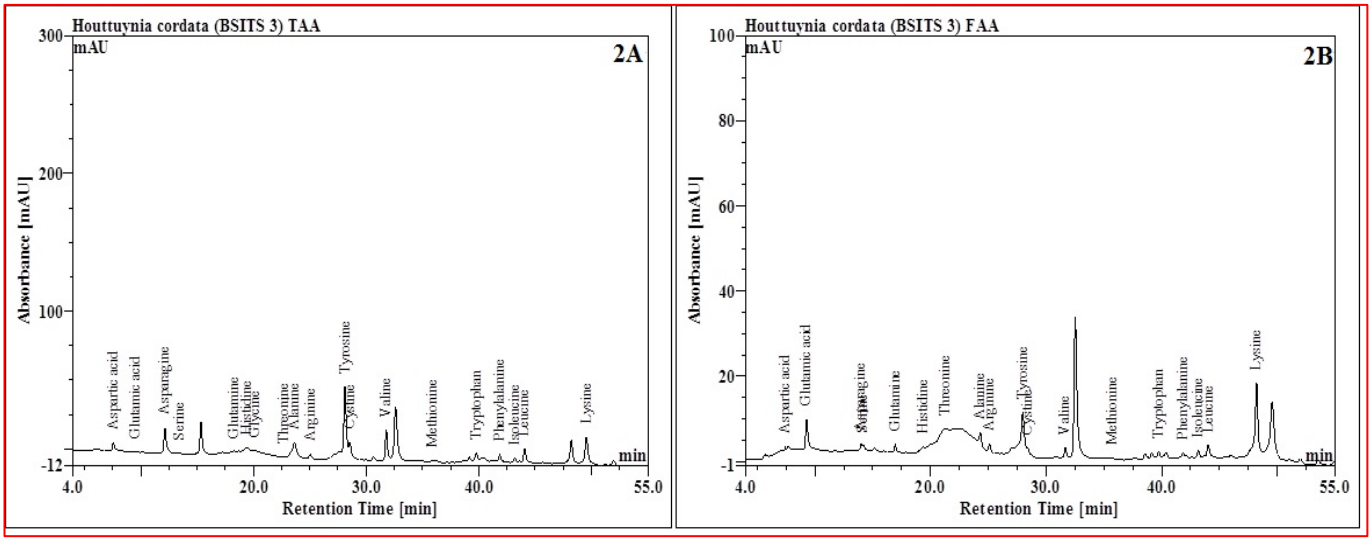

Figure 2: HPLC chromatogram of total (2A) and free (2B) amino acid in *H. cordata*

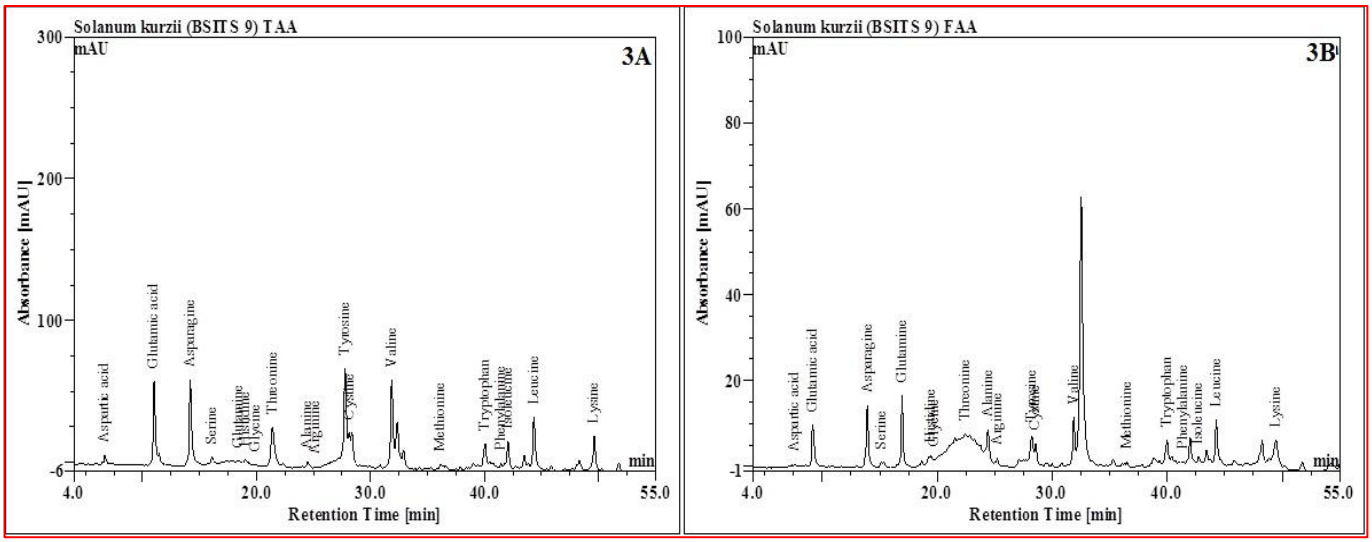

Figure 3: HPLC chromatogram of total (3A) and free (3B) amino acid in *S. kurzii*

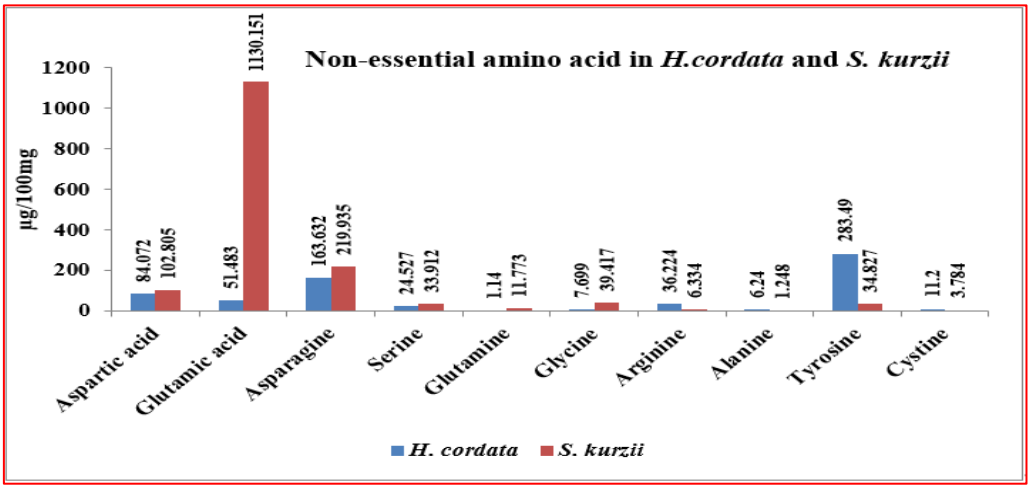

Figure 4: Comparative analysis of non-essential amino acid in *H. cordata* and *S. kurzii*

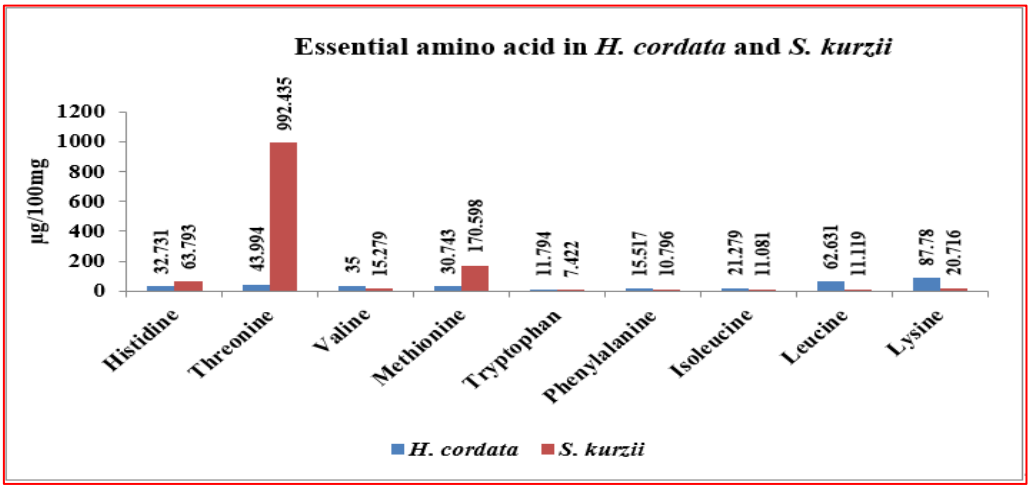

Figure 5: Comparative analysis of essential amino acid in *H. cordata* and *S. kurzii*

Table 1: Estimation of individual amino acid by HPLC

Name	*Houttuynia cordata*		*Solanum kurzii*	
	Free amino acid (µg/100mg)	**Total amino acid (µg/100mg)**	**Free amino acid (µg/100mg)**	**Total amino acid (µg/100mg)**
Aspartic acid	39.671±3.43	84.072±2.87	8.465± 0.78	102.805±9.88
Glutamic acid	29.544±1.78	51.483±4.08	183.262±11.76	1130.151±8.79
Asparagine	3.932±0.68	163.632±11.98	43.827± 2.64	219.935±5.68
Serine	11.614±1.12	24.527±1.59	28.561± 1.99	33.912±3.97
Glutamine	0.343±0.08	1.140±0.67	3.821±0.56	11.773±3.91
Histidine	26.882±1.63	32.731±3.97	29.040±2.98	63.793±2.55
Glycine	Not detected	7.699±0.55	1.308±0.78	39.417±3.86
Threonine	31.905±3.58	43.994±5.23	707.011±11.76	992.435±11.85
Arginine	1.927±0.58	36.224±1.19	3.917±0.98	6.334±0.48
Alanine	2.876±0.99	6.240±0.89	0.441±0.09	1.248±0.57
Tyrosine	76.548±2.79	283.490±12.57	2.801±0.77	34.827±3.27
Cystine	3.088±0.81	11.200±2.54	2.040±0.62	3.784±0.75
Valine	6.484±0.47	35.000±3.77	1.421±0.39	15.279±2.97
Methionine	14.577±1.44	30.743±2.89	44.396±1.67	170.598±8.94
Tryptophan	9.391±0.91	11.794±1.08	2.205±0.87	7.422±0.64
Phenylalanine	2.971±0.66	15.517±2.98	1.343±0.93	10.796±2.64
Isoleucine	0.596±0.08	21.279±3.09	0.916±0.08	11.081±6.11
Leucine	0.931±0.09	62.631±4.11	3.295±0.38	11.119±2.09
Lysine	51.248±2.11	87.780±6.08	7.716±0.49	20.716±2.97
Total (µg/mg)	**3.14**	**10.11**	**10.75**	**28.87**

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.

CONCLUSION

The present study provides a comprehensive comparative analysis of individual amino acid profiles in two wild edible plants from the North-East region of India, *H. cordata* and *S. kurzii*, using HPLC. The results reveal notable interspecies variation in both free and total amino acid contents. *Solanum kurzii* exhibited substantially higher concentrations of essential amino acids such as glutamic acid, threonine, methionine, and lysine, particularly in its total amino acid fraction, highlighting its superior nutritional potential. On the other hand, *Houttuynia cordata* showed higher levels of tyrosine and lysine in the free amino acid form, which are known for their roles in neurotransmitter synthesis and immune function, respectively. Statistical analyses, including one-way ANOVA and Pearson correlation, confirmed the significance of the observed differences, indicating distinct biochemical profiles between the two species. The strong correlations between free and total amino acid contents within each plant suggest consistent amino acid accumulation patterns.

These findings underscore the nutritional importance of wild edible plants as alternative sources of essential amino acids. They may serve as valuable dietary supplements, especially in regions with limited access to conventional protein sources. Additionally, the amino acids identified in these plants are associated with a range of health benefits, including anti-inflammatory, immune-modulatory, and neuroprotective functions. Therefore, *H. cordata* and *S. kurzii* may be promoted not only as food but also for their functional and nutraceutical properties. Further studies on bioavailability and clinical efficacy are recommended to validate their health-promoting potential.

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Author Contributions

Basundhara Pillai: Experimental Work Carried Out. Tapan Seal: Designed the study, Drafted the Manuscript, Statistical Analysis and Interpreted the Results

Abbreviation

HPLC: High Performance Liquid Chromatography, WEPs: Wild edible plants, OPA: o-Phthalaldehyde, $Na_2B_4O_7{\cdot}10H_2O$: Sodium tetraborate decahydrate, Na_2HPO_4: Sodium phosphate dibasic, DAD: Diode array detector, PCA: Principal Component Analysis, SEM: Standard Error Mean.

Conflict of interest

The authors declared no conflict of interest.

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