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Amino acid composition and nutritional significance of *Oenanthe Linearis* wall. ex DC: A wild leafy vegetable from Meghalaya

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Abstract
Oenanthe linearis Wall. ex DC., locally known as Jatira in Meghalaya, is a traditional wild leafy vegetable valued for its health benefits and unique flavor. This study presents a comprehensive evaluation of its amino acid profile using both ninhydrin-based colorimetric and HPLC techniques. The total amino acid content was 91.16 µg/mg, with 10.92 µg/mg present as free amino acids and 80.24 µg/mg as bound forms. Notably, threonine (1033.76 µg/100 mg), aspartic acid (122.92 µg/100 mg), and asparagine (119.88 µg/100 mg) were predominant, underscoring its high nutritional potential. Essential amino acids including histidine, lysine, valine, and threonine, alongside bioactive non-essential amino acids such as glutamic acid and serine, contribute to the plant's therapeutic promise. The amino acid profile supports its roles in protein synthesis, immune modulation, neurotransmission, and metabolic regulation. The predominance of bound amino acids further suggests enhanced bioavailability upon digestion. These findings highlight O. linearis as a valuable component in traditional diets and a promising candidate for functional food development and nutritional security in resource-limited regions.

Keywords: *Oenanthe linearis*, wild edible plant, amino acid profile, HPLC,

Introduction

Amino acids are the fundamental building blocks of proteins and are essential to virtually every biological process in plants, animals, and humans. Of the 20 standard amino acids, nine are considered essential for humans because they cannot be synthesized endogenously and must be obtained through the diet [20]. These include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Amino acids play critical roles beyond protein synthesis; they serve as precursors for neurotransmitters, hormones, and enzymes, regulate immune responses, and participate in metabolic pathways such as the urea cycle and gluconeogenesis [21]. Therefore, the availability of a balanced amino acid profile in food is vital for maintaining health and preventing nutritional deficiencies, especially in regions where animal-derived protein sources are limited or unaffordable.

Wild edible plants (WEPs) have gained considerable attention as underutilized but highly nutritious resources with the potential to address food and nutrition insecurity, particularly in rural and tribal communities. These plants are often rich in micronutrients, fiber, antioxidants, and essential amino acids, making them valuable complements or alternatives to conventional crops [2]. Unlike cultivated vegetables, many wild leafy greens are resilient to environmental stresses, require minimal inputs for growth, and are naturally adapted to local ecosystems, contributing to agro-biodiversity and ecological sustainability [7].

WEPs are especially important in traditional food systems, where they serve as critical sources of protein and micronutrients during periods of seasonal food scarcity [5]. Several ethnobotanical studies have reported the presence of diverse amino acids in wild species consumed by indigenous populations in Asia, Africa, and Latin America. For instance, wild leafy vegetables such as *Chenopodium album*, *Amaranthus spp.*, and *Portulaca oleracea* have been found to contain substantial levels of both essential and non-essential amino acids, demonstrating their potential in enhancing dietary amino acid intake in plant-based diets [11, 6].

In the context of Northeast India, particularly Meghalaya, the rich biodiversity supports a wide array of wild edible plants traditionally used by local communities. These species are not only culturally significant but also serve as functional foods with therapeutic properties. However, despite their traditional usage, many WEPs remain scientifically underexplored, especially regarding their detailed amino acid composition. A systematic biochemical evaluation of these plants is essential to understand their nutritional roles and promote them as sustainable dietary resources in public health nutrition and food diversification strategies [16].

The present study focuses on *Oenanthe linearis* Wall. ex DC., commonly known as Jatira in Meghalaya, a wild leafy vegetable widely consumed by indigenous tribes. Although appreciated for its refreshing taste and medicinal attributes, limited scientific data exist on its biochemical constituents. This study aims to analyze and characterize its free and total amino acid composition using ninhydrin assay and High-Performance Liquid Chromatography (HPLC), with the goal of elucidating its nutritional value and exploring its potential role in addressing protein-energy malnutrition in resource-constrained settings.

Material and methods

1. 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).

2. Plant materials

Oenanthe linearis were collected from north-eastern zone of India. The plant samples were authenticated at our office and voucher specimens (BSITS 5) 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.

3. Determination of amino acids

3.1 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. was employed [17]. Sample preparation and analysis of plant materials: (a) for free amino acids: a portion of powdered raw material (1gm) was extracted with 5ml aqueous solution of 1 N hydrochloric acid was added and kept in an ultrasonic bath at room temperature for 3 h and (b) for the sum of amino acids: a sample was extracted with an aqueous solution of 6 N hydrochloric acid was added and placed in a thermostat at 110°C. The hydrolysis was carried out for 24 h.

Then, 2 ml of the centrifuged extract/hydrolyzate was evaporated, washing 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 (-NH2) of amino acids, resulting in the formation of a purple-colored 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 [17].

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

3.2.1 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.

3.2.2 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) [8]. 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.

3.2.3 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 [4]. 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 [4].

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.

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), were used. SPSS software (version 11.0 for Windows) was used to conduct statistical analysis.

In this study, a simple and rapid HPLC method was optimized and validated for the determination of free and total amino acids in *Oenanthe linearis*. The method enabled the quantification of 19 amino acids within a total run time of 62 minutes, utilizing a high-resolution C18 column (250 × 4.6 mm, 5 µm) and an optimized separation gradient. Fig. 1 presents the standard chromatogram of the 19 free amino acids following pre-column derivatization with o-phthalaldehyde (OPA).

Results

Table 1: Estimation of free and total amino acids in *Oenanthe linearis* by HPLC

Name	Free amino acid (µg/100mg)	Total amino acid (µg/100mg)	Bound amino acid (µg/100mg)
Aspartic acid	85.231±1.75	122.922±9.87	37.691±2.87
Glutamic acid	21.287±2.66	31.460±2.09	10.173±4.34
Asparagine	7.227±0.74	119.881±5.97	112.654±9.05
Serine	13.803±1.11	33.366±1.05	19.563±4.08
Glutamine	0.593±0.55	9.783±0.97	9.190±0.47
Histidine	18.038±1.04	52.635±1.64	34.596±2.95
Glycine	Not detected	23.858±2.86	23.858±2.86
Threonine	47.352±1.87	1033.757±15.87	986.404±15.08
Arginine	0.098±0.004	0.900±0.07	0.801±0.06
Alanine	0.935±0.08	22.338±1.54	21.403±1.11
Tyrosine	0.268±0.09	3.794±0.72	3.526±0.64
Cystine	0.085±0.005	1.390±0.88	1.306±0.46
Valine	0.544±0.06	9.497±0.63	8.952±0.78
Methionine	0.260±0.08	1.674±0.48	1.414±0.97
Tryptophan	0.242±0.04	1.050±0.66	0.808±0.05
Phenylalanine	2.331±0.77	4.985±0.65	2.654±0.34
Isoleucine	0.172±0.03	2.352±0.59	2.180±0.67
Leucine	0.382±0.06	4.746±0.38	4.364±0.54
Lysine	2.595±0.59	10.376±1.84	7.781±0.77
Total (µg/mg) by HPLC	2.01	14.91	12.900
Total (Ninhydrin assay) µg/mg	10.92	91.16	80.24

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 amino acid composition of *Oenanthe linearis* was analyzed using both colorimetric (ninhydrin assay) and chromatographic (HPLC) techniques (Fig. 2-3). The ninhydrin assay revealed a free amino acid content of 10.92 µg/mg and a total amino acid content of 91.16 µg/mg, indicating a high proportion of bound amino acids, which become bioavailable upon digestion. Estimation of individual amino acids by HPLC further corroborated these findings. The total concentration of amino acids identified by HPLC was 14.91 µg/mg, of which 2.01 µg/mg were free amino acids and 12.90 µg/mg were present in bound form.

Among the non-essential amino acids, aspartic acid was the most abundant, with a total concentration of 122.92 µg/100 mg, of which 85.23 µg/100 mg were in the free form and 37.69 µg/100 mg bound. Similarly, asparagine exhibited high levels (119.88 µg/100 mg total), largely in the bound form (112.65 µg/100 mg), with only 7.23 µg/100 mg detected as free. Threonine, an essential amino acid, was the most predominant overall, with a striking total of 1033.76 µg/100 mg, comprising 47.35 µg/100 mg free and 986.40 µg/100 mg bound, highlighting its significance in nutritional and functional aspects.

Other notable amino acids include histidine (52.64 µg/100 mg total, 18.04 µg/100 mg free), serine (33.37 µg/100 mg total, 13.80 µg/100 mg free), and glutamic acid (31.46 µg/100 mg total, 21.29 µg/100 mg free). Amino acids such as glycine, alanine, valine, and phenylalanine were present in moderate amounts, while several others, methionine, cystine, tryptophan, tyrosine, and isoleucine, were found in trace but nutritionally relevant quantities. The levels of branched-chain amino acids (BCAAs), including leucine (4.75 µg/100 mg), isoleucine (2.35 µg/100 mg), and valine (9.50 µg/100 mg), although modest, contribute to the overall quality of protein present in the plant.

The data clearly indicate that *O. linearis* contains a diverse and balanced amino acid profile, with a predominance of bound forms, suggesting enhanced release and utilization during digestion. This underscores its potential as a sustainable source of dietary amino acids, particularly in regions dependent on wild edible plants for nutrition.

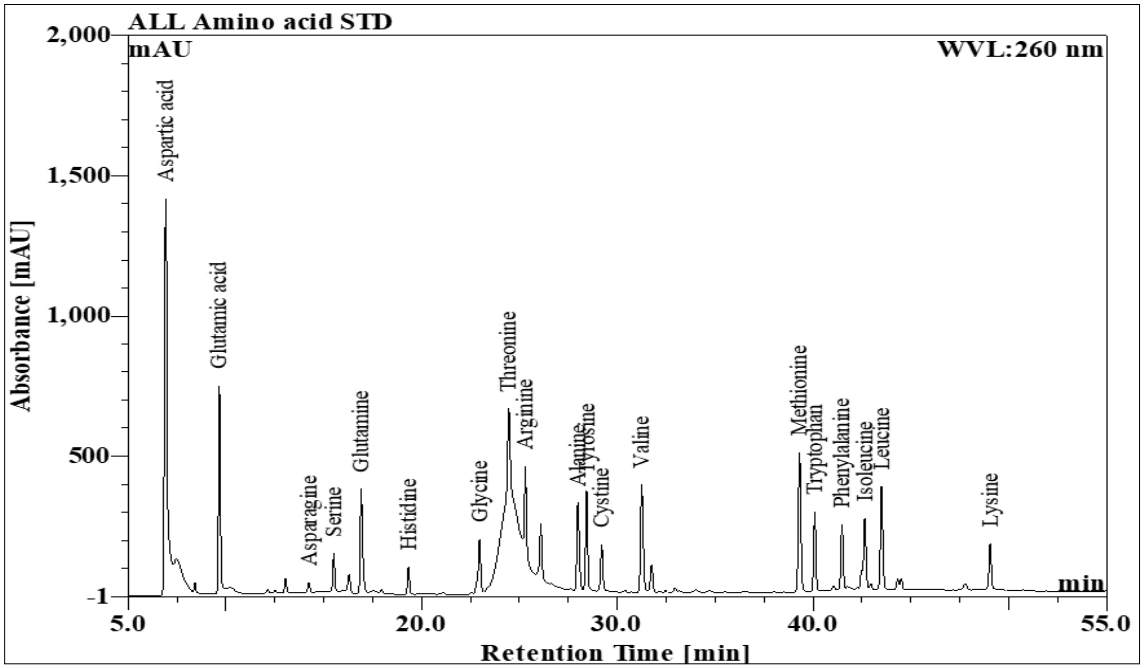

Fig. 1: HPLC chromatogram of standard amino acid

Discussion

Oenanthe linearis Wall. ex DC, commonly referred to as Jatira in Meghalaya, is a wild leafy vegetable traditionally consumed by indigenous communities for its refreshing flavor and health-promoting properties. A comprehensive analysis of its amino acid composition, employing both colorimetric (ninhydrin assay) and chromatographic (HPLC) techniques, revealed a total amino acid content of 91.16 µg/mg, with 10.92 µg/mg as free amino acids and 80.24 µg/mg as bound amino acids. This suggests a predominance of proteinogenic compounds that become bioavailable post-digestion or processing, enhancing the plant's nutritional utility.

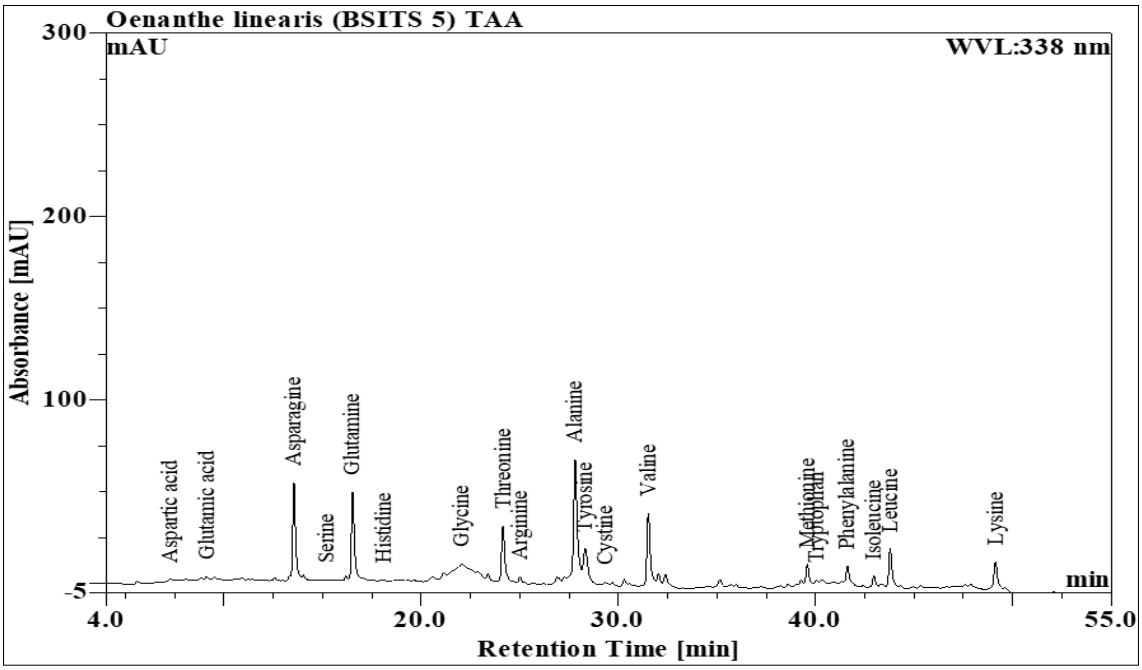

Fig. 2: HPLC chromatogram of total amino acid

Among the non-essential amino acids, aspartic acid (122.92 µg/100 mg total) and asparagine (119.88 µg/100 mg total) were notably abundant. Aspartic acid plays a central role in the urea cycle and energy metabolism, facilitating the synthesis of other amino acids and nucleotides (Wu, 2009). Asparagine is vital for nitrogen transport and stress response in plants and may modulate immune functions in humans [9]. The high asparagine content could reflect *O. linearis*' adaptive resilience to environmental stresses, as well as its potential therapeutic value during physiological stress or malnutrition.

The most prominent essential amino acid was threonine, with a remarkably high total content of 1033.76 µg/100 mg, of which 986.40 µg/100 mg was in bound form. Threonine is indispensable for mucin production in the gastrointestinal tract, immune system function, and protein synthesis,

making it particularly important in vegetarian diets where animal protein is scarce [19].

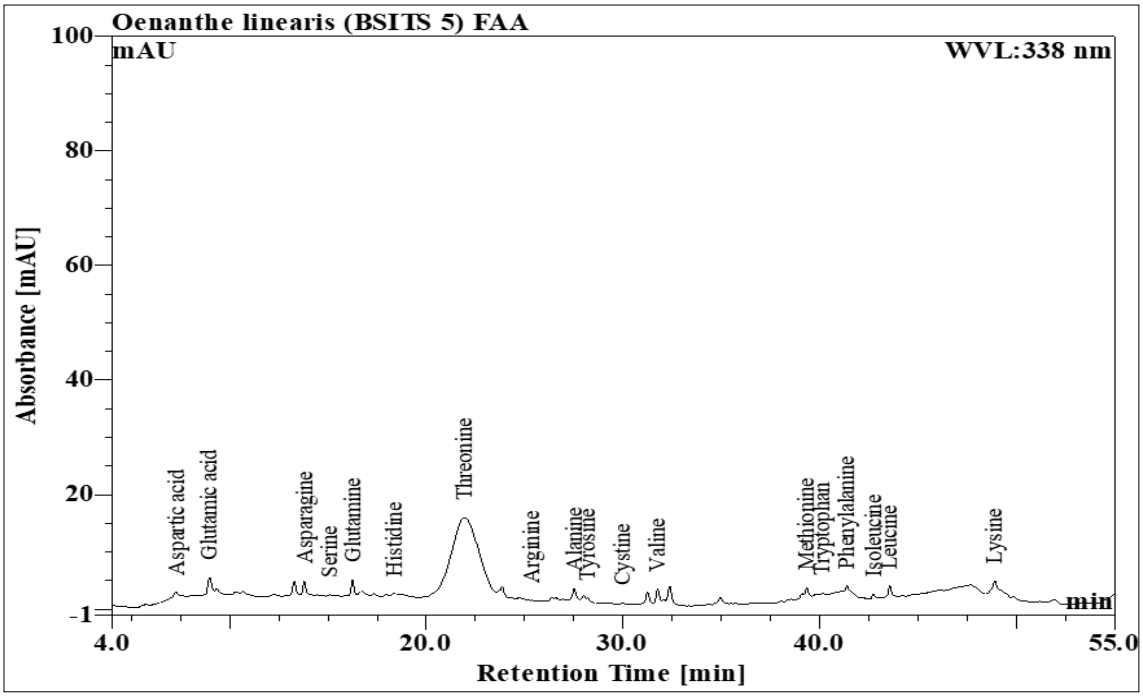

Fig.3: HPLC chromatogram of free amino acid

Histidine (52.64 µg/100 mg total; 34.60 µg/100 mg bound) was also present in appreciable amounts. It contributes to hemoglobin structure, metal ion chelation, and histamine biosynthesis, playing a crucial role in immune and inflammatory responses [1]. Additionally, glutamic acid (31.46 µg/100 mg total), a key neurotransmitter and metabolic intermediate, underscores the plant's potential neuroprotective role [15].

Other amino acids such as serine (33.37 µg/100 mg), glycine (23.86 µg/100 mg), and alanine (22.34 µg/100 mg) contribute to diverse metabolic pathways. Serine is essential for phospholipid and sphingolipid biosynthesis [3], glycine serves in collagen synthesis and as a cytoprotective agent [13], and alanine supports glucose metabolism.

Although glutamine was found in moderate amounts (9.78 µg/100 mg total), its known benefits for gut health and immune modulation add to the functional value of *O. linearis* [12]. Among essential amino acids, lysine (10.38 µg/100 mg), valine (9.50 µg/100 mg), and phenylalanine (4.99 µg/100 mg) were identified. Lysine is critical for calcium absorption, collagen formation, and tissue repair [21], while valine and phenylalanine contribute to muscle metabolism and neurotransmitter synthesis, respectively.

The presence of branched-chain amino acids (BCAAs), isoleucine, leucine, and valine, although in modest quantities, is significant given their role in muscle protein synthesis, energy regulation, and recovery, especially under conditions of physical exertion or malnutrition [18].

Sulfur-containing amino acids, such as methionine (1.67 µg/100 mg) and cystine (1.39 µg/100 mg), were found in trace levels. Methionine is indispensable for methyl group donation and antioxidant defense through glutathione synthesis [10], while cystine contributes to protein structure and redox homeostasis. Tryptophan (1.05 µg/100 mg), a serotonin precursor, though present in small amounts, is essential for sleep, mood regulation, and cognitive health [14].

Despite the relatively lower free amino acid content (2.01 µg/mg), the dominance of bound amino acids (12.90 µg/mg) suggests that *O. linearis* can effectively serve as a dietary source of bioavailable amino acids post-ingestion. This highlights its relevance in functional nutrition and traditional diets, particularly in nutritionally vulnerable and rural populations dependent on wild edible plants for protein and micronutrient intake.

In conclusion, the amino acid profile of *Oenanthe linearis* supports its classification as a nutritionally rich leafy vegetable with therapeutic promise. Its composition aligns with essential metabolic, structural, and regulatory functions, warranting its promotion in dietary diversification strategies and potential integration into nutraceutical and public health nutrition frameworks.

Correlation study

To understand the biochemical relationships among the different amino acid fractions in *Oenanthe linearis*, a correlation analysis was performed between free amino acids, bound amino acids, and total amino acids. The total amino acid content is the sum of the free and bound forms, and studying the correlation between these components helps identify which amino acids contribute most significantly to the total pool and how amino acid distribution may influence nutritional bioavailability.

A moderate positive correlation was observed between the free amino acid content and the total amino acid content across all amino acids (Pearson correlation coefficient $r = 0.666$, $R^2 = 0.443$, $p < 0.01$). This indicates that, although free amino acids contribute to the total pool, they are not the dominant form for most amino acids in *O. linearis*. This is expected, as amino acids typically exist in protein-bound forms and are released upon digestion or processing.

A strong and statistically significant positive correlation was found between bound amino acid content and total amino acid content ($r = 0.999$, $R^2 = 0.998$, $p < 0.001$). This nearly

perfect correlation confirms that bound amino acids overwhelmingly contribute to the total amino acid content, suggesting that protein hydrolysis during digestion will play a key role in nutrient availability. The significance level indicates an extremely reliable association.

A weak correlation was noted between free and bound amino acid concentrations ($r = 0.258$, $R^2 = 0.067$, $p > 0.05$), which was statistically non-significant. This suggests that the presence of free amino acids is not directly proportional to the bound forms, reflecting independent regulation or metabolic roles in plant physiology. Free amino acids may fluctuate with stress, storage, or metabolic demand, while bound forms are more conserved within structural or enzymatic proteins.

The very high correlation ($r = 0.999$) between bound and total amino acids signifies that the nutritional potential of *O. linearis* lies predominantly in its bound amino acid reservoir, which can be liberated upon cooking or digestion.

The free amino acid pool, although nutritionally important for immediate absorption and bioactivity, constitutes a minor portion and shows low correlation with the bound form, implying distinct physiological roles.

The moderate correlation between free and total amino acids indicates that while free amino acids are contributors to total amino acid content, they do not determine it.

The correlation study underscores the nutritional richness of *Oenanthe linearis*, emphasizing its potential as a proteinogenic, amino acid-dense leafy vegetable. The dominance of bound amino acids, coupled with their significant contribution to total content, supports its value as a dietary protein source, especially in regions relying on wild edibles for nutritional security. The poor correlation between free and bound fractions also highlights the importance of processing or digestion in releasing the full amino acid potential of this species.

Conclusion

The present study provides a comprehensive assessment of the amino acid composition of *Oenanthe linearis* Wall. ex DC., a wild leafy vegetable traditionally consumed by indigenous communities in Meghalaya. Using both ninhydrin assay and High-Performance Liquid Chromatography (HPLC), the findings revealed a high total amino acid content (91.16 µg/mg), with a predominance of bound amino acids (80.24 µg/mg), and a smaller fraction present in free form (10.92 µg/mg). Among individual amino acids, threonine, aspartic acid, and asparagine were notably abundant, with threonine being the most prominent essential amino acid.

The correlation analysis highlighted a strong and significant association between bound and total amino acid content ($r = 0.999$), reinforcing the nutritional importance of bound protein fractions, which become bioavailable upon digestion or thermal processing. The weak correlation between free and bound amino acids indicates distinct physiological roles, with free amino acids potentially reflecting metabolic status or stress responses. Overall, the amino acid profile of Oenanthe linearis underscores its high nutritional quality, functional food potential, and suitability as a plant-based protein source, particularly in nutritionally vulnerable regions. Its inclusion in traditional diets contributes to dietary diversity, protein intake, and public health nutrition. These findings support the promotion of *O. linearis* as a valuable component of food security strategies and encourage its integration into functional and nutraceutical food development.

Declaration of competing interest

The authors have no conflict of interest to declare.

Author contributions

Tapan Seal: Designed the study, Drafted the manuscript, Statistical analysis and Interpreted the results
Basundhara Pillai: Experimental work carried out
Sudeshna Datta: Experimental work carried out

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References

1. Brosnan ME, Brosnan JT, Histidine metabolism function. Journal of Nutrition,2020:150:2570–2575. doi: 10.1093/jn/nxaa079.
2. Burlingame B, Charrondiere R, Mouille B. Food composition is fundamental to the cross-cutting initiative on biodiversity for food and nutrition. Journal of Food Composition and Analysis,2009:22(5):361–365. doi: 10.1016/j.jfca.2008.11.007.
3. de Koning TJ, Snell K, Duran M, Berger R, Poll-The BT, Surtees R. *et al* L-serine in disease development. Biochemical Journal,2003:371(3):653–661.
4. de Sousa Fontes VM, Colombo Pimentel T, Martins da Silva AB, Suely Madruga M, Magnani M, Dos Santos Lima M. *et al* An improved method for determining free amino acids by RP-HPLC/DAD with o-phthalaldehyde derivatization: method evaluation in beers and wines. Food Chemistry, 2024, 435, 137591. doi: 10.1016/j.foodchem.2023.137591.
5. Ghosh-Jerath S, Singh A, Magsumbol MS, Lyngdoh T, Kamboj P, Goldberg G. *et al* Contribution of indigenous foods towards nutrient intakes and nutritional status of women in the Santhal tribal community of Jharkhand, India. Public Health Nutrition,2016:19(12):2256–2267. doi: 10.1017/S1368980016000318.
6. Grivetti LE, Ogle BM, Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutrition Research Reviews,2000:13(1):31–46. doi: 10.1079/095442200108728990.
7. Heywood VH, Overview of agricultural biodiversity and its contribution to nutrition and health. In: Fanzo J, Hunter D, Borelli T, Mattei F eds. Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health. Routledge, 2013, 35–67.
8. Hu Z, Li L, Yuan Y, Yue T. Ultrasensitive simultaneous determination of twenty-one amino acids and amines in culture media, red wine and beer. Food Chemistry,2014:158:56–65. doi: 10.1016/j.foodchem.2014.02.098.
9. Lea PJ, Sodek L, Parry MAJ, Shewry PR, Halford NG, Asparagine in plants. Annals of Applied Biology,2007:150(1):1–26.
10. Lu SC, Glutathione synthesis. Biochimica et Biophysica Acta - General

Subjects,2013:1830(5):3143–3153. doi: 10.1016/j.bbagen.2012.09.008.
11. Nesamvuni C, Steyn NP, Potgieter MJ, Nutritional value of wild, leafy plants consumed by the Vhavenda. South African Journal of Science,2001:97(12):51–54.
12. Newsholme P. Glutamine metabolism: nutritional clinical significance. Journal of Nutrition,2003:133(61):2040–2045.
13. Razak MA, Begum PS, Viswanath B, Rajagopal S. Multifarious beneficial effect of nonessential amino acid, glycine: a review. Oxidative Medicine Cellular Longevity, 2017, 1716701.
14. Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM, L-tryptophan: basic metabolic functions, behavioral research therapeutic indications. International Journal of Tryptophan Research,2009:2:45–60.
15. Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC, Glutamate metabolism in the brain focusing on astrocytes. Advances in Neurobiology,2014:11:13–30. doi: 10.1007/978-3-319-08894-5_2.
16. Seal T, Chaudhuri K, Pillai B, Chakrabarti S, Auddy B, Mondal T. *et al* Wild edible plants of Meghalaya State in India: nutritional, minerals, antinutritional, vitamin content and toxicity studies. Pharmacognosy Magazine,2020:16(1):142–151. doi: 10.4103/pm.pm_369_19.
17. Shafaei A, Ab Halim NH, Zakaria N, Ismail Z. Analysis of free amino acids in different extracts of *Orthosiphon stamineus* leaves by high-performance liquid chromatography combined with solid-phase extraction. Pharmacognosy Magazine,2017:13(l4):385–391. doi: 10.4103/0973-1296.216337.
18. Shimomura Y, Yamamoto Y, Bajotto G, Sato J, Murakami T, Shimomura N, *et al* Nutraceutical effects of branched-chain amino acids on skeletal muscle. Journal of Nutrition,2006:136(2):29S–532. doi: 10.1093/jn/136.2.529S.
19. Tang Q, Tan P, Ma N, Ma X. Physiological functions of threonine in animals beyond nutrition metabolism. Nutrients,2021:13(8):2592.
20. Wu G. Amino acids metabolism, functions, nutrition. Amino Acids,2009:37(1):1–17. doi: 10.1007/s00726-009-0269-0.
21. Wu G. Functional amino acids in nutrition and health. Amino Acids,2013:45(3):407–411. doi: 10.1007/s00726-013-1500-6.