

## Comprehensive analysis of free and total amino acids in *Chloroxylon swietenia* DC.: Implications for nutritional and functional food potential

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### Abstract

Amino acid profiling is a fundamental approach for assessing the nutritional quality and functional significance of wild edible plants. The present investigation evaluated the free amino acids (FAA), total amino acids (TAA), and bound fractions of *Chloroxylon swietenia* to determine its protein nutritional potential. Quantitative analysis ( $\mu\text{g}/100\text{ mg}$  dry weight) revealed substantial variation among individual amino acids and between their free and total forms. Among the free amino acids, glycine ( $43.616\ \mu\text{g}/100\text{ mg}$ ) was predominant, followed by asparagine ( $14.615\ \mu\text{g}/100\text{ mg}$ ), histidine ( $9.663\ \mu\text{g}/100\text{ mg}$ ), and lysine ( $8.784\ \mu\text{g}/100\text{ mg}$ ). The presence of free essential amino acids such as histidine and lysine enhance the immediate nutritional relevance of the plant. Serine and methionine were not detected in free form, suggesting rapid metabolic utilization or incorporation into proteins. Total amino acid content was markedly higher than the free fraction, indicating substantial protein-bound reserves. Alanine exhibited the highest total concentration ( $150.005\ \mu\text{g}/100\text{ mg}$ ), followed by cystine ( $120.509\ \mu\text{g}/100\text{ mg}$ ), glutamine ( $86.289\ \mu\text{g}/100\text{ mg}$ ), glycine ( $84.834\ \mu\text{g}/100\text{ mg}$ ), methionine ( $73.080\ \mu\text{g}/100\text{ mg}$ ), and arginine ( $69.987\ \mu\text{g}/100\text{ mg}$ ). Essential amino acids, including threonine, valine, isoleucine, leucine, lysine, phenylalanine, histidine, methionine, and tryptophan, were present in appreciable quantities, suggesting a relatively balanced amino acid spectrum. The bound fraction constituted the major proportion of most amino acids, particularly alanine, cystine, glutamine, methionine, and arginine, reflecting significant incorporation into structural and storage proteins. Overall, the findings indicate that *C. swietenia* possesses promising nutritional attributes and may serve as a valuable supplementary protein source among wild edible plants.

**Keywords:** *Chloroxylon swietenia*, wild edible plant, amino acid profiling, free amino acids (FAA), total amino acids (TAA), functional food potential

### Introduction

Wild edible plants (WEPs) have long played a significant role in the dietary and healthcare systems of indigenous communities across the globe, particularly in biodiversity-rich regions such as Northeast India. These plants serve as valuable sources of macro- and micronutrients and are rich in diverse bioactive compounds that contribute to health promotion and disease prevention (Rumicha *et al.*, 2025)<sup>[14]</sup>. In addition to vitamins, minerals, and phenolic constituents, amino acids represent one of the most fundamental biochemical components determining the nutritional quality of plant-based foods (Wu, 2009)<sup>[18]</sup>.

Amino acids are the primary building blocks of proteins and are indispensable for numerous physiological processes including enzymatic activity, hormone synthesis, neurotransmission, immune defense, and cellular repair mechanisms (Wu, 2009; Young and Pellett 1994)<sup>[18,19]</sup>. Based on nutritional classification, amino acids are categorized into essential and non-essential types. Essential amino acids cannot be synthesized by the human body and must be supplied through dietary intake, whereas non-essential amino acids, although synthesized endogenously, are crucial for maintaining metabolic homeostasis (Young and Pellett 1994)<sup>[19]</sup>. The evaluation of amino acid composition, therefore, provides critical insight into the protein quality and overall nutritional value of plant resources.

Furthermore, the assessment of both free and total amino acids enhances the understanding of a plant's functional and nutritional significance. Free amino acids are directly bioavailable and participate in rapid metabolic and physiological responses, while total amino acids, including

those incorporated into protein structures, reflect the plant's long-term protein nutritional contribution (Friedman 2004)<sup>[6]</sup>. Comprehensive amino acid profiling thus serves as an important biochemical parameter for evaluating wild edible plants as potential functional foods and nutraceutical resources.

*Chloroxylon swietenia* DC., belonging to the Rutaceae family, is a medium-sized deciduous tree attaining a height of 9–15 m with a girth of 50.0–80.0 cm and a spreading crown. The species is native to India and Sri Lanka and is commonly known as Ceylon Satinwood or East Indian Satinwood. It is widely recognized in traditional medicine for its diverse therapeutic applications. The Malasar tribes of Coimbatore district, Tamil Nadu, use leaf paste for the treatment of wounds, burns, cuts, skin diseases, fungal infections, and rheumatism. Various plant parts are traditionally employed in snakebite management. The stem bark is reputed for treating common cold, cough, ophthalmic infections, cataract, and wounds, and is used as an astringent. Dried stem bark, either alone or combined with sesame oil derived from *Sesamum indicum*, is applied externally to relieve itching. A paste prepared from leaves and roots is used orally and topically for headache relief. Despite its ethnomedicinal importance and traditional consumption, there is limited scientific information regarding the amino acid composition of *C. swietenia*. Considering its dual role as a wild edible and medicinal species, systematic investigation of its free and total amino acid profile is essential. Such profiling would provide scientific validation of its nutritional value, clarify its protein quality, and explore its potential as a supplementary source of essential amino acids. Moreover, understanding its

amino acid spectrum may contribute to the development of functional foods and nutraceutical formulations, thereby strengthening the scientific basis for its traditional utilization (Rumicha *et al.*, 2025; Wu, 2009; Young and Pellett 1994; Friedman 2004) <sup>[14,18,19,6]</sup>.

## Materials 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,  $\beta$ -mercaptoethanol were purchased from Sigma-Aldrich. HPLC-grade water, acetonitrile and methanol were purchased from Spectrochrom, India. Hydrochloric acid (HCl), sodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), and sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_4$ ) were purchased from Merck (Darmstadt, Germany).

### 2. Plant Materials

Plant materials *viz.* *Chloroxylon swietenia* were collected from the adjoining areas of Debrigarh Wildlife Sanctuary, Bargarh district, Odisha. The plant is called as *Bheru* in local dialect. The samples were taxonomically authenticated at our institute, and voucher specimen (BSITSMD 001) was 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.

### 3. Determination of Amino Acids

#### 3.1. 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 (Shafaei *et al.*, 2017) <sup>[15]</sup>.

Sample preparation and analysis of plant materials:

- 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.
- 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  $\mu\text{m}$  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 ( $-\text{NH}_2$ ) 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 (Shafaei *et al.*, 2017) <sup>[15]</sup>.

#### 3.2 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 Datta *et al.*, 2025 <sup>[3]</sup>. 50  $\mu\text{l}$  of the amino acid standard and samples (hydrolysed and non-hydrolysed) were mixed with 100  $\mu\text{l}$  of borate buffer (pH = 9.5) and 300  $\mu\text{L}$  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 Datta *et al.*, 2025 <sup>[3]</sup>. 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  $\mu\text{l}$  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 Datta *et al.*, 2025 <sup>[3]</sup>.

Before introducing the standard and working solutions into the HPLC apparatus, a filtration step was undertaken using a 0.45  $\mu\text{m}$  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.

### Results and Discussion

The amino acid composition of *Chloroxylon swietenia* was evaluated in terms of free amino acids (FAA), total amino acids (TAA), and bound fractions ( $\mu\text{g}/100$  mg dry weight). Significant quantitative variation was observed among

individual amino acids and between their free and protein-bound forms, reflecting both immediate metabolic availability and long-term nutritional potential. In present study, a simple and rapid HPLC method was optimized and validated for the determination of free and total amino acids in *Chloroxylon swietenia*. 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 and Fig. 2 showed the HPLC chromatogram for free and total amino acids after pre-column derivatization with o-phthalaldehyde (OPA). Table 1 presents the quantities of individual amino acids in *Chloroxylon swietenia*.

**Table 1:** Estimation of amino acid in *C. swietenia* by HPLC

	FAA	TAA	Bound
Amino acid	μg/100mg	μg/100mg	μg/100mg
Aspartic acid	1.490±0.97	13.311±1.55	11.821±0.89
Glutamic acid	7.075±0.65	31.841±3.98	24.767±2.97
Asparagine	14.615±1.11	30.482±5.97	15.867±2.34
Serine	ND	ND	ND
Glutamine	0.498±0.07	86.288±8.77	85.790±4.89
Histidine	9.663±0.64	18.013±2.56	8.350±1.99
Glycine	43.616±2.11	84.833±6.99	41.218±4.97
Threonine	2.548±0.49	21.635±2.98	19.087±2.16
Arginine	4.226±0.88	69.986±6.99	65.761±1.08
Alanine	3.697±0.72	150.005±11.34	146.309±11.43
Tyrosine	ND	ND	ND
Cystine	5.507±0.32	120.508±9.87	115.002±9.45
Valine	0.189±0.09	57.416±4.98	57.228±2.65
Methionine	0.000	73.079±3.78	73.080±5.88
Tryptophan	1.701±0.66	3.498±0.98	1.798±0.99
Phenylalanine	5.267±0.94	18.456±1.11	13.189±1.65
Isoleucine	0.902±0.08	11.615±2.96	10.714±2.87
Leucine	0.739±0.09	10.511±2.66	9.773±0.87
Lysine	8.784±0.32	26.767±1.48	17.984±1.76

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).

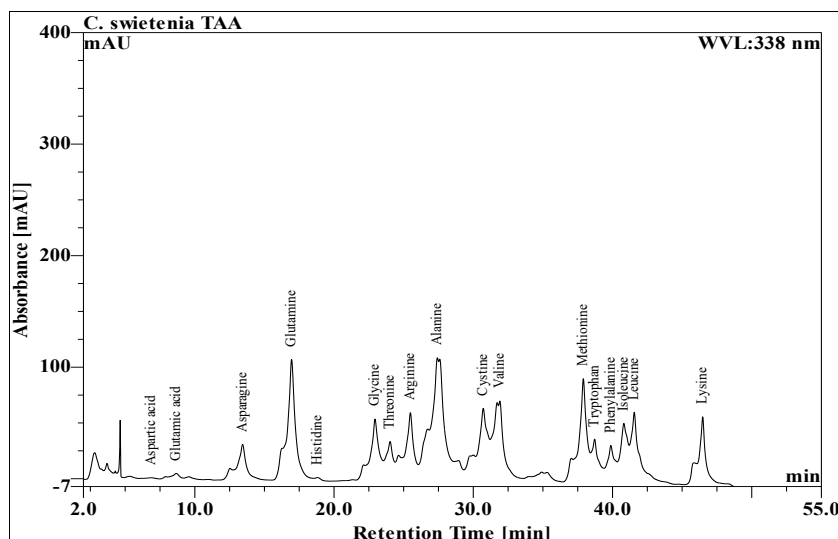
**ND: not detected**

**FAA: Free amino acid, TAA: Total amino acid**

The free amino acid pool was dominated by glycine (43.616 μg/100 mg), followed by asparagine (14.615 μg/100 mg), histidine (9.663 μg/100 mg), and lysine (8.784 μg/100 mg). Free amino acids serve as critical intermediates in nitrogen metabolism, stress responses, and osmotic regulation in plants (Forde and Lea 2007) [5]. The predominance of glycine aligns with findings in other medicinal plants such as *Moringa oleifera* and *Ocimum sanctum*, where glycine was also reported as one of the major free amino acids contributing to metabolic flexibility and stress resilience (Singh *et al.*, 2020, Chaudhary *et al.*, 2020) [16, 2]. Asparagine's relatively high concentration

suggests active nitrogen storage and transport, a pattern observed in medicinal species like *Withania somnifera* and *Asparagus racemosus* (Kohli *et al.*, 2023; Khanna *et al.*, 2006) [9, 8].

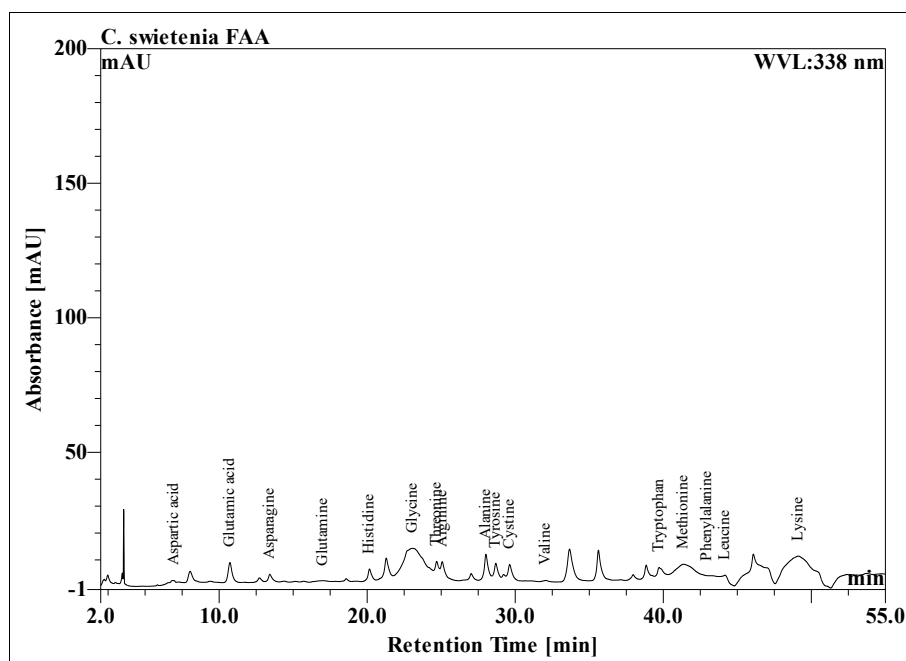
Free essential amino acids like histidine and lysine enhance immediate nutritional quality: histidine supports hemoglobin synthesis and neurotransmission, while lysine is vital for protein biosynthesis and immune modulation (Wu 2009) [18]. Serine and methionine were undetectable in free form, likely due to rapid incorporation into proteins or metabolic conversion. Similar absence of free serine was reported in leaf tissues of *Centella asiatica*, indicating species-specific amino acid utilization patterns (Kunjumon *et al.*, 2022) [10].



**Fig 1:** HPLC chromatogram of *C. swietenia* for total amino acids

Total amino acid content was substantially higher than free forms, consistent with reports from other medicinal plants where bound protein reserves dominate the amino acid pool (Ghosh Dasgupta *et al.*, 2014) [7]. Alanine exhibited the highest total concentration (150.005  $\mu\text{g}/100\text{ mg}$ ), followed by cystine (120.509  $\mu\text{g}/100\text{ mg}$ ), glutamine (86.289  $\mu\text{g}/100\text{ mg}$ ), and glycine (84.834  $\mu\text{g}/100\text{ mg}$ ). Compared to *M. oleifera* leaves (reported alanine  $\sim 102\text{ }\mu\text{g}/100\text{ mg}$ ) and *Ocimum basilicum* ( $\sim 90\text{--}110\text{ }\mu\text{g}/100\text{ mg}$ ), *C. swietenia* showed higher alanine and cystine levels, indicating a potentially richer profile of structural and sulfur-containing amino acids (Singh *et al.*, 2020, Chaudhary *et al.*, 2020: Nadeem *et al.*, 2022) [16, 2, 12]. Cystine and methionine are sulfur-containing amino acids

crucial for antioxidant defense and protein stability (Künstler *et al.*, 2020) [11]. The high cystine content in *C. swietenia* compares favorably with medicinal species like *Eclipta prostrata* and *Phyllanthus emblica*, which also exhibit significant sulfur amino acids enhancing their nutraceutical value (Timalsina *et al.*, 2021, Prananda *et al.*, 2023) [17, 13]. Essential amino acids including threonine, valine, isoleucine, leucine, lysine, phenylalanine, histidine, methionine, and tryptophan were present in appreciable quantities. The balanced essential amino acid spectrum mirrors findings in *Asparagus racemosus* and *Withania somnifera*, where the presence of branched-chain and aromatic amino acids contributes to high protein quality indices (Kohli *et al.*, 2023; Khanna *et al.*, 2006) [9, 8].



**Fig 2:** HPLC chromatogram of *C. swietenia* for free amino acids

The bound fraction, calculated as the difference between TAA and FAA, constituted the major proportion for most amino acids, reflecting significant involvement in protein structures. Alanine (146.309  $\mu\text{g}/100\text{ mg}$ ), cystine (115.002  $\mu\text{g}/100\text{ mg}$ ), and glutamine (85.790  $\mu\text{g}/100\text{ mg}$ ) had particularly high bound levels. This pattern is consistent with medicinal plants like *Centella asiatica* and *Withania somnifera*, where bound fractions predominate and contribute to sustained nutrient release during digestion (Kunjumon *et al.*, 2022; Ghosh Dasgupta *et al.*, 2014) [10, 7]. The presence of both essential and non-essential amino acids in *C. swietenia* underscores its nutritional potential. Amino acids like arginine are involved in nitric oxide synthesis and immune modulation (Wu, 2009) [18], while glutamic acid and glycine play roles in neurotransmission and antioxidant defense (Aguayo-Cerón *et al.*, 2023) [1]. Branched-chain amino acids such as valine, leucine, and isoleucine support muscle metabolism and tissue repair. The predominance of protein-bound amino acids suggests that the plant could serve as a sustained protein source upon digestion, while the appreciable free amino acid content supports rapid metabolic assimilation. The relatively balanced spectrum of essential amino acids enhances the value of *C. swietenia* as a supplementary protein source, particularly when compared to other wild

edible and medicinal plants with lower essential amino acid proportions [FAO, 1991]. Its amino acid profile, especially the high sulfur-containing and branched-chain amino acids, indicates a promising functional food and nutraceutical potential.

## Conclusion

The present study demonstrates that *Chloroxylon swietenia* possesses a nutritionally significant and well-distributed amino acid profile comprising both free and protein-bound fractions. The predominance of glycine, asparagine, histidine, and lysine in the free amino acid pool indicates immediate metabolic and physiological relevance, while the substantially higher total amino acid content confirms the presence of considerable structural and storage protein reserves.

Notably, the appreciable levels of essential amino acids, including lysine, methionine, valine, isoleucine, leucine, threonine, phenylalanine, histidine, and tryptophan, suggest a relatively balanced essential amino acid spectrum in comparison with other medicinal and wild edible plants. The high proportion of sulfur-containing amino acids such as cystine and methionine further enhances its nutritional importance, as these are frequently limiting in plant-based diets.

The dominance of the bound amino acid fraction indicates that *C. swietenia* can serve as a sustained source of dietary protein upon digestion, while the presence of free amino acids supports rapid metabolic assimilation. Collectively, these findings validate the protein nutritional potential of *C. swietenia* and support its consideration as a functional wild edible plant with promising nutraceutical applications. Further studies on protein digestibility, amino acid scoring patterns, and bioavailability would strengthen its candidacy as a supplementary plant-based protein resource.

#### Declaration of competing interest

The authors have no conflict of interest to declare.

#### Author contributions

**Manas Ranjan Debta:** Collected and identified plant

**Materials:** Experimental work carried out

**Basundhara Pillai:** Experimental work carried out

**Tapan Seal:** Designed the study, drafted the manuscript, Statistical analysis and Interpreted the results

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