Quantitative Analysis of Free Amino Acids In Euphorbiaceae Species Of Menal forest of Rajasthan

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Abstract:
This study quantitatively analyzed total free amino acids in three Euphorbiaceae species — *Euphorbia hirta*, *Euphorbia caducifolia*, and *Ricinus communis* — from Bhilwara, Rajasthan. Using ninhydrin-based colorimetric methods, the research found that *Ricinus communis* had the highest amino acid content, followed by *E. caducifolia* and *E. hirta*. The observed differences likely reflect species-specific metabolism or environmental adaptation. The study highlights the biochemical potential of these plants for nutraceutical and pharmaceutical applications.

Keywords: Euphorbiaceae, Biochemical estimation, phytochemistry, Amino acid quantification, Medicinal values.

INTRODUCTION:

The Euphorbiaceae family is one of the most diverse groups of angiosperms, containing between 6,000 and 7,500 species across approximately 200 to 300 genera, primarily found in tropical and subtropical areas (Christenhusz, 2017; Wurdack, 2021; Yang, 2024). Its members showcase a range of growth forms including herbs, shrubs, and cacti-like succulents, many of which secrete milky latex that contains defensive compounds (Agbo, 2022; Karaaslan *et al*., 2023). A distinctive taxonomic characteristic is the cyathium inflorescence present in Euphorbia (Steinmann, 2019). Economically and medicinally, species like *Ricinus communis* (castor oil plant) and certain Euphorbia species (E. hirta, E. caducifolia) are extensively used in industry and therapy (Ogunmoyole & Ajiboye, 2021; Sharma & Singh, 2023; Lee *et al*., 2022). Recent research has underscored their rich phytochemical composition, which includes alkaloids, terpenoids, flavonoids, and amino acids, highlighting their promise in ethnopharmacology and the development of nutraceuticals (Patel, 2022; Liu *et al*., 2023; Zhao *et al*., 2024; Patil *et al*., 2025). Despite this diversity, there is a scarcity of quantitative studies on free amino acids within Euphorbiaceae species, indicating a need for targeted biochemical research.

Amino acids are essential biochemical substances in plants, acting as the basic building blocks of proteins and playing vital roles in plant metabolism, nitrogen uptake, growth, and how plants respond to stress. Besides their involvement in protein formation, amino acids serve as precursors for various secondary metabolites like phenolics, alkaloids, and glucosinolates, which affect plant development and ecological relationships. The photometric ninhydrin method for estimating amino acids, introduced by Moore and Stein in 1948, continues to be a key technique in biochemical research. Lewis *et al*. (1970) explored the production of amino acids in ***Euphorbia hirta***, ***Euphorbia caducifolia***, and ***Ricinus communis*** highlighting their physiological origins and importance. In plant systems, the majority of amino acid synthesis takes place in plastids, although mitochondria, peroxisomes, and the cytosol also play roles in their generation (Mukhtar *et al*., 2022). Amino acid transport involves intricate translocation pathways through xylem and phloem, facilitating growth and nutrient distribution among roots, leaves, and developing tissues (Staveckienė *et al*., 2024). These compounds are crucial for tolerating abiotic stress and for reproductive development. From the perspective of human health, amino acids aid metabolic processes, help regulate immune responses, and act as antioxidants, which can assist in alleviating conditions such as diabetes, obesity, and arthritis (Mezhlumyan *et al*., 2022).

Phytochemical investigations of the Euphorbiaceae family reveal that various plant parts—roots, stems, leaves, and flowers—harbor unique metabolites that are significant for both pharmacological and nutraceutical applications. The roots of ***Euphorbia hirta*** and ***E. caducifolia*** produce alkaloids, tannins, terpenoids, and amino acids, whereas the stems of ***E. tithymaloides, E. turcomanica,*** and ***Ricinus communis*** are abundant in triterpenes, steroids, phytosterols, and flavonoids. The leaves of ***E. hirta, E. parviflora,*** and ***E. stracheyi*** show elevated concentrations of flavonoids, glycosides, and diterpenoids, which support their antioxidant and medicinal properties. The flowers of ***E. pulcherrima*** and ***R. communis*** contain flavonoids, phenolics, and amino acids that are associated with antimicrobial functions and stress response. In summary, each organ of the plant contributes specifically to the bioactive composition of the family, although there is a lack of comprehensive quantitative assessments of amino acids, highlighting the need for more advanced metabolomic studies.

Despite significant advancements in global and national studies profiling amino acids in cultivated and medicinal plants, the Euphorbiaceae family comprises a diverse range of angiosperms that hold considerable phytochemical and pharmacological value. Various parts of these plants yield unique bioactive compounds such as alkaloids, terpenoids, flavonoids, phenolics, and amino acids, which are essential for plant physiology, stress resilience, and promoting human health. Although their traditional and industrial applications are well documented, thorough quantitative research on amino acids remains scarce. Progressing research with metabolomic and molecular methods will be vital for revealing amino acid dynamics, enhancing the scientific foundation for ethnomedicinal applications, and encouraging the development of new nutraceutical and pharmaceutical products.

2. REVIEW OF LITERATURE

The latex extracted from *Euphorbia caducifolia* has received interest for its historical applications in wound healing, which is backed by scientific research into its bioactive characteristics. Goyal, Nagori, and Sasmal (2012) performed extensive investigations to assess the wound healing properties of this species' latex. Their findings revealed notable in vitro and in vivo wound healing effects, including improved tissue regeneration and faster wound closure, suggesting its potential therapeutic role in wound treatment Additionally, phytochemical studies have uncovered several bioactive compounds within the latex, as like 3,7,11,15-tetra-methyl-2-hexadecene-1-ol, 5,9-hepta-decadienoate, and methyl palmitate, which may play a role in its antimicrobial and wound healing effects. These results reinforce the traditional medicinal use of E. caducifolia latex for managing wounds and skin infections.

The research conducted by Prasad and Chandra (2018) offers an in-depth examination of the profiles of antioxidants & amino acids in wild edible medicinal plants from the Himalayan state of Uttarakhand. By employing High-Performance Liquid Chromatography (HPLC), the authors uncovered a variety of amino acids and antioxidant substances present in these plants, emphasizing their nutritional and therapeutic value. The results highlight the importance of these wild plants as significant sources of essential nutrients and bioactive compounds, which support their traditional medicinal applications in the area.

Bhushan (1981) created a method using paper chromatography for the simultaneous detection of sugars and free amino acids in plant tissues. This method, which appeared in Fresenius' Zeitschrift für analytische Chemie, became an important resource for examining the biochemical makeup of plants. By utilizing paper chromatography, Bhushan successfully separated and recognized various free amino acids and sugars found in plant tissues, thereby enhancing the understanding of their metabolic profiles. This approach has played a crucial role in plant biochemistry research, providing a relatively straightforward and efficient means for the qualitative analysis of these compounds.

Ahmad, Singh, and Kumar (2017) examined the phytochemical content and antimicrobial properties of extracts from *Euphorbia hirta*. According to their findings, this plant is abundant in a number of bioactive substances, including terpenoids, alkaloids, flavonoids, saponins, steroids, and sterols. Methanol extracts demonstrated notable antibacterial and antifungal effects against pathogens including Proteus mirabilis, Listeria monocytogenes, Aspergillus niger, and Aspergillus fumigatus, while extracts using chloroform and hexane exhibited moderate activity. These results support the traditional medicinal applications of E. hirta and underscore its potential as a source of antimicrobial substances. Additional research is suggested to isolate and characterize the active compounds for therapeutic use.

According to a review by Salem *et al*. published in Plant Foods for Human Nutrition in 2015, artichoke leaf extract (*Cynara scolymus*) has notable pharmacological properties and health advantages. This extract is abundant in polyphenolic compounds, which provide significant antioxidant effects that safeguard cells from oxidative harm. It demonstrates protective qualities for the liver, promotes liver regeneration, and aids in reducing cholesterol levels, benefiting cardiovascular health. Furthermore, it enhances the production of bile, facilitating digestion and the absorption of fats while relieving digestive issues. The majority of these advantages are linked to bioactive compounds such as cynaropicrin, which also exhibit anti-inflammatory effects and may have anti-cancer potential. Although the findings are encouraging, the authors suggest that additional clinical trials are necessary to fully determine its therapeutic efficacy (Salem *et al*., 2015).

Huang *et al*. (2016) investigated the role that root exudates play in *Ricinus communis's* buildup of copper (Cu) during Cu stress. The study demonstrated the potential of castor plants for use in phytoremediation by showing that they can retain large amounts of copper in both their roots and shoots. Cu buildup in the roots was positively correlated with elevated levels of low molecular weight organic acids, particularly tartaric, citric, and succinic acids, as revealed by the examination of root exudates. Additionally, the profiles of amino acids in the exudates changed based on varying Cu concentrations, signaling adaptive biochemical reactions to metal stress. In conclusion, the work highlights the importance of organic acids and amino acids in root exudates in enhancing Cu tolerance and accumulation, providing insight into the mechanisms of castor plant adaptation to metal stress.

The biochemical and molecular characteristics of *Ricinus communis's* sucrose and amino acid transporters were investigated by Williams *et al*. (1996). Through biochemical assays and molecular methodologies, the research identified a sucrose-H^+ symporter along with two amino acid transporters that have a wide range of substrate specificity. Functional analysis showed that the sucrose transporter mainly functions in cotyledons and roots, while the amino acid transporters are found in leaves, stems, and roots, suggesting their involvement in both source and sink tissues. These transporters play a crucial role in the effective distribution of carbon and nitrogen within the plant, aiding in growth, metabolism, and stress responses. The findings offer essential insights for improving nutrient uptake and the potential genetic enhancement of crops.

Weston, Hall, and Williams (1995) used isolated membrane vesicles to investigate the transport of amino acids in *Ricinus communis* roots. They found that basic amino acids like lysine and arginine are transported by a voltage-driven uniport mechanism, while neutral amino acids like glutamine and isoleucine are carried by a proton symport mechanism that depends on both a pH gradient and the membrane potential. They discovered a number of transporters that preferred neutral, basic, or acidic amino acids, each with a distinct substrate specificity. The pH sensitivity of the transport carriers was highlighted by the discovery that the efficiency of transport was higher at pH 5.5 than at pH 7.5. This research offers important insights into the molecular mechanisms of amino acid absorption in castor roots, contributing to a better understanding of nutrient transport and plant metabolism.

3. MATERIALS AND METHODS:

Free amino acids were estimated using the procedure outlined by Moore and Stein (1948). Ninhydrin reagent was made using two separate solutions: Solution B had 0.8 g of reagent-grade stannous chloride ($SnCl_2$) dissolved in 500 ml of citrate buffer at pH 5.0, and Solution A had 20 g of ninhydrin dissolved in 500 ml of methyl cellosolve. These solutions were mixed just before use to preserve the activity of the reagent and to ensure optimal color formation during the reaction with amino acids. Leaf, stem, and root samples were gathered, shade-dried, and then oven-dried at temperatures between 50°C and 55°C until a stable weight was reached to prevent the loss of thermolabile metabolites. About 200 mg of the dried material was ground in 10 millilitres of 80% ethanol, centrifuged for 10 minutes at 10,000 rpm, and then extracted again using 10 millilitres of 80% ethanol. The supernatants that were gathered were mixed together. Five millilitres of chloroform and distilled water were added to the extract in order to remove chlorophyll and other colours. After shaking and letting the mixture settle, the top layer of water was removed for examination.

One millilitre of the aqueous phase was mixed with one millilitre of freshly made ninhydrin reagent in order to develop colour. For twenty minutes, the resultant mixture was boiled in a boiling water bath. Five millilitres of 50% isopropanol were added as a diluent once it had cooled. A 2375 Double Beam Spectrophotometer, which gauges the strength of the purple complex (Ruhemann's purple) produced by the interaction between ninhydrin and free amino acids, was used to measure the absorbance at 570 nm.

A standard curve created with alanine was used for quantification, and the amount of free amino acids present was expressed in milligrammes per gramme of dry weight. Samples from three different organs—leaves, stems, and roots—were analysed for each type of plant. To ensure the accuracy, uniformity, and reproducibility of the results across all plant parts, each organ sample was processed using three biological replicates (collected from three different plants) and three technical replicates (repeated measurements of the same extract).

4. RESULT:

TOTAL FREE AMINO ACIDS (mg/g dry weight) IN VARIOUS ORGANS OF SELECTED species FROM Menal forest RAJASTHAN

S. No.	Name of species	Root	Stem	Leaf	Total in entire plant
1.	***Euphorbia hirta***	1.78	2.27	4.73	8.78
2.	***Euphorbia caducifolia***	2.12	1.52	9.44	13.08
3.	***Ricinus communis***	3.65	4.33	9.99	17.97

The data show distinct interspecific and organ-specific variations in the distribution of total free amino acids (mg/g dry weight) in the root, stem, and leaf of three species chosen from Rajasthan's Menal forest. The highest total amino acid content (17.97 mg/g) was found in Ricinus communis, with the leaf having the highest concentration (9.99 mg/g), followed by the stem (4.33 mg/g) and root (3.65 mg/g). ***Euphorbia caducifolia*** occupied an intermediate position with a total of 13.08 mg/g, characterized by a strikingly high accumulation in the leaf (9.44 mg/g) compared to relatively lower values in the root (2.12 mg/g) and stem (1.52 mg/g). Although a similar distribution pattern was seen, *Euphorbia hirta* had the lowest total free amino acid content (8.78 mg/g), with the leaf having the highest concentration (4.73 mg/g), followed by the stem (2.27 mg/g) and root (1.78 mg/g). A consistent trend across all three species indicates that leaves are the primary sites of free amino acid accumulation, which may reflect their higher metabolic activity and involvement in protein synthesis and nitrogen metabolism. Stems generally possessed moderate amounts, while roots contained the least, suggesting a gradient of amino acid concentration corresponding to the physiological roles of these organs.

CONCLUSION:

The overall analysis demonstrates that ***Ricinus communis*** is the richest source of free amino acids among the studied species, while ***Euphorbia hirta*** contains the least. The consistent dominance of leaves across all species highlights their critical role in amino acid biosynthesis and storage, reflecting their importance as key metabolic centers in plant nitrogen economy and overall growth.

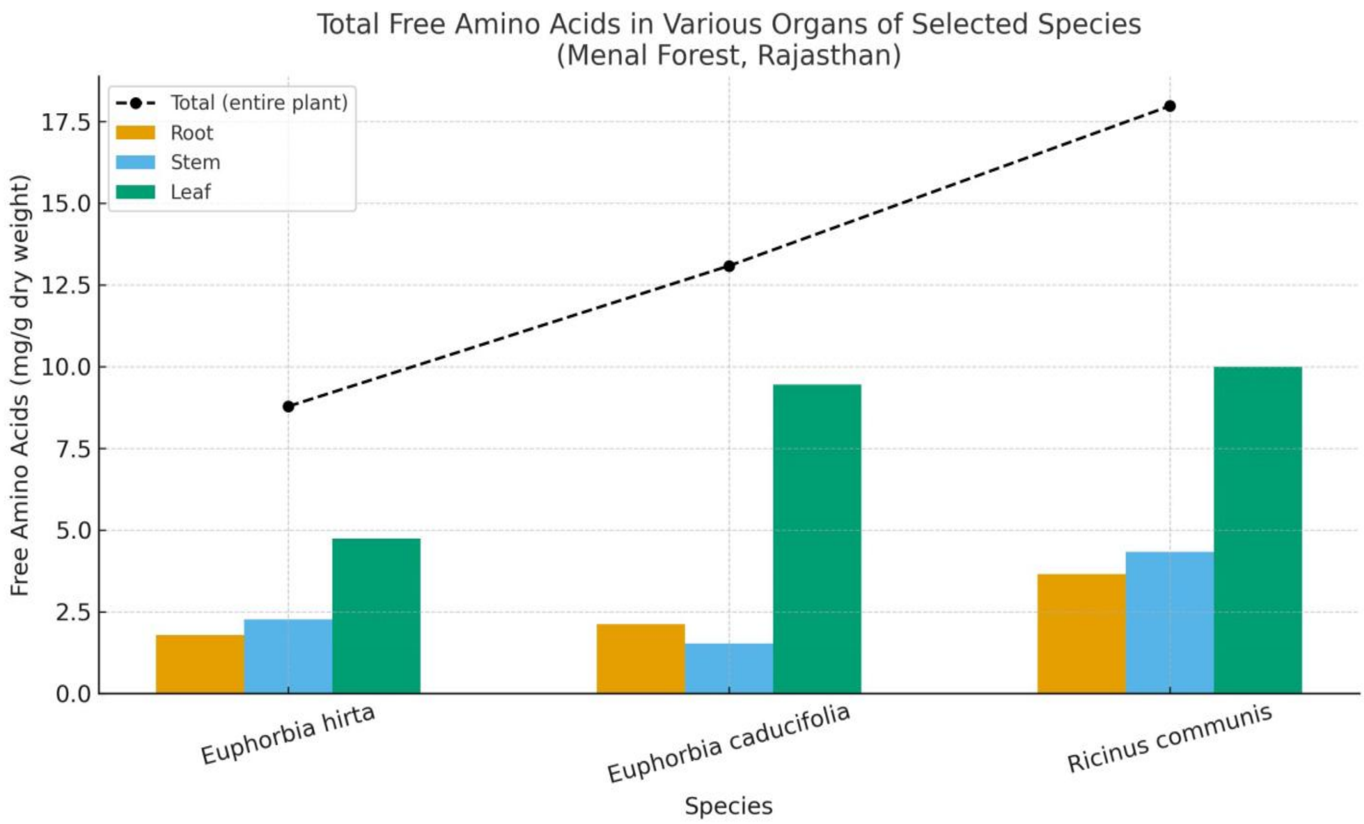
1) Discussion

The present investigation on the total free amino acid content in different organs of three selected species from the Menal forest of Rajasthan—***Euphorbia hirta***, ***Euphorbia caducifolia***, and ***Ricinus communis***—reveals significant interspecific differences and organ-specific patterns that reflect their physiological and ecological adaptations. All three species' leaves have a consistently greater quantity of free amino acids, indicating that these organs serve as the main locations for protein turnover, nitrogen uptake, and amino acid production. The carbon skeletons required for the production of amino acids are provided by photosynthetic processes in the metabolically active tissues known as leaves, and their high levels suggest an active nitrogen economy that is vital for growth and development.

Among the species studied, ***Ricinus communis*** exhibited the greatest total free amino acid content, indicating a more efficient nitrogen metabolism and possibly a greater capacity for protein synthesis compared to the two Euphorbia species. This higher accumulation may also be linked to its robust growth habit and ecological versatility, allowing it to adapt to the semi-arid conditions of the Menal forest. ***Euphorbia caducifolia*** showed a moderate total amino acid content but displayed a remarkable concentration in the leaf tissue, which may represent a physiological strategy to maintain osmotic balance and metabolic flexibility in response to environmental stress, such as drought or high temperature. ***Euphorbia hirta***, in contrast, recorded the lowest values across all organs, reflecting either a relatively slower metabolic rate or a more conservative nitrogen utilization strategy.

The organ-wise distribution pattern, where **stems** generally contained moderate amounts and **roots** the least, highlights the gradient of amino acid translocation and utilization within the plant body. Roots, being primarily responsible for absorption and anchorage, exhibit lower metabolic activity in amino acid synthesis, while stems function as conduits for transport and storage, thus exhibiting intermediate levels. This hierarchical distribution supports the concept that free amino acids are synthesized predominantly in photosynthetically active tissues and subsequently mobilized to other organs as required.

Overall, the findings underscore the ecological significance of amino acid accumulation as an adaptive mechanism in plants inhabiting dry tropical forests like Menal. The pronounced leaf dominance in amino acid content may serve as a biochemical buffer against abiotic stress by facilitating osmoprotection, nitrogen storage, and rapid metabolic responses. These results not only provide insight into the nitrogen metabolism of the studied species but also contribute valuable baseline data for understanding the physiological ecology of native plants in semi-arid regions of Rajasthan.



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