International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Comparative Seasonal Profiling of Total Free Amino Acids in Euphorbia Indica and Euphorbia Microphylla Growing in Semi-Arid Ecosystems of Rajasthan, India

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Abstract: ***The present study was carried out to estimate the total free amino acid content of various parts of Euphorbia indica and Euphorbia microphyll collected from the Bassi Wildlife Sanctuary area during rainy season. The aim of the study was to understand the organ-specific variation and metabolic adaptability of these wild species to the semi-arid environments. The dry weight (dw) of free amino acids in the leaves, stems and roots was mg/g. Euphorbia indica leaves had the following: 47.56 mg/g dw followed by stem (31.63 mg/g dw) and root (26.83 mg/g dw). A similar trend was observed in Euphorbia microphylla where it accumulated mainly in the leaves (43.66 mg/g dw) followed by the stem (39.75 mg/g dw) and root (36.80 mg/g dw). The results showed that leaves. Seasonal precipitation and good weather during the rainy season probably resulted in the formation of amino acids in both species. The study emphasizes the adaptive biochemical reactions and potential therapeutic significance of these plants as amino acids, free act as an important precursor for number of physiologically active chemicals. These results are based on the baseline biochemical data of naturally occurring species of Euphorbia from the Bassi Wildlife Sanctuary area of Rajasthan.***

Keywords: Free amino acids, seasonal variation, Euphorbia indica, Euphorbia microphylla, plant metabolism, ecophysiology, semi-arid ecosystem.

1. Introduction

The over 6,000–7,500 species spread across 200–300 genera, the Euphorbiaceae family is one of the biggest and most varied groupings of angiosperms, with most of them found in tropical and subtropical areas (Christenhusz, 2017; Wurdack, 2021; Yang, 2024). Members of Euphorbiaceae are morphologically extremely diverse, including herbs, shrubs and cactus-like succulents. Several species have the characteristic of producing milky latex rich in protective bioactive chemicals (Agbo, 2022; Karaaslan et al., 2023). The cyathium inflorescence is a characteristic taxonomic feature of Euphorbia (Steinmann, 2019). Recent studies have emphasized the great phytochemical diversity of the Euphorbiaceae family, pointing to the presence of important bioactive compounds such as alkaloids, flavonoids and amino acids. The presence of these metabolites suggests the potential for significant ethnopharmacological applications and nutraceutical development of Euphorbiaceae species (Patel, 2022; Liu et al., 2023; Zhao et al., 2024; Patil et al., 2025). However, the high diversity of the family Euphorbiaceae presents a lack of quantitative studies on free amino acids in species of the Euphorbiaceae, thus requiring more specific biochemical investigations. Amino acids are the basic building blocks proteins, but also important components of Nitrogen and plant metabolism absorption, development and stress response. Amino acids, in addition to their role in protein synthesis, are also precursors for secondary metabolites, such as alkaloids, phenolics and glucosinolates, which influence plant growth and ecological relationships. The photometric method for the estimation of amino acids by ninhydrin was developed by Moore & Stein (1948).

Most amino acids in plant systems are synthesized in plastids (Mukhtar et al., 2022). Synthesis of amino acids also takes place in peroxisomes, mitochondria, and the cytosol. Complex translocation pathways in phloem and xylem mediate amino acid transport to roots, leaves and growing tissues, thereby supporting growth and nutrient distribution (Staveckienė et al., 2024). Lewis et al. (1970) studied the production of amino acids in Ricinus communis, Euphorbia caducifolia and Euphorbia hirta. The physiological sources and significance were emphasized. Prasad & Chandra studied the antioxidant and amino acid composition of wild medicinal plants of Uttarakhand Himalayas using HPLC research and reported their important nutritional and medicinal potential.

Weston and Williams (1995) used isolated membrane vesicles to investigate the flow of amino acids in Ricinus communis roots. They revealed that neutral amino acids, such as glutamine and isoleucine, are transported via a proton symport process that is dependent on both a pH gradient and membrane potential, whereas basic amino acids, such as lysine and arginine, are transported via a voltage-dependent uniport mechanism. Bhushan (1981) created a method for employing paper chromatography to

simultaneously detect sugars and free amino acids in plant tissues. This method, which was initially described in Fresenius' Zeitsc hrift für Analytical Chemie, developed into a useful instrument for researching the biochemical makeup of plants. By successfully extracting and identifying various free amino acids and carbohydrates using paper chromatography, Bhushan advanced our understanding of the metabolic properties of plant tissues.

2. Materials and Methods

Study Area

The present research was conducted at Bassi Wildlife Sanctuary, located in the semi-arid region of Rajasthan, India, approximately between latitudes 24.92° and 25.00° N and longitudes 74.42° and 74.68° E. The area is known for its high summer temperatures, low annual rainfall, and distinct seasonal variations, including winter, summer, and monsoon. Because the sanctuary is home to a variety of xerophytic and herbaceous plants that have adapted to arid environmental conditions, it is suitable for ecological and biochemical investigations on native plant species.

Plant Collection

Fresh specimens of Euphorbia indica and Euphorbia microphylla were collected from their natural habitats within Bassi Wildlife Sanctuary throughout a number of seasons. As soon as the plant materials were gathered, they were carefully separated into leaves, stems, and roots. The samples were shade-dried and then oven-dried at a regulated temperature until they attained a constant weight after being thoroughly cleaned to remove any dust or soil particles that might have adhered to them. The materials were dried, ground into a fine powder, and stored in airtight containers for further biochemical analysis.

Determination of Free Amino Acid Content

Free amino acids were estimated using the technique outlined by Moore and Stein (1948).

The ninhydrin reagent was made using two distinct solutions: Eight grams of reagent-grade stannous chloride ($SnCl_2$) were dissolved in 500 milliliters of pH. Citrate buffer in solution at pH 5.0 B. A included 20 g of ninhydrin and 500 cc of methyl cellosolve. These solutions were mixed just before use in order to preserve the reagent's activity and ensure the best possible color change during the amino acid reaction. Samples of leaves, stems, and roots were collected, dried in the shade, and then oven-dried at temperatures between 50°C and 55°C until a stable weight was reached in order to prevent loss in thermolabile metabolites.

The dried material was ground into roughly 200 mg in ten milliliters of 80% ethanol, centrifuged for ten minutes at 10,000 rpm, and then extracted again using ten milliliters of 80% ethanol. The supernatants that were gathered were mixed together. Five milliliters of chloroform and distilled water were added to the extract to remove chlorophyll and other colors. After shaking the mixture and letting it settle, the top layer of water was removed for analysis. One milliliter of the liquid phase and one milliliter of freshly made ninhydrin reagent were mixed to produce color. After that, the mixture is cooked for twenty minutes in a bath of boiling water. Five milliliters of 50% isopropanol were added as a diluent once it had cooled.

A 2375 Double Beam Spectrophotometer was used to measure the absorbance of 570 nm in order to determine the intensity complex of purple light (Ruhemann's purple), which is produced when free amino and ninhydrin interact. Using a standard curve created with alanine, the amount of free amino acids present was calculated in milligrams per gram of dry weight. For each type of plant, samples from three different organs- leaves, stems, and roots- were analyzed using a usual curve. To guarantee accuracy and consistency across all plant parts, duplicates (obtained from measurements of the reproducibility of the same findings samples from three employing a standard curve) were processed using three biological and three technical replicates (repeated extract). Three distinct plants made up each organ sample.

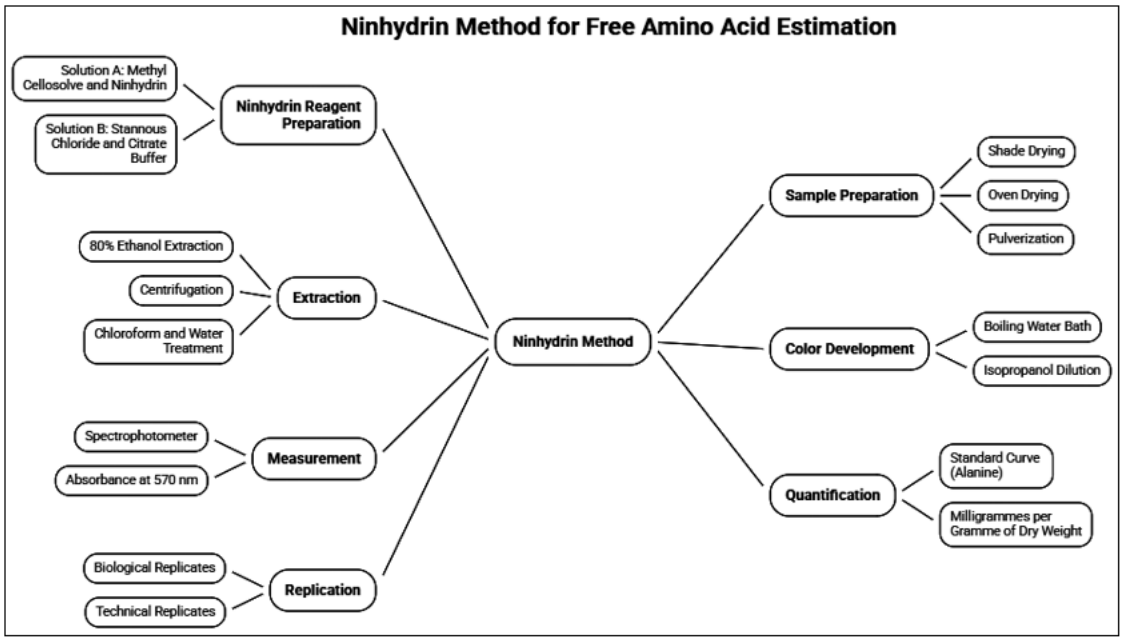

Figure 1: Overview of the Ninhydrin-Based Method of Free Amino Acid Estimation

3. Result

Table 1: Total Free Amino Acids (mg/g dw) (rainy) in Different Plant Parts of Selected Euphorbiaceae Species from Bassi Wildlife Sanctuary, Rajasthan

S. No.	Name of the plant species	Leaf	Stem	Root	Total Average
1	Euphorbia indica	47.56	31.63	26.83	47.43
2	Euphorbia microphylla	43.66	39.75	36.80	23.68

During the rainy season, different plant sections of the selected species of the Forest, Rajasthan, showed different total free amino acid concentrations (mg/g dry weight) (Table 1). In both species, leaf, show far higher quantities of amino acids than root and stem tissues, indicating that photosynthetically active organs are intricate in active Increase metabolic and physiological activities.

With an average total value of 47.43 mg/g dw, the leaf of Euphorbia indica had the highest attentiveness of free and total amino acids (47.56 mg/g dw), followed by stem (31.63 mg/g dw) and root (26.83 mg/g dw). The higher buildup of amino acids in leaves may be caused by enhanced vegetative growth during the wet season, increased protein synthesis, and active nitrogen metabolism. During this period, favourable climatic conditions and increased moisture availability likely promote the synthesis of amino acids and their distribution across aerial plant tissues.

Similarly, Euphorbia microphylla showed notable differences in plant parts. The leaf had the highest concentration of amino acids (43.66 mg/g dw), followed by the root (36.80 mg/g dw) and stem (39.75 mg/g dw). When compared to Euphorbia indica, this species had a relatively balanced distribution of amino acids among vegetative organs, indicating efficient food distribution and adaptable metabolic activity in forest habitats. There aren't many comparative biochemical studies on wild Euphorbia species from Rajasthan's Menal Forest region, which results in the current work. A lot of prior research has concentrated on taxonomic descriptions or therapeutic qualities, but little is known about the seasonal distribution of amino acids in various plant organs. The study shows how different organ and species accumulate different amounts of free amino acids throughout the natural rainy season. Future ecological, physiological, phytochemical, and pharmacological research on naturally occurring medicinal plants from semi-arid forest settings may benefit from these discoveries.

4. Discussion

The current study showed that the total free amino acid content of different Euphorbia indica plant sections fluctuated significantly over the rainy season. The leaf has the maximum amount of amino acids (47.56 mg/g dw), followed by the stem (31.63 mg/g dw) and root (26.83 mg/g dw), with an average value of 47.43 mg/g dw. The greater accumulation of free amino acids in leaf tissues indicates increased metabolic and physiological activity in photosynthetically active organs.

Since leaves are regarded to be the primary sites for protein production and nitrogen assimilation, higher amino acid concentrations in leaves may suggest increased enzymatic activity and active cellular metabolism during the rainy season. Amino acid synthesis and nitrogen uptake may have been enhanced by the favorable environment and enough soil moisture during that time. Furthermore, free amino acids help plants maintain physiological equilibrium in the face of changing environmental conditions by acting as metabolic intermediates and osmo protectants. Stem and root tissues contain less amino acids than leaves, which may suggest that these organs have less biosynthetic activity. However, the substantial amino acid content of roots indicates that they are involved in the translocation and nutrient absorption of the plant system. The current study is essential since there is a dearth of biochemical information on naturally occurring Euphorbia indica and Euphorbia microphylla from the Bassi Wildlife Sanctuary region. The seasonal fluctuations in free amino acid levels of both species reflect their metabolic adaptations to semi-arid conditions. Higher amino acid accumulation may also increase their therapeutic value because amino acids are precursors for several physiologically active secondary metabolites.

5. Conclusion

Total free amino acid levels in the various tissues of Euphorbia indica and Euphorbia microphylla fluctuated considerably during the rainy season in the Bassi Wildlife Sanctuary. The greatest buildup of amino acids was seen in the leaves of both species, suggesting increased metabolic activity. These results demonstrate the two Euphorbia species' metabolic plasticity and point to their possible medicinal utility in semi-arid environments.

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