**Quantitative Analysis of Total Free Amino Acids and Their Pharmacological Implications in Wild Solanaceae Species from Menal Forest, Rajasthan, India**

ABSTRACT

This study assesses the total free amino acid content in five wild Solanaceae species—Datura fastuosa, Datura innoxia, Physalis angulata, Physalis minima, and Solanum virginianum—collected from the Menal Forest region of Chittorgarh, Rajasthan. Using the ninhydrin colorimetric method following acid hydrolysis, both interspecific and organ-specific variations were observed. Among the species, Datura innoxia exhibited the highest amino acid concentrations in leaves (68.09 mg/g), stems (54.07 mg/g), and roots (35.08 mg/g), suggesting strong metabolic activity and adaptive responses to semi-arid conditions. Solanum virginianum and Datura fastuosa also showed substantial foliar amino acid levels, supporting their traditional use in treating liver disorders, infections, and neurological issues. In contrast, Physalis angulata and Physalis minima had lower amino acid contents, particularly in roots and stems, although they retain medicinal value due to other active compounds like withanolides and flavonoids. Overall, leaves consistently recorded the highest amino acid levels across all species, underlining their role in biosynthesis. The findings highlight the nutritional and therapeutic significance of these underutilized wild taxa, reinforcing their relevance in pharmaceutical and nutraceutical applications.

Key Words:Solanaceae, Phytochemistry, Quantitative amino acid analysis, Wild medicinal plants, Ethnomedicine.

INTRODUCTION

The Solanaceae family, comprising nearly 90 genera and over 3,000 species, is a widely distributed group of flowering plants known for its ethnobotanical, nutritional, and pharmacological significance. Among its largest genera, *Solanum* includes more than 2,000 species and exhibits a broad geographical range across tropical and subtropical regions of Africa, Australia, China, India, and the Americas, including the Himalayan belt and parts of southern and eastern India. Many Solanaceae species, including *Datura*, *Physalis*, and *Solanum*, are recognized for their reservoir of bioactive phytochemicals, such as alkaloids, steroidal saponins, terpenoids, flavonoids, lignans, sterols, polyphenols, fatty acids, coumarins, and vitamin C, which contribute to their substantial nutraceutical and pharmaceutical relevance (Lim, 2012; Sharma et al., 2021).

Amino acids are indispensable biochemical compounds in plants, serving as the foundational units of proteins and playing critical roles in plant metabolism, nitrogen assimilation, growth, and stress response. In addition to their role in protein synthesis, amino acids function as precursors for numerous secondary metabolites such as phenolics, alkaloids, and glucosinolates, influencing both plant development and ecological interactions. Moore and Stein (1948) pioneered the photometric ninhydrin method for amino acid estimation, which remains a standard technique in biochemical analyses. Lewis et al. (1970) investigated the biosynthesis of amino acids in *Datura stramonium*, emphasizing their physiological origin and significance. In plant systems, amino acid biosynthesis occurs predominantly in plastids, though mitochondria, peroxisomes, and cytosol also contribute to their production (Mukhtar et al., 2022). The transportation of amino acids involves complex translocation pathways via xylem and phloem, supporting growth and nutrient allocation between roots, foliage, and developing tissues (Staveckienė et al., 2024). These compounds are also important in abiotic stress tolerance and reproductive development. From a human health perspective, amino acids support metabolic function, regulate immune responses, and act as antioxidants, contributing to the mitigation of disorders such as diabetes, obesity, and arthritis (Mezhlumyan et al., 2022).

Phytochemical studies on Solanaceae species have documented the presence and variation of amino acids across taxa. Patel et al. (2011) observed significant amino acid fluctuations during fruit maturation in *Physalis minima*, while Karpagasundari and Kulothungan (2014) reported amino acid and secondary metabolite presence in *P. minima* leaves through chromatographic profiling. Mezhlumyan et al. (2022) confirmed the hypoglycemic potential of *Physalis angulata* and *P. alkekengi* proteins, correlating with their amino acid content. In *Datura innoxia*, biochemical analysis by Nagarkar (2021) revealed allergenicity potential linked to pollen amino acid composition. Recent studies in *Solanum* (Pęksa et al., 2021; Mukhtar et al., 2022) and eggplant variants have highlighted genotype- and development-specific amino acid profiles. Additionally, Staveckienė et al. (2024) examined amino acid changes during fruit maturation in *Solanum* species.

Although global and national studies have made significant progress in profiling amino acids in cultivated and medicinal Solanaceae, comprehensive biochemical investigations of wild species from the semi-arid ecosystems of Rajasthan remain limited. The present study seeks to address this gap by estimating total free amino acids in five wild Solanaceae species—*Datura fastuosa*, *Datura innoxia*, *Physalis angulata*, *Physalis minima*, and *Solanum virginianum*—collected from the Menal Forest region of Chittorgarh, Rajasthan. Using acid hydrolysis followed by the ninhydrin colorimetric method, this research aims to evaluate the nutritional, ecological, and pharmacological relevance of these underexplored wild taxa.

MATERIAL AND METHODS

The Moore and Stein (1948) method was employed for the estimation of free amino acids. To prepare the ninhydrin reagent, two separate solutions were freshly prepared: Solution A comprised 20 g of ninhydrin dissolved in 500 ml of methyl cellosolve, while Solution B consisted of 0.8 g of reagent-grade stannous chloride (SnCl₂) dissolved in 500 ml of citrate buffer (pH 5.0). These two solutions were combined immediately prior to use to maintain reagent activity and ensure optimal color development during the reaction with amino acids. **Leaf, stem and root samples** were collected, shade-dried, and then oven-dried at 50–55°C until a constant weight was achieved to avoid loss of thermolabile metabolites. About 200 mg of this dried material was homogenized in 10 ml of 80% ethanol, centrifuged at 10,000 rpm for 10 minutes, and re-extracted with another 10 ml of 80% ethanol. The two supernatants were pooled. To remove chlorophyll and other pigments, 5 ml each of chloroform and distilled water were added to the extract. The mixture was shaken and allowed to separate, and the upper aqueous phase was collected for analysis.

For the **color development,** 1 ml of the aqueous phase was treated with 1 ml of freshly prepared ninhydrin reagent. The reaction mixture was heated in a boiling water bath for 20 minutes. After cooling, 5 ml of 50% isopropanol was added as a diluent. Absorbance was recorded at 570 nm using a **2375 Double Beam Spectrophotometer,** which detects the intensity of the purple-colored complex (Ruhemann’s purple) formed by the reaction between ninhydrin and free amino acids.

Quantification was performed using a standard curve prepared with alanine, and the free amino acid content was expressed in mg/g dry weight. For each plant species, samples from three different organs—leaf, stem, and root—were analyzed. Each organ sample was processed with three biological replicates (collected from three individual plants) and three technical replicates (repeated measurements of the same extract), ensuring accuracy, consistency, and reproducibility of the data across all plant parts.

RESULTS

Total free amino acids were estimated in different organs—leaf, stem, and root—of five selected Solanaceae species of the same age collected from the Menal Forest region, Chittorgarh, Rajasthan. The results (Table 1) reveal clear interspecific and organ-specific variations in amino acid content. Among the species studied, *Datura innoxia* consistently exhibited the highest amino acid concentrations across all organs, particularly in leaves (68.09 mg/g dry weight), stems (54.07 mg/g), and roots (35.08 mg/g), indicating its superior metabolic activity and potential adaptability to the semi-arid forest environment. *Solanum virginianum* and *Datura fastuosa* also showed relatively high amino acid levels in leaves (57.07 mg/g and 56.03 mg/g, respectively), stems (46.03 mg/g and 49.05 mg/g), and roots (31.05 mg/g and 28.09 mg/g), reflecting their biochemical richness and possible roles in stress tolerance and physiological functions. Conversely, the lowest amino acid content was found in *Physalis angulata* (48.06 mg/g in leaves) and *Physalis minima* (35.04 mg/g in stems and 20.03 mg/g in roots), which may be due to differences in metabolic pathways or ecological adaptations. Notably, leaves of all species contained the highest amino acid concentrations, underscoring their primary role in amino acid biosynthesis and overall plant metabolism. The average total free amino acid content across all organs was highest in *Datura innoxia* (52.41 mg/g), followed by *Solanum virginianum* (44.72 mg/g), and lowest in *Physalis angulata* (35.38 mg/g). These findings highlight the nutritional and pharmacological potential of these wild Solanaceae species, supporting their traditional uses and encouraging further exploration of their bioactive compounds for pharmaceutical and nutraceutical applications. Among the five Solanaceae species studied, *Datura innoxia* demonstrated the highest amino acid content, supporting its established use in traditional medicine for treating pain, asthma, inflammation, and muscle spasms, owing to its antispasmodic, anti-inflammatory, and analgesic properties. *Solanum virginianum*, a well-known Ayurvedic herb (Kantakari), is widely used in managing respiratory disorders, liver ailments, and bacterial infections, and is a key component of classical formulations like Dashmool and Chyawanprash. *Datura fastuosa* (syn. *D. metel*) is valued for its effectiveness in treating neurological conditions such as epilepsy and convulsions, supported by the presence of alkaloids like scopolamine and atropine. *Physalis angulata*, though lower in amino acid content, is traditionally employed by tribal communities for its antimalarial, anti-asthmatic, and anti-inflammatory benefits. Lastly, *Physalis minima*, despite its relatively low biosynthetic profile, holds ethnomedicinal importance in the treatment of fever, hepatitis, and urinary tract infections, likely due to its rich reservoir of withanolides and flavonoids.

Top of Form

Bottom of Form

**Table 1: Total free amino acids (mg/g dry weight) in different organs of selected Solanaceae species from the Menal Forest region, Chittorgarh, Rajasthan**

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| **S. No.** | | **Name of species** | | | | | **Leaf** | | **Stem** | | | **Root** | | **Total in**  **entire plant** | |
| 1. | | *Datura fastuosa* | | | | | 56.03 | | 49.05 | | | 28.09 | | 44.39 | |
| 2. | | *Datura innoxia* | | | | | 68.09 | | 54.07 | | | 35.08 | | 52.41 | |
| 3. | | *Physalis angulate* | | | | | 48.06 | | 36.03 | | | 22.04 | | 35.38 | |
| 4. | | *Physalis minima* | | | | | 55.07 | | 35.04 | | | 20.03 | | 36.71 | |
| 5. | | *Solanum virginianum* | | | | | 57.07 | | 46.03 | | | 31.05 | | 44.72 | |
|  | |  | | | | |  | |  | | |  | |  | |
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**Fig 1: Graph of Total Free Amino Acids (Mg/G Dry Weight) In Different Organs of Selected Solanaceae Species from Menal Forest, Chittorgarh**

DISCUSSION

The study revealed notable interspecific and organ-specific differences in total free amino acid content among five wild Solanaceae species from the Menal Forest. *Datura innoxia* showed the highest concentrations in all plant parts—leaves (68.09 mg/g), stems (54.07 mg/g), and roots (35.08 mg/g)—indicating strong metabolic activity and potential adaptation to semi-arid conditions. Its high foliar amino acid levels reflect active biosynthesis, aligning with its known pharmacological uses in managing pain, inflammation, and respiratory disorders. *Solanum virginianum* and *Datura fastuosa* also recorded substantial amino acid content, particularly in leaves, supporting their medicinal roles in treating liver ailments, infections, and neurological conditions. Conversely, *Physalis angulata* and *Physalis minima* showed lower amino acid levels, especially in roots and stems. Despite this, they remain important ethnomedicinal plants, likely due to other active constituents such as withanolides and flavonoids.

Across all species, leaves consistently had the highest amino acid content, highlighting their central role in biosynthesis. On average, *D. innoxia* ranked highest (52.41 mg/g), followed by *S. virginianum* (44.72 mg/g), and *P. angulata* lowest (35.38 mg/g), reinforcing the nutritional and therapeutic potential of these wild taxa.

CONCLUSION

This study highlights the biochemical diversity of selected Solanaceae species from the Menal Forest, with Datura innoxia emerging as the richest in total free amino acids. Its established medicinal use is supported by its superior metabolic profile. Solanum virginianum and Datura fastuosa also exhibit promising amino acid content, while Physalis species, though comparatively lower, hold phytochemical significance due to other bioactive constituents. Leaf tissues were found to be the most metabolically active across all species, reaffirming their central role in biosynthesis.

Although the medicinal potential of Solanaceae is well documented, there has been limited organ-specific quantification of free amino acids in wild populations from semi-arid forest regions like Menal. This study addresses that gap by providing foundational data on the amino acid content of underexplored species. These findings support continued biochemical exploration of wild Solanaceae for potential pharmacological and nutraceutical applications.

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REFERENCE

Moore, S., & Stein, W. H. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry, 176*(1), 367–388.

Lewis, O. A. M., Nieman, E., & Munz, A. (1970). Origin of amino acids in *Datura stramonium* seeds. *Annals of Botany, 34*(4), 843–848.

Lim, T. K. (2012). Edible medicinal and non-medicinal plants: Volume 3, Fruits. Springer Science & Business Media.Patel, P. R., et al. (2011). Physiochemical changes in sunberry (*Physalis minima* L.) fruit during growth and ripening. *Fruits, 66*(1), 37–47.

Karpagasundari, C., & Kulothungan, S. (2014). Analysis of bioactive compounds in *Physalis minima* leaves using GC-MS, HPLC, UV-VIS, and FTIR techniques. *Journal of Pharmacognosy and Phytochemistry, 3*(4), 196–201.

Sharma, M., Dhaliwal, I., Rana, K., Delta, A. K., & Kaushik, P. (2021). Phytochemistry, pharmacology, and toxicology of *Datura* species—A review. *Antioxidants, 10*(8), 1291.

Nagarkar, S. S. (2021). Biochemical analysis of pollen grains of *Datura innoxia* L. for screening its allergenicity.

Pęksa, A., Miedzianka, J., Nemś, A., & Rytel, E. (2021). The free-amino-acid content in six potato cultivars through storage. *Molecules, 26*(5), 1322.

Mezhlumyan, L. G., Khikmatullaev, I. L., Rakhimova, S. K., Narbutaeva, D. A., Yusupova, S. M., & Aripova, S. F. (2022). Amino-acid composition and hypoglycemic properties of proteins from *Physalis alkekengi* and *P. angulata*. *Chemistry of Natural Compounds, 58*(1), 187–189.

Mukhtar, Z. G., Özer, D., Karataş, F., & Saydam, S. (2022). Amino acid contents of some eggplant species grown in different regions. *Journal of the Institute of Science and Technology, 12*(2), 857–869.

Staveckienė, J., Medveckienė, B., Vaštakaitė-Kairienė, V., Kulaitienė, J., & Jarienė, E. (2024). Amino acid changes during maturation in *Solanum* fruit. *Agriculture, 14*(6), 802.

Ozeer, F. Z., Nagandran, S., Wu, Y. S., Wong, L. S., Stephen, A., Lee, M. F., ... & Sarker, M. M. R. (2024). A comprehensive review of phytochemicals of *Withania somnifera* (L.) Dunal (Solanaceae) as antiviral therapeutics. *Discover Applied Sciences, 6*(4), 187.

Sharma, M. K., Sharma, R., Arora, D., & Kanche, M. S. (2024). Beyond blossoms: Ethnobotany’s insight into the diversity of angiosperm families. *Shineeks Publishers*.

Desai, H. S., Singh, S., Agarwal, V., & Barot, M. (2025). Ethnobotanical review of wild edible plants. In *Exploring Traditional Wild Edible Plants* (pp. 1–26). CRC Press.

Arora, A., Jain, S., & Paliwal, V. (2025). Tyrosine-derived alkaloid diversity in some cultivated species of poppies (*Genus Papaver*): A review. *Natural Products: Phytochemistry, Botany, Metabolism of Alkaloids, Phenolics and Terpenes*, 1–40.

Ma, X., Zhang, C., Yang, L., Hedges, S. B., & Zhong, B. (2025). New insights on angiosperm crown age based on Bayesian node dating and skyline fossilized birth-death approaches. *Nature Communications, 16*(1), 2265.