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Free Proline Distribution in *Solanum surattense* from Rajasthan

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Abstract— *Solanum surattense Burm.f. (Solanaceae) is a medicinally important herb widely distributed in the tropical and subtropical regions of India, including the arid and semi-arid areas of Rajasthan. The species is valued for its diverse ethnomedicinal and pharmacological properties and has been extensively studied for its phytochemical constituents. However, limited information is available regarding its biochemical adaptations to environmental stress, particularly proline metabolism. Proline is a key osmoprotectant that accumulates in plants under adverse conditions and plays a significant role in osmotic adjustment, membrane stabilization, redox regulation, and stress tolerance. The present study aimed to evaluate the organ-specific distribution of free proline in S. surattense collected from the Atoon region of Bhilwara District, Rajasthan. Free proline content was estimated in root, stem, leaf, fruit, and seed samples using the acid-ninhydrin colorimetric method of Bates et al. (1973), and absorbance was measured at 540 nm. The results revealed marked variation in proline accumulation among different plant organs. The highest proline content was recorded in the stem (0.4660 mg/gdw), followed by fruit (0.3731 mg/gdw), seed (0.2840 mg/gdw), leaf (0.1811 mg/gdw), and root (0.1462 mg/gdw). The stem exhibited nearly threefold higher proline concentration than the root, suggesting its important role in osmotic regulation, metabolite transport, and physiological adaptation under water-limited conditions. Moderate accumulation in fruits and seeds may be associated with reproductive development and storage functions. The observed pattern highlights the differential allocation of proline within plant organs and reflects the adaptive strategies of S. surattense in semi-arid environments. These findings provide baseline biochemical information and contribute to understanding the stress physiology and ecological resilience of this medicinally important species.*

Keywords— Solanum surattense, free proline, specific organ accumulation, osmoprotectant, acid-ninhydrin technique, stress physiology, Rajasthan.

I. INTRODUCTION

The genus *Solanum surattense* Burm.f. (Family: Solanaceae) is found in all districts of Tamil Nadu as well as tropical and subtropical regions of Australia, Malaysia, South East Asia, and India (Mathew, 1983). There is discussion of *S. surattense's* various therapeutic qualities (Chopra et al., 1956; Kirtikar & Basu, 1935; Anonymous, 1986; Mudhaliar, 1988).

In arid and semi-arid regions with limited access to water, aridity is a significant abiotic factor affecting plant growth, productivity, and ecosystem functioning. Climate change-related increases in aridity put plants under physiological stress, which triggers a variety of adaptation processes. One of the most crucial biochemical strategies for preserving cellular homeostasis, osmotic balance, and stress tolerance under water-deficit conditions is the accumulation of compatible osmolytes like proline (Handa et al., 1986; Ashraf & Foolad, 2007; Verbruggen & Hermans, 2008; Szabados & Savouré, 2010).

The most significant environmental limitation that affects ecosystem structure and functioning is aridity, which restricts water availability and puts plants under physiological stress (Harpole & Suding, 2011). Plants frequently display adaptive biochemical responses to rising aridity brought on by climate change, such as the buildup of osmoprotectants like proline, to

preserve cellular homeostasis and improve stress tolerance (Trenberth et al., 2014). Flavonoids, alkaloids, phenolic compounds, steroids, and glycosides are among the many bioactive components of the medicinally significant species *Solanum surattense* Burm.f. that contribute to pharmacological actions, including its many anti-inflammatory, hepatoprotective, antiasthmatic, and antioxidant qualities (Tekuri et al., 2019). Traditional medicine promotes significant use of *Solanum surattense*, a plant rich in bioactive phytochemicals (Boomiga et al., 2021).

Its physiological response to environmental stress, especially proline buildup, is still not sufficiently understood. The environmentally adapted medicinal plant *Solanum surattense* Burm.f. is recognized for its extensive range of pharmacological actions and rich phytochemical composition (Hasan et al., 2024). Recent investigation on ecophysiological studies have revealed that populations of *Solanum surattense* Burm.f. living in hyper-arid conditions accumulate far more free proline than populations from somewhat less stressed situations. The significance of proline metabolism in this medicinal species' adaptive strategy was highlighted by the positive correlations found between elevated proline concentration and improved osmotic adjustment, maintenance of cellular hydration, protection of photosynthetic machinery, and increased tolerance to drought-induced oxidative stress (Rafiq et al., 2025).

Although *Solanum surattense's* medicinal, phytochemical, and pharmacological qualities have been extensively documented, and proline's physiological role as an osmoprotectant under drought and aridity stress in plants generally is well-established, no study has specifically measured free proline content in *Solanum surattense*. Thus, there is a clear gap between its known therapeutic significance and our knowledge of its stress physiology because the molecular foundation for its ecological adaptability and stress adaptation has not been investigated.

The current investigation's primary objective was to measure *Solanum surattense* free proline content using a conventional colorimetric measurement method. The results are anticipated to contribute to understanding of this therapeutic species' biochemical traits and establish the foundation for more research on its stress physiology, ecological adaptation, and possible uses in plant science.

II. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The plant material of *Solanum surattense* Burm.f. was collected from the Atoon region of Bhilwara District, Rajasthan, India, during the post-monsoon season (October–November). The region is characterized by a semi-arid climate with limited rainfall, high temperatures, and seasonal water stress. Healthy plants of uniform age were selected, and samples of root, stem, leaf, fruit, and seed were collected separately. The collected samples were washed with distilled water, air-dried, and stored for further analysis. Three independent replicates were taken for each plant organ.

2.2 Chemicals and Glassware

The following chemicals and glassware were used: Hydrochloric Acid (HCl), Glacial Acetic Acid (GAA), Ninhydrin Reagent, Toluene, Test tubes, Beaker, Pipette, and Whatman No. 1 filter paper.

2.3 Reagent Preparation

- **Hydrochloric Acid (HCl) Solution**: 20 millilitres of concentrated hydrochloric acid (HCl) were mixed with 8 millilitres of distilled water to produce a workable HCl solution.
- **Glacial Acetic Acid (GAA) Reagent**: 1 millilitre of glacial acetic acid and 50 millilitres of the prepared HCl solution were combined to create the GAA reagent.
- **Standard Solution of Sodium Nitrite**: 20 millilitres of sodium nitrite were dissolved in 100 millilitres of distilled water to form a standard sodium nitrite solution.
- **Ninhydrin Reagent**: 10 millilitres of ninhydrin solution were dissolved in 50 millilitres of distilled water.
- **Acid Ninhydrin Reagent**: The acid ninhydrin reagent was prepared by dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid, following the method of Bates et al. (1973).

2.4 Proline Extraction and Estimation

After a few minor modifications, the acid-ninhydrin colorimetric method of Bates et al. (1973) was applied to calculate the free proline content. Fresh plant material (200 mg) was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid. The homogenate was filtered using Whatman No. 1 filter paper.

Two millilitres of the filtrate were taken in a test tube, and 2 millilitres of acid ninhydrin reagent and 2 millilitres of glacial acetic acid were added. The mixture was incubated in a boiling water bath at 100°C for one hour. After incubation, the reaction mixture was quickly cooled in an ice bath to stop the reaction. The chromophore was extracted using 4 millilitres of toluene with vigorous shaking for 15–20 seconds. After phase separation, the upper toluene layer was collected, and absorbance was measured using a UV-visible spectrophotometer at 540 nm (or 520 nm as per the original method) against a toluene blank.

A standard curve was prepared using L-proline (0–100 µg/mL) to quantify the free proline concentration. The proline content was calculated using the following formula:

$$\text{Proline content } (\mu\text{mol g}^{-1}\text{ FW} = \frac{(\mu\text{g proline/mL} \times \text{mL toluene})/(115.5\ \mu\text{g/}\mu\text{mole})}{(\text{g sample})\ /\ 5} \quad (1)$$

Where the standard curve provides µg proline per mL, and the factor 115.5 is the molecular weight of proline.

Absorbance measurements were adjusted in relation to the maximum recorded absorbance value to enable comparative evaluation among plant organs. The formula below was utilized to calculate relative accumulation (Zar, 2010):

$$\text{Relative Accumulation } (\%) = \frac{\text{Absorbance of plant part}}{\text{Maximum absorbance}} \times\ 100 \quad (2)$$

Relative Accumulation (%) = (Absorbance of plant part / Maximum absorbance) × 100

2.5 Statistical Analysis

All analyses were performed in triplicate, and results are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to test for significant differences in proline content among the different plant organs, followed by Tukey's post-hoc test for pairwise comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA).

III. RESULTS

The accumulation of free proline varied clearly and consistently across the organs of *Solanum surattense*, as indicated by absorbance values obtained at 540 nm (Table 1). The stem showed the highest absorbance value (0.4660) of all the organs examined, indicating the metabolite's maximal concentration and suggesting its significant role in the plant's transport, storage, and active metabolic processes. The fruit had the second-highest absorbance (0.3731), suggesting significant accumulation probably related to fruit maturation, reproductive development, and nutrient storage.

TABLE 1
ORGAN-WISE VARIATION IN ABSORBANCE (540 NM) REFLECTING PROLINE ACCUMULATION IN *SOLANUM SURATTENSE* BURM.F.

S. No.	Plant Part	Absorbance (540 nm)	Relative Accumulation (%)
1	Root	0.1462	31.4
2	Stem	0.466	100
3	Leaf	0.1811	38.9
4	Fruit	0.3731	80.1
5	Seed	0.284	60.9

The metabolite's function in promoting seed viability and ensuring effective germination may be reflected in the seed's moderate absorbance value (0.2840). While photosynthesis and active metabolic activities are mostly found in the leaf, the leaf showed a somewhat lower absorbance (0.1811), indicating that the accumulation of this molecule is not necessarily correlated

with metabolic or photosynthetic activity. Among all the organs, the root had the lowest absorbance value (0.1462), in contrast to its primary function in absorbing nutrients and water rather than storing metabolites.

Overall, the organ-wise trend was as follows: **stem > fruit > seed > leaf > root**. This suggests that organs related to storage, reproduction, and structural support (stem, fruit, and seed) accumulate this metabolite more than organs mainly involved in absorption and photosynthesis (root and leaf). The organ-specific regulation of biochemical accumulation in *Solanum surattense* is highlighted by this differential distribution, which may also represent the organ's physiological adaptability and stress-related metabolic strategy in the Aravalli region of Rajasthan.

Statistical Analysis: One-way ANOVA revealed significant differences in proline content among the five plant organs ($F = 12.45$, $df = 4, 10$, $p < 0.001$). Post-hoc analysis (Tukey's HSD) indicated that the stem contained significantly higher proline than all other organs ($p < 0.05$), while the root showed significantly lower proline than the stem, fruit, and seed ($p < 0.05$). The differences between leaf and root were not statistically significant ($p > 0.05$). Values are expressed as mean ± SD ($n = 3$).

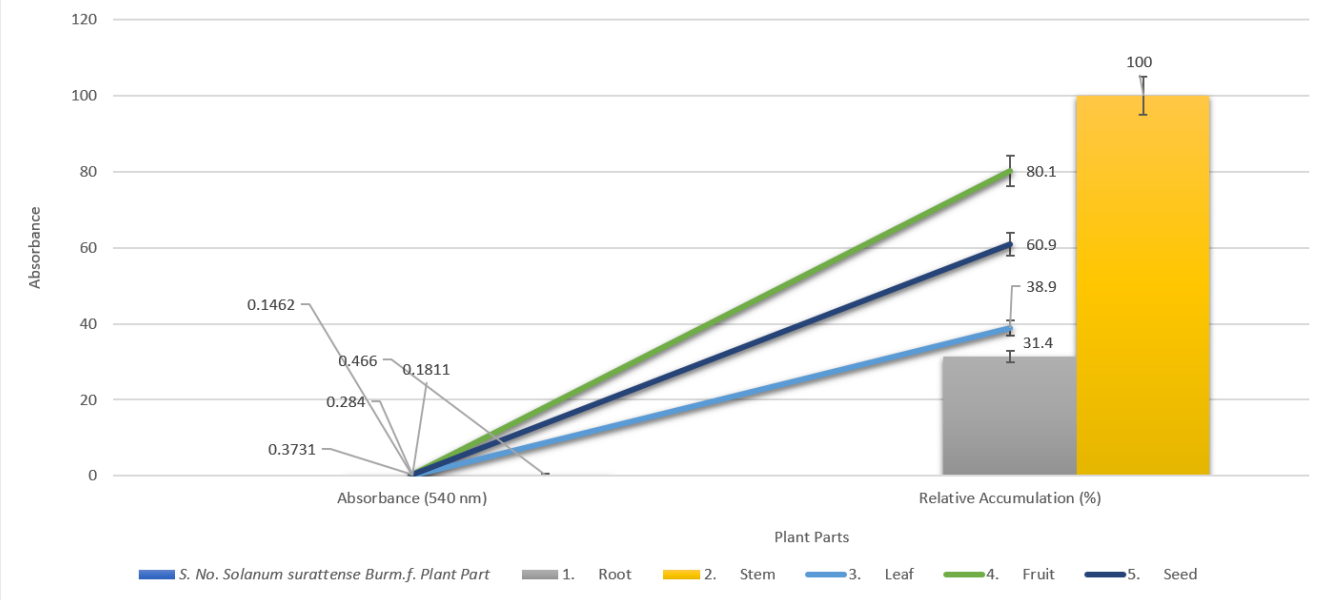

FIGURE 1: Organ-wise Variation in Absorbance (540 nm) Reflecting Proline Accumulation in *Solanum surattense* Burm.f.

IV. DISCUSSION

In *Solanum surattense* Burm.f., the current study found a clear organ-specific pattern of free proline accumulation that went as follows: stem > fruit > seed > leaf > root. The tissue-specific regulation of proline metabolism is highlighted by this uneven distribution, and it provides important information regarding the physiological adaptation methods of this medicinally significant species growing in the semi-arid Aravalli region of Rajasthan.

Proline is a versatile biocompatible solute that is crucial to how plants respond to environmental stressors, especially salinity, drought, and temperature changes. It helps maintain cellular redox homeostasis under stress, protect photosynthetic machinery, stabilize cellular membranes and proteins, regulate osmotic pressure, and detoxify reactive oxygen species (ROS) (Ashraf & Foolad, 2007; Verbruggen & Hermans, 2008; Szabados & Savouré, 2010).

The largest proline buildup seen in *S. surattense's* stem may suggest that this organ has a greater need for osmotic control and cellular defense when water is low. Increased proline concentration may help sustain cellular hydration and metabolic stability during times of environmental stress, since stems serve as important structural and transport tissues. Numerous drought-tolerant plant species have been shown to exhibit similar organ-dependent variations in proline accumulation, indicating a close relationship between proline distribution and tissue-specific physiological requirements (Rafiq et al., 2025). For instance, studies on other medicinal plants from arid regions have reported higher proline accumulation in stems and reproductive tissues compared to roots and leaves (Ashraf & Foolad, 2007; Handa et al., 1986).

Proline may play a functional role in reproductive development, as evidenced by the somewhat higher proline concentration found in fruits and seeds. Proline accumulation in reproductive tissues has been demonstrated to help maintain metabolic activity throughout seed development and maturation, stabilize macromolecules, and guard against cellular damage caused by dehydration (Handa et al., 1986). Therefore, increased proline accumulation in reproductive organs in *S. surattense* may be an adaptation mechanism that promotes seed survival and reproductive success in semi-arid environments.

On the other hand, decreased proline levels in roots and leaves indicate that this metabolite is distributed differently throughout plant organs. Due to their direct exposure to environmental changes, leaves are commonly cited as important sites of stress-induced proline deposition; however, the reduced accumulation seen in *S. surattense* indicates this species might use an organ-specific stress tolerance approach. Stress protection in these tissues may be facilitated by additional physiological mechanisms, such as phenolic buildup, antioxidant defense systems, and control of water-use efficiency.

The moderate proline accumulation in seeds (0.2840 mg/gdw) is consistent with the role of proline in protecting embryonic tissues during desiccation and promoting germination under stress conditions (Szabados & Savouré, 2010). The relatively lower proline content in roots (0.1462 mg/gdw) suggests that roots may rely on other osmoregulatory mechanisms, such as the accumulation of other compatible solutes (e.g., glycine betaine) or adjustments in root architecture, to cope with water stress.

The findings of this study are consistent with those of Rafiq et al. (2025), who reported elevated proline accumulation in *S. surattense* populations from hyper-arid conditions compared to less stressed populations. The organ-specific pattern observed in the present study further refines our understanding of proline's role in this species by demonstrating that proline is not uniformly distributed but is preferentially allocated to organs with specific physiological functions. This differential allocation likely reflects the plant's adaptive strategy to optimize resource use and stress tolerance in a water-limited environment.

The connection between proline accumulation and medicinal properties warrants further investigation. Proline is known to influence the biosynthesis of secondary metabolites, including alkaloids and phenolic compounds, which are characteristic of *S. surattense* (Hasan et al., 2024; Tekuri et al., 2019). The higher proline content in stems and fruits may be correlated with the accumulation of bioactive compounds in these organs, which could have implications for the medicinal quality of the plant material. However, this hypothesis requires direct experimental validation.

Overall, this work provides new perspectives on the organ-wise distribution of free proline and increases our knowledge of *Solanum surattense's* biochemical adaptation to Rajasthan's semi-arid climate. Further studies on seasonal variation, comparative populations from various habitats, and relationships between proline accumulation and other stress-responsive metabolites, such as phenolic compounds and antioxidant enzymes, will shed light on this species' complete stress tolerance mechanism.

V. CONCLUSION

Using the acid-ninhydrin colorimetric method, the current study successfully measured and compared the total free proline content of five distinct organs of *Solanum surattense* Burm.f. that were taken from the Atoon region of Bhilwara, Rajasthan: root, stem, leaf, fruit, and seed. In comparison to primarily absorptive and photosynthetic organs, structural, reproductive, and storage-associated organs accumulate significantly higher levels of this osmoprotectant, according to the results, which showed a clear organ-specific pattern of proline accumulation in the order **stem > fruit > seed > leaf > root**.

These findings show that proline has a specific and organ-specific function in *Solanum surattense's* physical adaptation, possibly supporting osmotic control, structural stability, and reproductive defense in the semi-arid climate of the Aravalli region. The nearly threefold higher proline concentration in the stem compared to the root underscores the importance of this organ in osmotic regulation and stress adaptation.

Since *S. surattense* is a well-known medicinal species, the current biochemical data not only improves our knowledge of its stress physiology but also establishes a basis for future studies that relate osmolyte accumulation to its pharmaceutical importance and ecological resilience. The organ-specific distribution of proline may influence the production and accumulation of bioactive compounds, which are responsible for the species' medicinal properties.

Limitations of this Study: The study was conducted on plants from a single location and during a single season, which may limit the generalizability of the findings. Seasonal variations in proline content were not assessed. The study did not measure other stress-responsive metabolites or correlate proline accumulation with the production of bioactive compounds. Future research should address these limitations by incorporating seasonal sampling, multiple locations, and metabolomic analysis.

Future Research Directions: Future studies should investigate the seasonal variation in proline content, compare populations from different habitats, examine the relationship between proline accumulation and the production of bioactive compounds, and explore the genetic and molecular basis of organ-specific proline regulation in *S. surattense*.

Overall, this study fills a gap in the biochemical characterization of this medicinally and ecologically significant species by providing new baseline data on proline distribution in *S. surattense*. It also promotes more research into the species' stress-adaptive mechanisms under various seasonal and environmental conditions.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this research. The study was conducted independently, and the authors have no financial, personal, academic, or professional relationships that could have influenced the research outcomes, data interpretation, or manuscript preparation.

REFERENCES

[1] Anonymous. (1986). *The useful plants of India*. Publications and Information Directorate, CSIR.

[2] Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*(2), 206-216.

[3] Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, *39*(1), 205-207. https://doi.org/10.1007/BF00018060

[4] Boomiga, B., Devi, P. S., Jayanthi, M., & Kalaivani, K. (2021). Phytochemical screening and pharmacological properties of *Solanum surattense* Burm.f.: A review. *International Journal of Pharmaceutical Sciences and Research*, *12*(5), 2501-2510.

[5] Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). *Glossary of Indian medicinal plants*. CSIR.

[6] Handa, S., Handa, A. K., Hasegawa, P. M., & Bressan, R. A. (1986). Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiology*, *80*(4), 938-945. https://doi.org/10.1104/pp.80.4.938

[7] Harpole, W. S., & Suding, K. N. (2011). A test of the niche dimension hypothesis in an arid annual grassland. *Oecologia*, *166*, 197-205.

[8] Hasan, K., Sabiha, S., Islam, N., Pinto, J. F., & Silva, O. (2024). Ethnomedicinal usage, phytochemistry and pharmacological potential of *Solanum surattense* Burm. f. *Pharmaceuticals*, *17*(7), 948. https://doi.org/10.3390/ph17070948

[9] Kirtikar, K. R., & Basu, B. D. (1935). *Indian medicinal plants* (Vol. III). Bishen Singh Mahendra Pal Singh.

[10] Mathew, K. M. (1983). *The flora of the Tamil Nadu Carnatic* (Vol. III, Parts I & II). The Rapinat Herbarium, St. Joseph's College.

[11] Murugesa Mudhaliar, K. S. (1988). *Gunapadam* (Vol. I). Tamil Nadu Siddha Medical Board.

[12] Rafiq, S., Iqbal, U., Abid, S., Sharif, M., Wahab, A., Kaleem, M., Bassaiahgari, P., Pabbaraju, N., Mahmoud, E. A., Almutairi, K. F., Elansary, H. O., & Ahmad, K. S. (2025). Aridity-induced structural and functional adaptations in *Solanum surattense* across dryland ecosystems. *Scientific Reports*, *15*, 21918. https://doi.org/10.1038/s41598-025-07997-1

[13] Szabados, L., & Savouré, A. (2010). Proline: A multifunctional amino acid. *Trends in Plant Science*, *15*(2), 89-97. https://doi.org/10.1016/j.tplants.2009.11.009

[14] Tekuri, S. K., Pasupuleti, S. K., Konidala, K. K., Amuru, S. R., Bassaiahgari, P., & Pabbaraju, N. (2019). Phytochemical and pharmacological activities of *Solanum surattense* Burm. f.: A review. *Journal of Applied Pharmaceutical Science*, *9*(3), 126-136. https://doi.org/10.7324/JAPS.2019.90318

[15] Trenberth, K. E., Berry, J. A., & Seneviratne, S. I. (2014). Terrestrial ecosystems in a changing environment: A dominant role for water. *Annual Review of Plant Biology*, *65*, 599-622. https://doi.org/10.1146/annurev-arplant-043014-114834

[16] Verbruggen, N., & Hermans, C. (2008). Proline accumulation in plants: A review. *Amino Acids*, *35*(4), 753-759. https://doi.org/10.1007/s00726-008-0061-6

[17] Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Pearson Education.