

# Comparative Phytochemical Profiling and Free Proline Accumulation in *Butea Monosperma* Var. *Lutea* and *Terminalia Arjuna*: Taxonomical and Stress Adaptive Significance

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## ABSTRACT

The biochemical characterisation of endangered angiospermic plants is still lacking, despite their considerable therapeutic and ecological importance. In this work, the phytochemical components and free proline concentration of *Terminalia arjuna* and *Butea monosperma* var. *lutea* are assessed and compared in relation to their taxonomical relevance. Important secondary metabolites identified by qualitative phytochemical screening using conventional biochemical techniques include alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds. The free proline content was evaluated using the ninhydrin approach as a gauge of stress adaption.

The findings demonstrated biochemical diversity among the chosen taxa by revealing discernible interspecific variation in phytochemical composition. Differential buildup of proline was seen in the two species, indicating differences in their physiological reactions to environmental stressors. *Terminalia arjuna* showed a more rich phytochemical profile, whereas *Butea monosperma* var. *lutea* showed a higher proline concentration, suggesting improved stress resistance.

The detected biochemical changes support the role of phytochemicals and free proline as useful tools in experimental taxonomy and stress physiology. Even though they cannot replace conventional morphological traits, these features provide helpful extra information for taxonomic assessment and conservation-focused botanical study of endangered angiospermic species.

Quantitative estimation revealed that free proline content was highest in leaves followed by stem and root in both species. However, *Butea monosperma* var. *lutea* consistently exhibited higher proline levels across all plant parts compared to *Terminalia arjuna*, indicating a superior stress adaptive potential.

**Keywords:** *Butea monosperma* var. *lutea*; *Terminalia arjuna*; phytochemical analysis; free proline; endangered angiosperms; experimental taxonomy; stress physiology

## 1. INTRODUCTION

Angiospermic plants, the biggest and most diverse group of plants, are vital to ecological balance, biodiversity, and human health (Raven, Evert & Eichhorn, 2013). Many angiosperms have significant medical, commercial, and ecological significance, but overexploitation, habitat destruction, and unfavorable environmental circumstances have put some species in danger of going extinct (IUCN, 2022;

Singh & Rawat, 2012). Therefore, for the conservation and sustainable use of endangered angiospermic plants, scientific documentation and biochemical characterization are essential (Jain, 1991; Ncube, Afolayan & Okoh, 2008).

In taxonomy, morphological and anatomical characteristics have historically been the main factors used to identify and categorize plants (Stace, 1989). However, biochemical and phytochemical criteria have become more significant in recent years as additional instruments in experimental taxonomy and systematic botany (Harborne, 1998; Swain, 1973). Glycosides, alkaloids, flavonoids, tannins, and saponins and phenolic chemicals are examples of secondary metabolites that differ considerably between plant species. Phytochemical profiling is a useful method for taxonomic evaluation and interspecific comparison since this variation frequently reflects underlying genetic variations and adaptive strategies (Harborne & Turner, 1984; Trease & Evans, 2002) (Figure 4 & 5).

Among the biological markers that have been studied the most in plant ecology and physiology studies is free proline. As a suitable osmolyte, proline builds up in plants under numerous abiotic stresses, including drought, salinity, and extremely high or low temperatures (Bates, Waldren & Teare, 1973; Ashraf & Foolad, 2007). It is essential for osmotic adjustment, protein and membrane stability, and oxidative stress defense (Szabados & Saviouré, 2010). Proline buildup has been regularly used as a measure of stress tolerance and adaptation capacity in plants because of its stress-responsive nature (Kavi Kishor et al., 2005). Proline's differential accumulation among species offers useful supplemental information for taxonomical and comparative investigations, despite the fact that it is not regarded as a primary taxonomic characteristic (Misra & Gupta, 2005).

*Butea monosperma* var. *lutea* and *Terminalia arjuna* are two angiospermic species with important ethnobotanical and medicinal uses (Kirtikar & Basu, 1935; Nadkarni, 2007). Both species differ significantly in morphology, habitat preference, and physiological responses, while being well-known for their ecological roles and therapeutic purposes (Kumar & Singh, 2015). Despite their importance, there are still few comparative studies that concentrate on their phytochemical composition and proline accumulation, especially in connection to taxonomy and stress resistance.

The current study compares *Terminalia arjuna* with *Butea monosperma* var. *lutea* in terms of phytochemical analysis and free proline quantification. The study aims to analyze the usefulness of proline content and phytochemicals as auxiliary tools in taxonomical interpretation and conservation-oriented botanical research on endangered angiospermic species, in addition to evaluating interspecific biochemical variation (Figure 1 & 2).



Figure 1- (A) *Terminalia arjuna* (B) *Butea monosperma* var. *lutea*

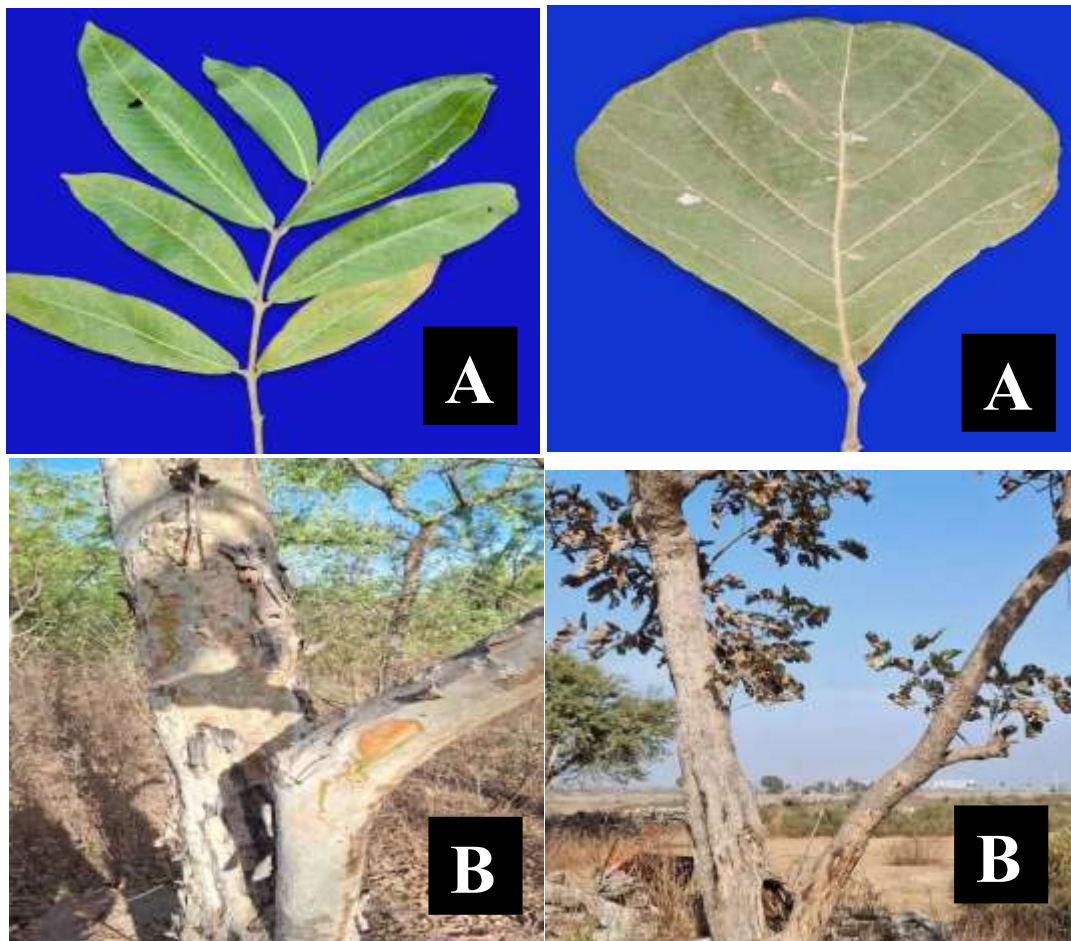




Figure 2- (A)*Terminalia arjuna*- (a) Leaf (b) Stem (c) Root (B) *Butea monosperma* var. *lutea*- (a) Leaf (b) Stem (c) Root

## 2. METHODS AND MATERIALS

### 2.1 Selection and Collection of Plant Material

During the active growth season, healthy, disease-free plant samples of *Terminalia arjuna* and *Butea monosperma* var. *lutea* were removed from their natural habitat. Mature plants exhibiting typical morphological traits were carefully chosen. Standard floras and taxonomic keys were used to authenticate the plant items that were gathered. Additionally, being employed for phytochemical analysis, New leaves were utilized for proline estimate.

### 2.2 Preparation of Plant Material

To get rid of dust and debris, the obtained Plant samples were carefully washed with distilled water after being under running tap water. After being shade-dried for seven to ten days at room temperature, the samples were ground into a powder using a mechanical grinder. For additional phytochemical investigation, the powdered the content was retained in airtight containers.

### 2.3 Preparation of Plant Extracts

Approximately 10 g of dried plant powder were extracted each using methanol as a solvent and cold maceration for 48 hours. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were further concentrated under reduced pressure. Until they were required for phytochemical screening, the concentrated extracts were stored at 4°C.

### 2.4 Qualitative Phytochemical Screening

Standard biochemical techniques were employed in a qualitative phytochemical analysis of the plant extracts to determine the presence of important secondary metabolites. The phytochemicals analyzed included alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids. The results were categorized as present (+), considerably present (++), or absent (–) based on color intensity and precipitate formation.

### 2.5 Calculation of Free Proline

Fresh leaf samples were used to determine the free proline content using the conventional ninhydrin technique. Fresh leaf tissue weighing around 0.5 g was homogenized in 3% sulfosalicylic acid before being filtered. The filtrate was incubated in a boiling water bath after reacting with glacial acetic acid and acidic ninhydrin reagent. After cooling the reaction mixture, toluene was used to extract the chromophore. A spectrophotometer was used to test the coloured solution's absorbance at 520 nm. A standard curve was

used to calculate the proline concentration, which was then represented as  $\mu\text{mol}$  proline per gram of fresh plant tissue weight.

## 2.6 Analysis of Statistics

Each experiment was conducted in triplicate, and the findings were expressed using the mean  $\pm$  standard deviation. A comparison between the two species was carried out in order to assess interspecific variation in proline content and phytochemical components.

## 3. RESULT

The proline content in different plant organs (leaves, Stem, Roots) of endangered angiospermic species collected from forest and wildlife sanctuary regions of south-central Rajasthan showed marked interspecific variation. In this study, a targeted comparative analysis was carried out between *Butea monosperma* var. *lutea* and *Terminalia arjuna* to evaluate physiological differences related to stress tolerance. *Butea monosperma* var. *lutea* was collected from **Bhanwariya Khurd village near Katunda Mod, Chittorgarh district**, while *Terminalia arjuna* the samples were gathered from the **Harni Mahadev forest area, Bhilwara district**. The variation that was noticed in proline accumulation reflects species-specific adaptive responses to environmental stress conditions prevailing in their respective habitats.

### Leaf Proline Content (Table 1 & Figure 3)

In leaves, *Butea monosperma* var. *lutea* exhibited a **high proline concentration (13.75 mg/gdw)**, whereas *Terminalia arjuna* showed the **lowest proline level (5.89 mg/gdw)** among the studied species. The elevated leaf proline content in *Butea* indicates a stronger osmotic adjustment mechanism, enabling better tolerance to drought and environmental stress conditions prevalent in semi-arid regions.

### Stem Proline Content (Table 1 & Figure 3)

Rhizome analysis showed that *Butea monosperma* var. *lutea* accumulated **9.15 mg/gdw of proline**, which was significantly more than that recorded in *Terminalia arjuna* (**5.24 mg/gdw**). This suggests that *Butea* maintains metabolic resilience even in underground storage organs, supporting sustained growth during prolonged stress periods.

### Root Proline Content (Table 1 & Figure 3)

Roots of *Butea monosperma* var. *lutea* contained **8.54 mg/gdw of proline**, while *Terminalia arjuna* had a markedly lower value (**2.34 mg/gdw**). Given that roots are in intimate contact with soil moisture deficit, higher proline accumulation in *Butea* roots reflects superior drought adaptability and protection of cellular structures.

### Whole-Plant Proline Accumulation (Table 1 & Figure 3)

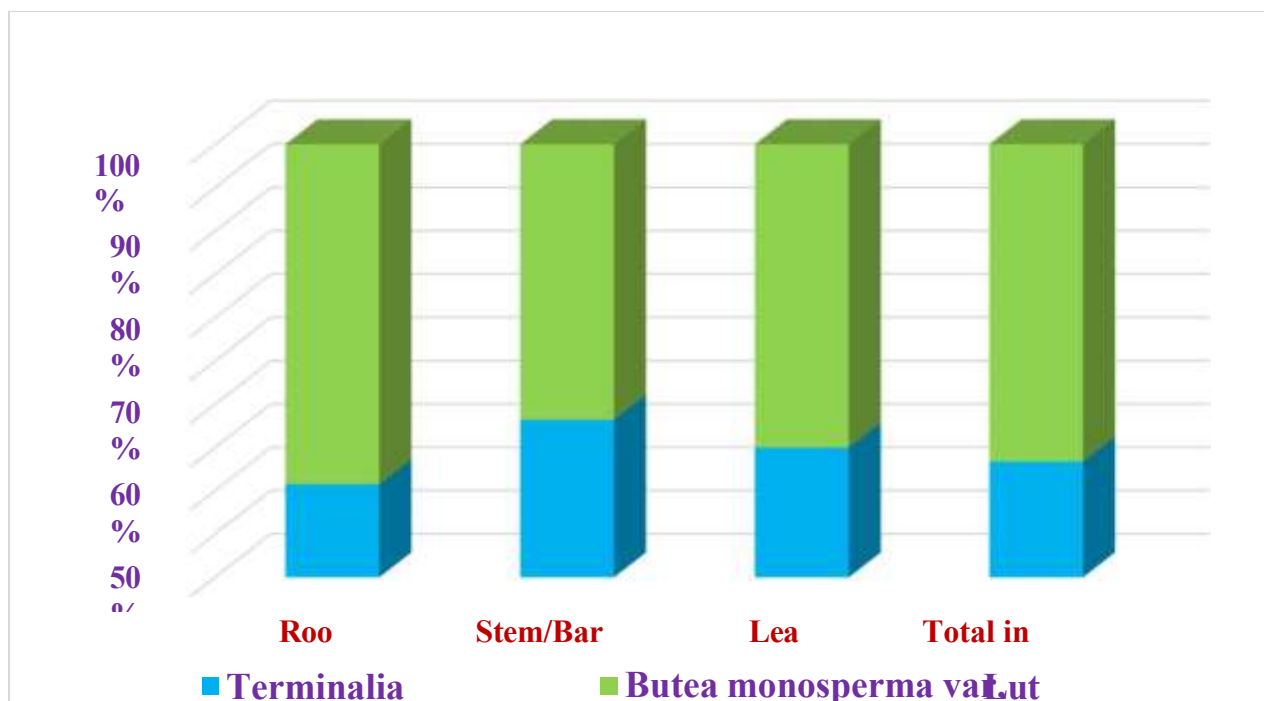
When total plant proline content was considered, *Butea monosperma* var. *lutea* recorded a **high cumulative value of 18.50 mg/gdw**, in contrast to *Terminalia arjuna*, which showed the **lowest overall proline content (6.76 mg/gdw)**. This three-fold difference underscores fundamental physiological divergence between the two species.



Table: - 1

**TOTAL FREE PROLINE (mg/gdw) IN VARIOUS PARTS OF *Butea monosperma* var. *lutea* AND *Terminalia arjuna***

| S.No. | Plant Parts           | <i>Butea monosperma</i> var. <i>lutea</i> | <i>Terminalia arjuna</i> |
|-------|-----------------------|-------------------------------------------|--------------------------|
| 1.    | Leaves                | 13.75                                     | 5.89                     |
| 2.    | Stem                  | 9.15                                      | 5.24                     |
| 3.    | Root                  | 8.54                                      | 2.34                     |
| 4.    | Total in entire Plant | 18.50                                     | 6.76                     |



**Figure: 3- TOTAL FREE PROLINE (mg/gdw) IN VARIOUS PARTS OF *Butea monosperma* var. *lutea* AND *Terminalia arjuna***

| S. No | Plant Part Name | Chemical Extract | Tanin | Saponin | Alkaloid | Flavonoid | Phenol | Terpenoid | Coumarin | Anthroquinone | Steroid |
|-------|-----------------|------------------|-------|---------|----------|-----------|--------|-----------|----------|---------------|---------|
| 1     | Stem            | Ethanol          | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 2     |                 | Methanol         | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 3     |                 | Chloroform       | -     | -       | -        | -         | -      | +         | -        | -             | +       |
| 4     |                 | Haxen            | -     | -       | -        | -         | -      | +         | -        | -             | +       |
| 5     | Leaves          | Ethanol          | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 6     |                 | Methanol         | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 7     |                 | Chloroform       | -     | -       | -        | -         | -      | +         | -        | -             | +       |
| 8     |                 | Haxen            | -     | -       | -        | -         | -      | +         | -        | -             | +       |
| 9     | ROOT            | Ethanol          | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 10    |                 | Methanol         | +     | +       | +        | +         | +      | +         | -        | -             | +       |
| 11    |                 | Chloroform       | -     | -       | -        | -         | -      | +         | -        | -             | +       |
| 12    |                 | Haxen            | -     | -       | -        | -         | -      | +         | -        | -             | +       |

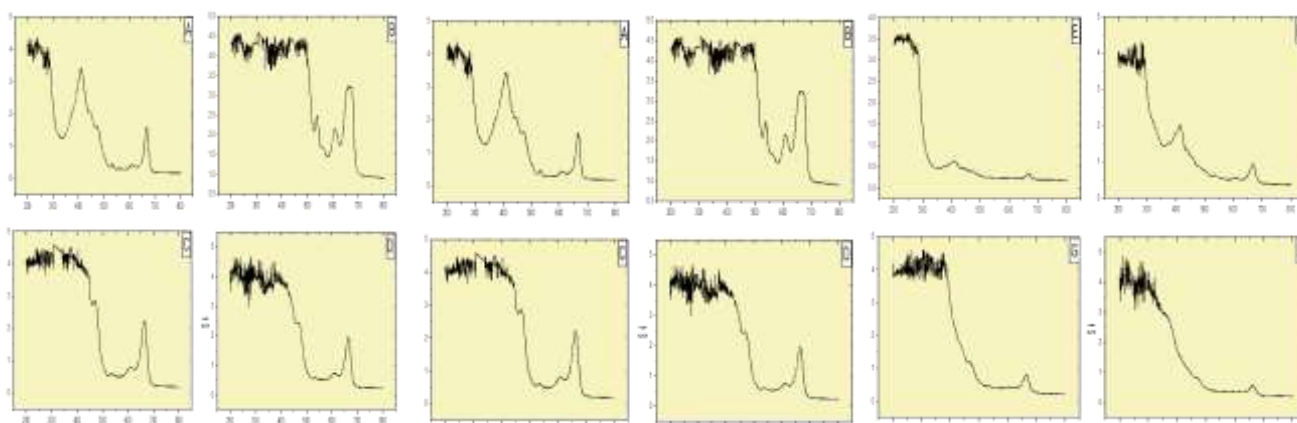
Figure 4- *Terminalia arjuna* Secondary Metabolites result

| S.No | Plant Part Name | Chemical Extract | Tanin | Saponin | Alkaloid | Flavonoid | Phenol | Terpenoid | Coumarin | Anthroquinone | Steroid |
|------|-----------------|------------------|-------|---------|----------|-----------|--------|-----------|----------|---------------|---------|
| 1    | Stem            | Ethanol          | +     | +       | +        | +         | +      | +         | +        | -             | +       |
| 2    |                 | Methanol         | +     | +       | +        | +         | +      | +         | +        | -             | +       |
| 3    |                 | Chloroform       | -     | -       | -        | -         | -      | +         | +        | -             | +       |

|    |        |                |   |   |   |   |   |   |   |   |   |
|----|--------|----------------|---|---|---|---|---|---|---|---|---|
| 4  |        | Haxen          | - | - | - | - | - | + | + | - | + |
| 5  | Leaves | Ethanol        | + | + | - | + | + | + | - | - | - |
| 6  |        | Methanol       | + | + | - | + | + | + | - | - | - |
| 7  |        | Chlorofoa<br>m | - | - | - | - | - | + | - | - | - |
| 8  |        | Haxen          | - | - | - | - | - | + | - | - | - |
| 9  | ROOT   | Ethanol        | + | + | - | + | + | + | - | - | - |
| 10 |        | Methanol       | + | + | - | + | + | + | - | - | - |
| 11 |        | Chlorofoa<br>m | - | - | - | - | - | + | - | - | - |
| 12 |        | Haxen          | - | - | - | - | - | + | - | - | - |

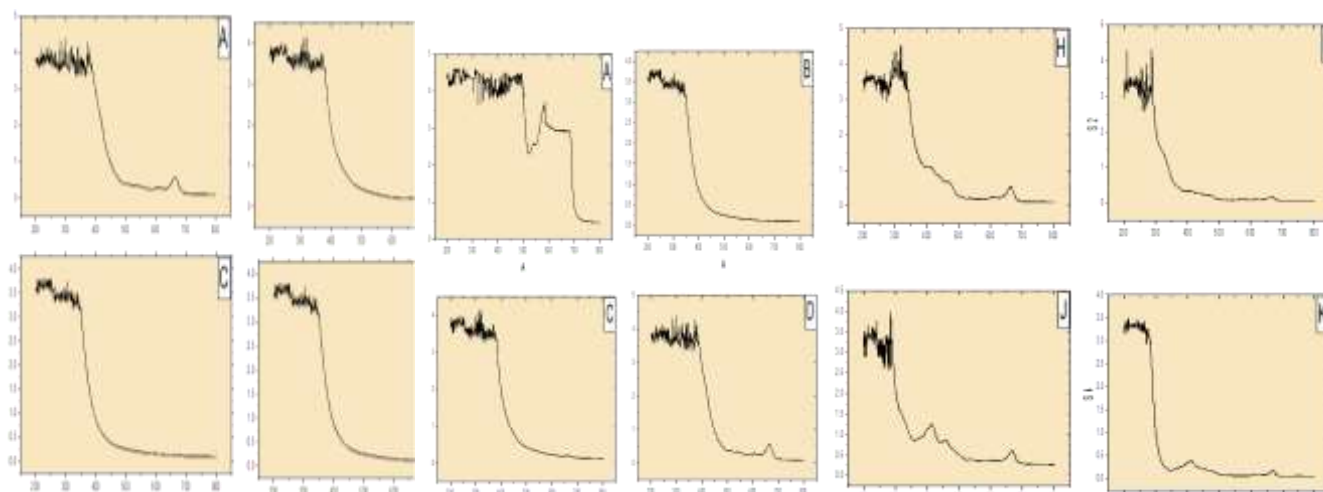
Figure 5 - *Butea monosperma* var. *lutea* Secondary Metabolites result

**FIGURE 6:** - THE PHYTOCHEMICAL STUDIES OF SECONDARY METABOLITES IN LEAVES, STEM & ROOT OF *BUTEA MONOSPERMA* VAR. *LUTEA* IN DIFFERENT T5 YPE OF SOLUTION



Extract :- (1) Ethanol (2) Methanol (3)Choloroform (4)Haxen

**FIGURE 7:** - THE PHYTOCHEMICAL STUDIES OF SECONDARY METABOLITES IN LEAVES, STEM & ROOT OF *TERMINALIA ARJUNA* IN DIFFERENT TYPE OF SOLUTION



**Extract :- (1) Ethanol (2) Methanol (3)Choloroform (4)Haxen**

#### 4. DISCUSSION

One well-known biochemical indicator of abiotic stress resistance is proline buildup, especially in situations including heat stress and drought. The current comparative analysis unequivocally shows that the proline concentration of *Terminalia arjuna* and *Butea monosperma* var. *lutea* varies significantly between species across several plant parts.

Proline concentrations in all plant sections of *Butea monosperma* var. *lutea* were consistently greater than those of *Terminalia arjuna*. A remarkably high proline concentration (13.75 mg/gdw) was found in the leaf tissue of *Butea monosperma* var. *lutea*, suggesting active osmotic correction and improved stress sensitivity in photosynthetically active tissues. Proline levels in *Terminalia arjuna* leaves, on the other hand, were much lower (5.89 mg/gdw), indicating a comparatively poorer metabolic response to environmental stress.

There were also notable differences in the proline content of the roots between the two species. *Terminalia arjuna* had the lowest proline content (2.34 mg/gdw), while *Butea monosperma* var. *lutea* collected 8.54 mg/gdw. *Butea monosperma* var. *lutea* has increased proline accumulation because its roots are immediately exposed to soil moisture deficiencies. At the rhizospheric level, *lutea* exhibits improved cellular defense mechanisms and drought awareness.

In a similar vein, *Butea monosperma* var. *lutea*'s stem/bark tissues have higher proline levels (9.15 mg/gdw) than *Terminalia arjuna*'s (5.24 mg/gdw). This implies that proline synthesis and transport throughout the plant body are made possible by *Butea monosperma* var. *lutea*'s effective systemic stress signaling mechanism.

*Butea monosperma* var. *lutea* (18.50 mg/gdw) demonstrated an almost three-fold increase accumulation than *Terminalia arjuna* (6.76 mg/gdw) when the total proline content of the entire plant was taken into account. This significant variation demonstrates species-specific physiological adaptations and shows that *Butea monosperma* var. *lutea* is relatively more resilient to the extended drought and heat stress that frequently occurs in the south-central Rajasthan region.

*Terminalia arjuna*'s comparatively mesic nature and restricted ability to adapt to xeric and semi-arid environments may be the cause of its decreased proline accumulation. According to ecophysiological data in the research area, decreased proline synthesis in *Terminalia arjuna* is likewise linked to increased membrane damage and decreased stress tolerance. (Table 1 & Figure 3)

#### 5. CONCLUSION

*Butea monosperma* var.'s free proline content was compared. Proline accumulation is a crucial biochemical sign of stress tolerance among endangered angiospermic species in south-central Rajasthan, as demonstrated by *lutea* and *Terminalia arjuna*.

In all plant organs, *Butea monosperma* var. *lutea* showed noticeably greater proline accumulation, suggesting better adaptation mechanisms against heat stress and drought. Increased proline levels in stem tissues, roots, and leaves indicate effective osmotic control, cellular defense, and metabolic resistance in challenging environmental circumstances.

*Terminalia arjuna*, on the other hand, continuously showed the lowest proline content, indicating a lack of stress adaptation and a greater vulnerability to temperature stress and moisture deficiency. Reduced ability for osmotic adjustment and membrane stability is further suggested by the low content of proline in roots and leaves. Overall, the results show that *Terminalia arjuna* needs relatively favorable microclimatic conditions for long-term growth, while *Butea monosperma* var. *lutea* has a stronger

physiological and biochemical foundation for survival under arid and semi-arid habitats. These findings highlight the significance of proline-based biochemical screening in determining endangered plant species' drought tolerance and conservation priority.

While *Butea monosperma* var. *lutea* can be regarded as a relatively resilient species appropriate for restoration programs in stress-prone landscapes, *Terminalia arjuna* should be given particular consideration by a conservation standpoint through habitat protection, assisted regeneration, and moisture conservation strategies.

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