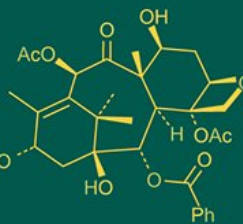
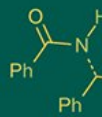


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Morphological, physicochemical and phytochemical characterization of *Butea Monosperma* (Lam) taub flowers collected from different locations of Jammu region

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Abstract

Butea monosperma (Lam.) Taub., sometimes referred to as Palash or Flame of the Forest, is a significant medicinal tree that is found all throughout the Indian subcontinent and is a member of the Fabaceae family. The blooms are prized for their natural hues, substantial nutritional content and variety of bioactive chemicals that provide important anti-oxidant and medicinal qualities. The current study "Morphological, Physico-chemical and Phytochemical Characterization of *Butea monosperma* (Lam.) Taub. Flowers Collected from Different Locations of Jammu Region," was carried out at the Division of Post Harvest Management, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-Jammu) during 2025-2026 due to their growing potential in functional foods, nutraceuticals and pharmaceutical applications. Four districts in the Jammu region viz., Kathua, Samba, Jammu and Udhampur were used to gather fresh flowers, which were then subjected to conventional analytical techniques. In addition to physico-chemical and phytochemical parameters like ash, crude protein, crude fat, titratable acidity, total sugars, reducing sugars, total phenolic content, ascorbic acid, total carotenoids and mineral composition, the study evaluated morphological features like flower color, dimensions, calyx characteristics, moisture content and visual quality.

For the examined quality parameters, the analysis showed significant diversity among the chosen regions. Ash content varied between 6.90 and 11.00 per cent, crude protein between 2.96 and 3.16 per cent, crude fat between 3.52 and 5.69 per cent, titratable acidity between 2.82 and 3.18 per cent, ascorbic acid between 6.92 and 10.76 mg/100g, total carotenoids between 3.81 and 4.45 mg/100g and total phenolic content between 18.20 and 41.70 mg GAE/100g. The flowers' potential as a useful natural source of bioactive components was demonstrated by their notable quantities of nutrients and anti-oxidant chemicals. The results show that *B. monosperma* flowers' nutritional and phytochemical content is greatly influenced by their geographic location and emphasize its appropriateness for use in the functional food, pharmaceutical, nutraceutical and natural colorant sectors.

Keywords: palash, physico-chemical characterization, phytochemical characterization, anti-oxidant activity, total phenolic content, carotenoids, ascorbic acid, bioactive compounds.

Introduction

Butea monosperma (Lam.) Taub., a medium-sized deciduous tree in the Fabaceae family, is often referred to as palash, dhak, tesu or the "Flame of the Forest". It is extensively dispersed in India, Nepal, Bangladesh, Sri Lanka, Myanmar and Thailand and is indigenous to South-east Asia and the Indian sub-continent. Tropical and sub-tropical regions are ideal for the species' growth, especially wastelands, open grasslands and dry deciduous woods. *B. monosperma* has drawn significant attention in both traditional medicine and contemporary scientific study due to its exceptional adaptability, ecological value and variety of therapeutic characteristics (Sindhia and Bairwa, 2010; Kumari *et al.*, 2022; Joshi *et al.*, 2024) [5, 12]. The tree is distinguished by its brilliant orange-red flowers that bloom during late winter and early spring, imparting a fiery appearance to the landscape and earning it the popular name "Flame of the Forest". *B. monosperma* has a significant role in Indian culture and traditional healthcare systems in addition to its decorative value. The plant is considered a useful

medicinal species in Nighantus and traditional Ayurvedic writings (Ahire *et al.*, 2020). Four types of Palash have been identified, according to Raj Nighantu: Rakta (red), Pita (yellow), Shweta (white) and Nila (blue). While the other types are somewhat uncommon, the Rakta cultivar is the most prevalent and widely used for both commercial and medical uses (Pawar *et al.*, 2019). The leaves are sewn into biodegradable disposable plates (patravali), symbolizing the plant's cultural and environmental significance, while the blooms are historically utilized to make natural colors for the Holi celebration (Wanjari *et al.*, 2016; Methaniya *et al.*, 2023) [6].

The ethno-botanical importance of palash is notably obvious among the indigenous and tribal populations of India. Various plant parts, including flowers, bark, leaves, seeds, roots and gum, have been utilized for generations to manage a wide range of diseases. Traditional healers utilize the plant in the treatment of skin problems, diarrhea, dysentery, 2.

Materials and Methods

Plant material and sampling

Fresh, fully opened, healthy and disease-free flowers of *Butea monosperma* (Lam.) Taub. were collected in the month of march from naturally growing trees in the Jammu region, Jammu and Kashmir, India. In order to prevent moisture loss and retain their biological components, flowers were hand plucked between 7:00 and 9:00 AM. For the study, only flowers with uniform color and maturity, free of insect infestation, mechanical damage, or any obvious symptoms of disease were chosen. Within two hours after being harvested, the flowers were transferred to the Post Harvest Management Laboratory of SKUAST-Jammu under ambient conditions in clean, perforated polyethylene bags. Flowers were air-dried at room temperature, gently cleaned with distilled water to get rid of dust and other surface impurities, and then sorted to get rid of samples that were damaged or wilted. For physical, biochemical, phytochemical and anti-oxidant investigations, representative samples were chosen at random and split into three repetitions. While the remaining samples were either shade-dried or kept at -20°C until further examination, fresh flowers were utilized right away for moisture, color and other quality evaluations.

Physical characterization

Flower color

A Hunter Lab Colorimeter (Hunter Associates Laboratory, USA) and the Royal Horticultural Society (RHS) Color Chart were used to visually assess the color of fresh palash flowers. Prior to measurement, conventional white and black tiles were used to calibrate the device. The values of L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were acquired by taking color readings at three distinct points on the petals. The representative color value was determined by taking the mean of three observations.

Flower length and width (CM)

A digital Vernier calliper (Aerospace, China) was used to measure the length and width of the flowers. Flower width was measured across the fully extended petals and flower length was measured from the base of the calyx to the tip of the standard petal. All measurements were recorded in centimetres (cm).

Moisture content of fresh flower (%)

The hot air oven technique was used to assess the fresh flower samples' moisture content. A fresh flower sample weighing around 5 g was precisely weighed into a moisture dish that had been previously weighed. The sample was then dried in a hot air oven at 105°C until a consistent weight was reached, signifying total moisture elimination. Following drying, the samples were reweighed after being chilled in a desiccator to stop atmospheric moisture absorption. The following formula was used to compare the weight loss before and after drying in order to determine the % moisture content:

$$\text{Moisture (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}] \times 100$$

Calyx characteristics

Both qualitative and quantitative criteria were used to assess the flowers' calyx features. Careful visual inspection was used to record qualitative findings, such as calyx color, pubescence and form. A digital Vernier calliper was used to measure the calyx diameter at its widest point and its length from base to apex for quantitative evaluation. The observations were utilized to evaluate the morphological difference among the flower samples and all measures were recorded in millimetres (mm).

Visual quality score

The overall visual quality of the fresh flowers was evaluated using a five-point hedonic scale based on their external appearance, color, freshness and overall market acceptability. The flowers were visually assessed immediately after collection, and scores were assigned according to their quality attributes. A score of 5 indicated excellent quality with fresh, bright, and undamaged flowers; 4 indicated good quality with only minor defects; 3 represented fair quality with noticeable signs of deterioration; 2 denoted poor qualities with considerable loss of freshness and appearance and 1 indicated unacceptable quality, where the flowers were severely deteriorated and unsuitable for further use.

Biochemical analysis

Crude fat

The Soxhlet extraction technique was used to assess the crude fat content of *Butea monosperma* flowers (Ranganna, 2001). A cellulose extraction thimble was filled with around 2-5 g of finely powdered, oven-dried floral material that had been precisely weighed. Petroleum ether was utilized as the extraction solvent and the thimble was put inside the Soxhlet extraction device. For six to eight hours, the extraction was done continuously until the solvent in the siphon tube became colorless, signifying that all of the sample's lipids had been extracted. The extraction flask holding the crude fat was put on a water bath to evaporate any remaining petroleum ether after the extraction was finished and the solvent was recovered by distillation. After that, the flask was dried for approximately an hour at 105°C in a hot air oven, or until a consistent weight was achieved. Following drying, the flask was precisely weighed and chilled in a desiccator to stop moisture absorption. The amount of crude fat that was removed from the sample was shown by the extraction flask's weight increasing. The weight difference between the flask before and after extraction was used to compute the crude fat content, which

was then represented as a percentage (%) based on dry weight.

Fat (%) = $\frac{\text{Weight of the flask after extraction} - \text{initial weight of flask}}{\text{Weight of sample}} \times 100$

Determination of titratable acidity

Alkali titration was used to calculate titratable acidity (TA), which was then represented as a percentage of citric acid (Ranganna, 2001). A 250 mL beaker was filled with 100 mL of carbon dioxide-free distilled water and around 1.0 g of coarsely crushed fresh flower sample. To ensure that the organic acids were completely extracted, the mixture was thoroughly agitated for ten minutes before being filtered using Whatman No. 1 filter paper. Two or three drops of phenolphthalein indicator were added to a conical flask containing an aliquot of 10.0 mL of the clear filtrate. The end point was determined by titrating the sample against standardized 0.1 N sodium hydroxide (NaOH) until a slight pink color remained for around 30 seconds. The titratable acidity was computed using the usual formula and represented as a percentage (%) of citric acid equivalent after the volume of NaOH drank was noted.

Determination of crude protein

The Kjeldahl technique was used to determine the crude protein content of the floral samples (Ranganna, 2001). A Kjeldahl digestion flask was filled with precisely 1.0 g of finely powdered dried flower sample. Concentrated sulfuric acid (H_2SO_4) was used to digest the sample while a catalyst combination of potassium sulphate (K_2SO_4) and copper sulphate (CuSO_4) was present. Digestion was carried out under carefully regulated heating until the solution became clear, signifying that all of the organic nitrogen had been converted to ammonium sulphate. The digest was sent to the distillation unit after cooling and being diluted with distilled water. The digest was then made extremely alkaline by adding an excess of sodium hydroxide (NaOH) solution, which released ammonia from the ammonium sulphate. A Kjeldahl distillation equipment was used to extract the released ammonia, which was then collected in a flask with 4% boric acid solution and an appropriate mixed indicator. Until the end point was achieved, the trapped ammonia was then titrated against standardized 0.1 N hydrochloric acid. The amount of acid used during titration was used to compute the nitrogen content, and the nitrogen percentage was multiplied by the conversion factor 6.25 to determine the crude protein concentration. The findings were given as a percentage (%) based on dry weight.

The total nitrogen (%) was calculated using the formula:

Total nitrogen (%) = $(V_1 - V_2) \times N \times 1.4007W$

where:

- V_1 = volume of HCl used for sample titration (mL)
- V_2 = volume of HCl used for blank (mL)
- N = normality of HCl
- W = weight of sample (g)

Crude protein (%) was then obtained by multiplying the nitrogen content by the conventional factor 6.25:

Crude Protein (%) = Nitrogen (%) $\times$ 6.25

Ash content

The standard technique was used to determine the total ash content of *Butea monosperma* flowers (Ranganna, 2001). A

pre-weighed silica crucible was filled with around 2.0 g of finely powdered dried flower sample. To char the sample and reduce spattering losses, the crucible was first slowly heated over a low flame. After that, it was moved to a muffle furnace that was kept at $550 \pm 5^\circ\text{C}$ and burned for four to six hours or until the organic matter had completely burned and turned into a light grey or white ash. The crucible was carefully taken out of the furnace after ashing, cooled in a desiccator to stop moisture absorption and then precisely weighed. The crucible was put back in the muffle furnace for further burning until a consistent weight was reached if carbon particles were still visible. The weight of the residue left over after burning was used to compute the ash content, which was then reported as a percentage (%) on a dry weight basis.

The following formula was used to represent the ash content as a percentage of dry weight:

Total ash (%) = $\frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$

Total and reducing sugars

Total sugar content of *Butea monosperma* flowers was estimated by the Anthrone method (Hedge and Hofreiter, 1962). The absorbance of the developed green color was measured at 620 nm using a UV-Visible spectrophotometer. Total sugar content was calculated using a glucose standard curve and expressed as g/100 g on a dry weight basis.

Reducing sugar content of *Butea monosperma* flowers was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The absorbance of the coloured reaction mixture was measured at 540 nm using a UV-Visible spectrophotometer. Reducing sugar content was calculated from a glucose standard curve and expressed as g/100 g on a dry weight basis.

Total phenolic content

Total phenolic content (TPC) of *Butea monosperma* flower extract was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). A sample of finely powdered flowers weighing about 0.5 g was extracted using 80% methanol and filtered through Whatman No. 1 filter paper. A 7.5% sodium carbonate solution was added after an aliquot of the extract was combined with Folin-Ciocalteu reagent and let to react for three to five minutes. After properly mixing the reaction mixture, it was incubated at room temperature for 30 minutes in the dark to allow for full color development.

Using a UV-visible spectrophotometer and a reagent blank, the absorbance of the resultant blue-colored solution was determined at 765 nm. Gallic acid was used to create a standard calibration curve, which was then used to determine the floral extract's total phenolic content. The results were expressed as milligrams of gallic acid equivalents (mg GAE/g) on a dry weight basis.

Ascorbic acid

Ascorbic acid content of *Butea monosperma* flowers was estimated by the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method (AOAC, 2022). A cold mortar and pestle were used to precisely weigh and homogenize a fresh flower sample weighing around 5 g with 3% metaphosphoric acid. Because metaphosphoric acid stabilizes ascorbic acid and keeps it from oxidizing throughout the extraction process, it acted as the extraction medium. To guarantee full ascorbic acid extraction, the homogenate was filtered through

Whatman No. 1 filter paper and the residue was repeatedly cleaned with tiny amounts of 3% metaphosphoric acid. The filtrate was collected in a volumetric flask, and the same extraction solution was used to increase the volume to a predetermined level.

An aliquot of the extract was put into a conical flask and titrated against a standardized 2,6-dichlorophenolindophenol (DCPIP) dye solution until a faint but noticeable pink color remained for about 15 seconds, at which time the titration was considered to have ended. The ascorbic acid concentration was computed using the previously established dye factor and the volume of dye eaten was noted. Mg of ascorbic acid per 100 g of fresh floral sample was used to express the results.

Mineral profile

The mineral profile of *Butea monosperma* flowers was determined following wet acid digestion of the dried flower samples. A nitric acid-perchloric acid combination was used to precisely weigh 0.5 g of finely powdered material into a digestion flask. The mixture was heated under controlled conditions until a clear, colorless solution was formed, signifying full digestion of the organic matter. The digest was filtered through Whatman No. 42 filter paper into a volumetric flask once it had cooled to room temperature. Deionized water was then added to the flask to bring its volume up to a known level. The amounts of important minerals, including calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), were measured using an Atomic Absorption Spectrophotometer (AAS) following calibration with suitable reference solutions. A flame photometer calibrated with standard sodium and potassium solutions was used to measure sodium (Na) and potassium (K) independently. Using the corresponding standard calibration curves, the concentration of each mineral was determined and reported as mg/100 g on a dry weight basis. Every calculation was done in triplicate, and the average results were shown.

Total carotenoids

Total carotenoid content of *Butea monosperma* flowers was determined following the method (Ranganna, 2010). Using a mortar and pestle, around 1 g of fresh floral material was coarsely homogenized with cooled acetone until the residue became colorless, signifying full carotenoid pigment extraction. To achieve maximal pigment recovery, the extract was filtered using Whatman No. 1 filter paper, and the residue was again extracted using fresh acetone. To eliminate the acetone phase, the combined filtrate was moved to a separating funnel filled with petroleum ether and distilled water was gradually added. After gathering the petroleum ether layer that contained the carotenoid pigments

and passing it through anhydrous sodium sulphate to eliminate any remaining moisture, the volume was increased to a predetermined level using petroleum ether. A UV-visible spectrophotometer was used to test the extract's absorbance at 450 nm using petroleum ether as the blank. Using the proper extinction coefficient, the total carotenoid content was computed and reported as mg/100 g on a fresh weight basis.

Statistical analysis

All experiments were conducted in triplicate, and the data obtained were expressed as mean $\pm$ standard deviation (SD). The experimental data were subjected to analysis of variance (ANOVA) under a Completely Randomized Design (CRD) to determine the significance of differences among treatments. The significance of treatment means was tested at the 5% probability level ($p \leq 0.05$), and mean comparisons were performed using the Least Significant Difference (LSD) test.

Results and discussions

Study area and geographical location of sampling sites

Butea monosperma flowers from four districts in the Jammu region viz., Jammu, Samba, Kathua and Udhampur were used in the current study (Figure 3.2). The Union Territory of Jammu and Kashmir's many agroclimatic zones are represented by these districts, which offer a variety of environmental conditions for assessing the morphological, physico-chemical and phytochemical traits of Palash flowers.

The sub-tropical plains of Jammu, Samba and Kathua are known for their warm summers, mild winters and moderate annual rainfall. Udhampur, on the other hand, has a milder sub-temperate environment with lower average temperatures throughout the flowering season due to its greater elevation. Different environmental circumstances that might affect plant development, blooming behavior and the synthesis of primary and secondary metabolites are created by the variations in altitude, temperature, rainfall and relative humidity among various places.

The manufacture of phenolic compounds, carotenoids, flavonoids, vitamins and other bioactive components in flowering plants is known to be regulated by environmental variables such as temperature, sun radiation, soil properties and moisture availability. Thus, the choice of these four districts offered a chance to evaluate the impact of agroclimatic and geographic diversity on the qualitative characteristics of *Butea monosperma* flowers. The current study's location-wise comparison offers useful baseline data for determining appropriate areas for the gathering and use of palash flowers for industrial, medical and nutritional purposes.

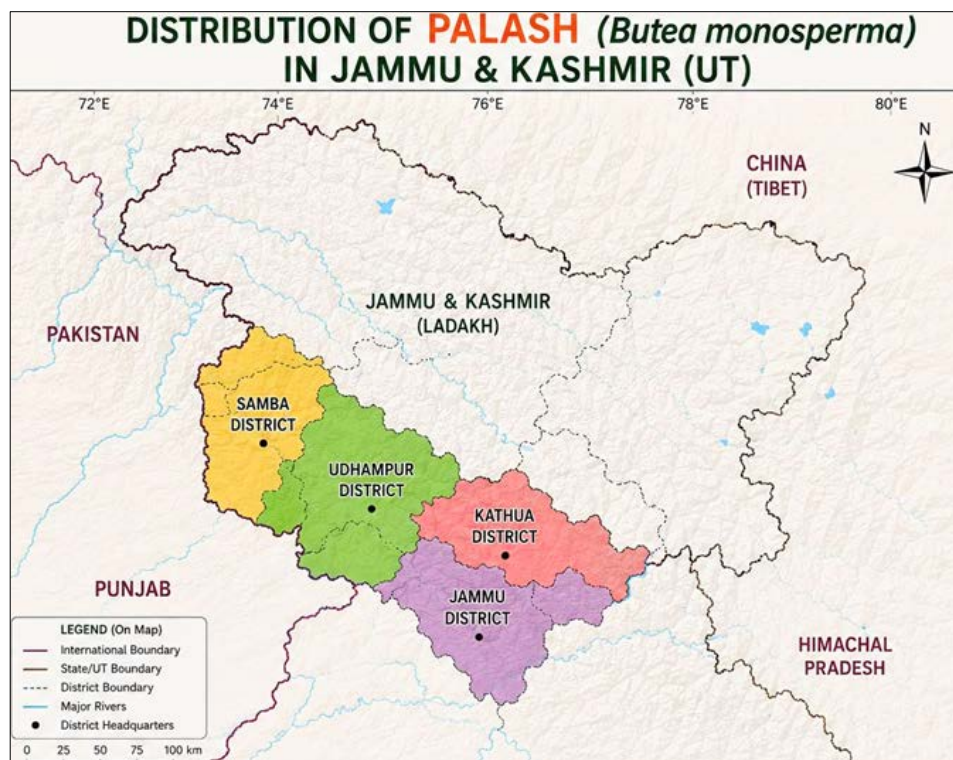
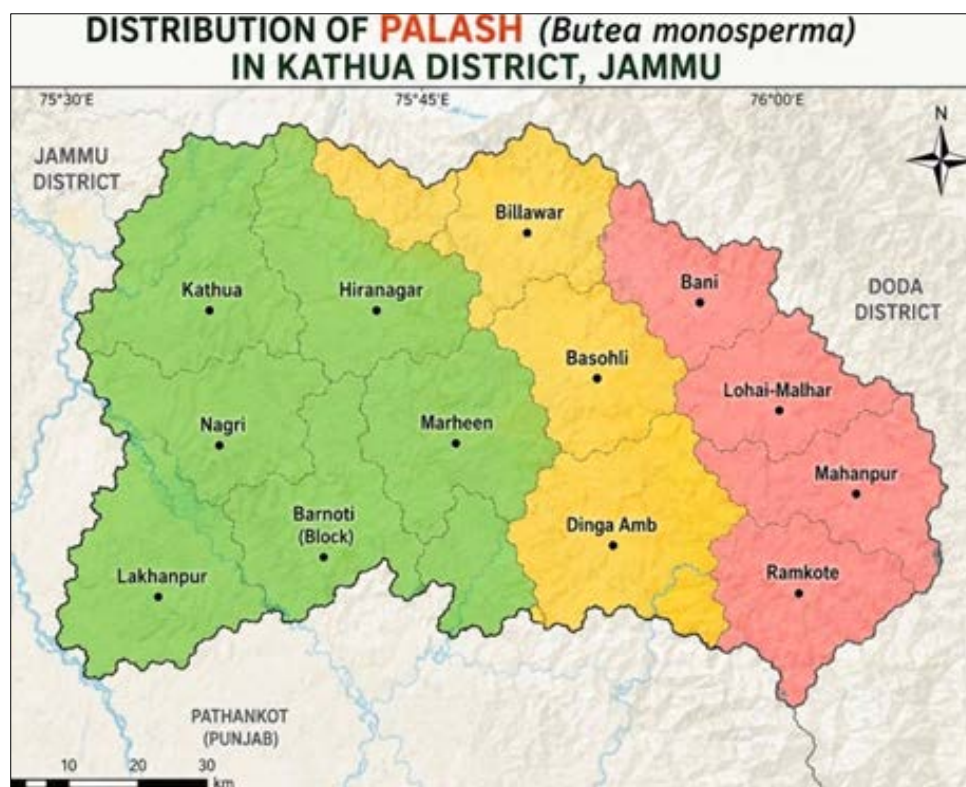


Fig 1: Map of Jammu and Kashmir showing the study area



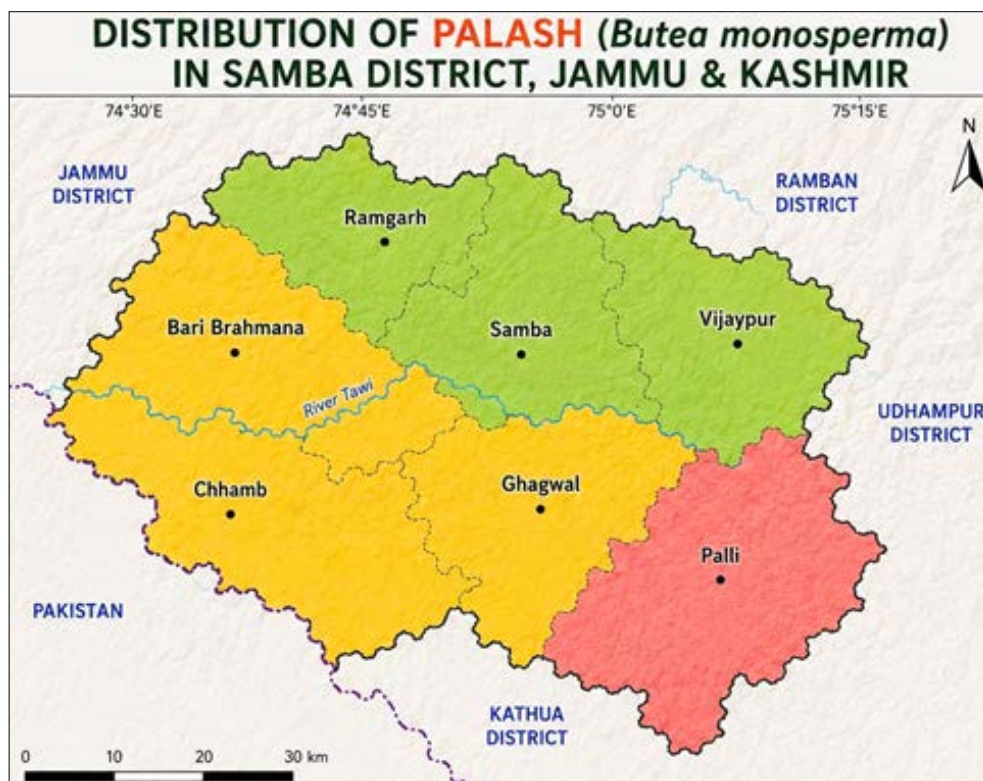




Fig 2: Sampling locations (Jammu, Samba, Kathua and Udhampur)

Morphological characteristics

Butea monosperma has thick racemes of vivid orange to flaming red blooms in late winter and early spring. Papilionaceous, meaty and extremely zygomorphic, the flowers have five petals namely, the standard, wings and keel as well as a campanulate calyx, which are characteristics of the Fabaceae family (Sindhia and Bairwa, 2010) [12]. The blooms have a distinctive beak-shaped corolla and a short pedicel, while the calyx is dark green, velvety and coated with small hairs. The nectar-rich blooms are a vital food source for birds and pollinating insects.

Physicochemical characteristics of *butea monosperma* flowers

The proximate analysis of *Butea monosperma* flowers revealed ash content (6.9-11%), crude protein (2.96-3.16%), crude fat (3.52-5.69%) and titratable acidity (2.82-3.18%), revealing a composition profile indicative of edible flowers with potential functional food uses (Table 1). Ash content reflects the mineral matter needed for bone health, enzyme cofactor function, with the highest values found in Samba (11%) and Jammu (10.5%), showing higher bioavailability of minerals in these districts.

Table 1: Physico-chemical characteristics of palash (*Butea monosperma*) flowers from different locations.

Physio-chemical properties of flower	Kathua	Samba	Jammu	Udhampur
Ash content (%)	6.9	11	10.5	8.7
Ascorbic acid (mg/100g)	10.76	10.66	6.92	9.48
Titrate acidity (%)	3.08	2.82	3.18	2.95
Total phenolic content (mg/100g)	18.2	30.7	41.7	22.7
Crude protein (%)	3.06	3.16	2.96	2.98
Oil content (%)	5.69	4.75	3.52	5.63
Total carotenoids (mg/100g)	4.45	3.93	3.81	4.01

The crude fat content varied greatly between sites (3.52-5.69%), with the highest values in Kathua (5.69%) and Udhampur (5.63%). This suggests that certain soil conditions and higher temperatures promote lipid formation and storage in floral tissues (Singer *et al.*, 2016) [13]. These lipids have several roles in flowers, such as serving as precursors of volatile signaling substances, energy storage molecules and structural membrane constituents (Borghi *et al.*, 2019). Crucially, palash flowers' oil content facilitates the possible extraction and use of lipid-soluble beneficial substances including fat-soluble vitamins and carotenoids (Fernandes *et al.*, 2018). The titratable acidity values (2.82-3.18%) show the presence of organic acids, most likely citric acid, malic acid and other weak acids that contribute

to the flowers' overall antioxidant potential by scavenging free radicals through their reducing properties (Kandyliis, 2022) [4].

A small but nutritionally significant addition to vitamin C consumption is the ascorbic acid content (6.92-10.76 mg/100 g). The presence of ascorbic acid in *B. monosperma* flowers, along with their high phenolic content, supports complementing anti-oxidant defense mechanisms, even if the amounts are lower than those in specific vitamin C-rich sources like citrus fruits (Gholivand and Piryaee, 2014). The greatest quantities of ascorbic acid were found in Kathua (10.76 mg/100 g) and Samba (10.66 mg/100 g), two locations with moderate to high water availability and comparatively mild temperatures that promote the

production of vitamin C. *Butea monosperma* flowers are a source of provitamin A molecules, which the body transforms into retinol and other active forms of vitamin A necessary for immune system function, eyesight, and epithelial tissue maintenance, according to the total carotenoid content (3.81-4.45 mg/100 g) (Blaner, 2020).

Conclusion

The current study, "Morphological, Physicochemical and Phytochemical Characterization of *Butea monosperma* (Lam.) Taub. Flowers Collected from Different Locations of Jammu Region," showed that *Butea monosperma* flowers are a rich source of nutrients and bioactive compounds with substantial potential for use in food, medicine and nutraceuticals. The morphological, physicochemical and phytochemical features of flowers taken from Kathua, Samba, Jammu and Udhampur varied noticeably, according to the study, suggesting that floral quality and composition are influenced by geographic location. In addition to having favorable physical attributes, the blossoms had significant concentrations of crude protein, crude fat, ash, sugars, ascorbic acid, carotenoids, phenolic compounds, and important minerals. The flowers' capacity to function as a useful natural source of antioxidants is demonstrated by their high total phenolic content, carotenoid concentration, and anti-oxidant activity. These bioactive components may support the traditional therapeutic applications of palash and help avoid illnesses connected to oxidative stress. The flowers' significance as a functional food ingredient is increased by the mineral analysis, which also verified that they contain nutritionally significant macronutrients and micronutrients. The observed variations between sites imply that the nutritional and phytochemical profile of *B. monosperma* flowers is influenced by geographic and environmental variables. Such data is helpful in determining the best places to gather premium flowers for use in industry and medicine.

Overall, *Butea monosperma* flowers are a promising neglected natural resource with significant nutritional and medicinal potential, according to the study's findings. Their usage in the production of medicinal preparations, nutraceutical formulations, functional foods, herbal items and natural colorants may encourage the sustainable use of this native species while producing goods with additional value. To improve their economic exploitation and broader use, more research on the separation of individual bioactive chemicals, evaluation of biological activity, product formulation and storage stability is advised.

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