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Agro-climatic influence on bioactive and nutritional composition of palash flowers for functional food innovation

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Introduction: Edible flowers are increasingly recognized as functional food ingredients for their nutritional value and bioactive compounds. *Butea monosperma* (palash), widely used in traditional medicine and culture in India, remains underexplored for its nutritional and phytochemical potential. This study aimed to evaluate the nutritional composition, mineral profile, and bioactive compounds of palash flowers collected from four districts in Bundelkhand, India, to assess their suitability for functional food development.

Methods: Fresh flowers were collected during the peak flowering season (February–April 2024) from Lalitpur, Banda, Hamirpur, and Jhansi. Samples were dried, powdered, and analyzed for proximate composition (ash, protein, fat, carotenoids, titratable acidity, ascorbic acid), total phenolics, and antioxidant activity (DPPH assay). Mineral content was quantified by ICP-OES, and bioactive compounds were identified by GC–MS. Functional groups were characterized by FTIR spectroscopy. Statistical analyses included ANOVA and correlation analyses of environmental factors with phytochemical variation.

Results: Proximate analysis revealed ash (5.1–9.2%), crude protein (1.16–1.36%), crude fat (1.72–3.89%), carotenoids (2.01–2.65 mg/100 g), and ascorbic acid (5.12–8.96 mg/100 g). Total phenolic content ranged from 16.4 to 39.9 mg/100 g and correlated with antioxidant activity (13.8–16.8% DPPH inhibition). Drought-stressed Hamirpur samples had the highest values. Mineral profiling indicated high calcium (4924–5,729 ppm), magnesium (2338–2,846 ppm), and iron (198–419 ppm), along with selenium, zinc, copper, and molybdenum. GC–MS identified diverse phytochemicals, including gallic acid, thymol, turmeronol B, colchicine derivatives, vitamin A palmitate, and melezitose. FTIR confirmed functional groups associated with polyphenols, carotenoids, and flavonoids.

Conclusion: The findings demonstrated that *Butea monosperma* flowers are rich in minerals, phenolics, and bioactive compounds, and that their moderate antioxidant potential is influenced by agro-climatic conditions. Their nutritional and phytochemical profiles support the valorization of palash flowers as sustainable functional food ingredients and nutraceuticals. Integrating indigenous floral resources into modern diets can enhance food security, promote cultural preservation, and contribute to sustainable nutrition.

KEYWORDS
antioxidant activity, *Butea monosperma*, functional food, mineral profile, palash flower, phenolic compounds

1 Introduction

Edible flowers have attracted considerable attention in food science and nutrition for their unique sensory attributes, nutritional composition, and abundance of bioactive phytochemicals with potential health benefits (Loizzo et al., 2016; Carboni et al., 2025). Beyond their traditional roles as natural food colorants or ornamental garnishes, edible flowers are increasingly recognized as functional ingredients that may reduce oxidative stress and the risk of chronic diseases (Pires et al., 2022; Prabawati et al., 2022; Purohit et al., 2022). In this context, the valorization of underutilized indigenous floral resources is gaining importance for sustainable nutrition and diversification of diets (Chen et al., 2018; Fakhri et al., 2022).

Butea monosperma (Lam.) Taub., commonly known as *palash*, *dhak*, or "flame of the forest," is a deciduous tree in the Fabaceae family, native to the Indian subcontinent and Southeast Asia (Kumari et al., 2022; Joshi et al., 2024). It is revered as sacred in Hindu culture and is renowned for its vibrant orange-red flowers, which bloom from late winter to early spring. Palash holds significant cultural, ecological, medicinal, and socioeconomic importance for rural and tribal communities in India (Sindhia and Bairwa, 2010; More et al., 2012). Its flowers have been widely used in traditional Ayurvedic, Unani, and Siddha medicine for their purported anti-inflammatory, hepatoprotective, antimicrobial, antidiabetic, wound-healing, and antioxidant properties (Sutariya and Saraf, 2015; Kaur et al., 2017).

The ethnobotanical importance of palash extends deeply into the lives of indigenous and tribal populations, especially in central and eastern India. Tribal communities, such as the Bheel, Vantangiya, and other forest-dwelling groups, have utilized palash flowers, leaves, bark, seeds, and gum to treat a wide range of health issues. These ailments include skin diseases, gastrointestinal disorders, diabetes, fever, hepatic dysfunction, helminthiasis, and menstrual irregularities (Jain et al., 2018; Srivastava et al., 2023). Furthermore, the flowers and immature fruits are consumed as traditional vegetables by tribal communities, providing essential micronutrients and phytochemicals during seasonal food scarcity (Patil et al., 2006; Varsha, 2011; Kumari et al., 2022). Palash leaves are also crafted into eco-friendly disposable plates (*patravali*), and the flowers are used as a natural dye source for the vibrant colors celebrated during Holi, India's festival of colors, reinforcing their cultural embeddedness (Methaniya et al., 2023).

The antioxidant capacity of palash flowers is attributed to their high phenolic content and free radical scavenging activity, positioning them as natural alternatives to synthetic antioxidants in food preservation and nutraceutical formulations (Loizzo et al., 2016). Moreover, the flowers have exhibited antidiabetic activity by inhibiting α-glucosidase and α-amylase enzymes, enhancing glucose tolerance, and modulating serum lipid profiles in experimental diabetes models (Loizzo et al., 2016). Anti-inflammatory, wound-healing, antimicrobial, and immunomodulatory activities, which corroborate their traditional medicinal uses (Sutariya and Saraf, 2015; Kumari et al., 2022).

Despite the considerable ethnobotanical relevance and pharmacological potential of *B. monosperma* flowers, comprehensive evaluation of their nutritional composition and phytochemical characteristics, especially in relation to food applications, remains limited (Kumari et al., 2022). Most existing studies have focused on isolated bioactive compounds or therapeutic properties, with insufficient attention to comprehensive proximate analysis, mineral profiling, and region-specific phytochemical variation, all of which are essential for advancing their use in functional food development.

The Bundelkhand region of central India has a semi-arid to subhumid climate, variable temperature regimes, limited and erratic precipitation patterns, and moderate relative humidity (Singh, 2020; Jatav, 2024). These environmental factors profoundly influence the biosynthesis and accumulation of primary and secondary metabolites in plants (Yang et al., 2018; Qaderi et al., 2023). Abiotic stresses are known to stimulate phenolic biosynthesis, carotenoid accumulation, and mineral uptake in flowering plants, resulting in significant intraspecific phytochemical diversity (Pant et al., 2022; Salam et al., 2023). Consequently, investigating palash flowers collected from different districts of Bundelkhand offers valuable insights into the interplay between environmental stress and phytochemical expression.

The present study aimed to comprehensively evaluate the physicochemical properties, mineral composition, bioactive phytochemical profile, and functional group characterization of *B. monosperma* flowers collected from four districts of Bundelkhand (Lalitpur, Banda, Hamirpur, and Jhansi). Analytical methods included proximate composition analysis, mineral quantification using inductively coupled plasma-optical emission spectrometry (ICP-OES), identification of volatile and semi-volatile bioactive compounds by gas chromatography–mass spectrometry (GC–MS), and functional group elucidation through Fourier-transform infrared spectroscopy (FTIR). This integrated approach provides a scientific basis for validating and promoting the sustainable use of this underexplored floral resource in food and nutraceutical applications.

2 Methodology

2.1 Plant material collection

Fresh flowers of *Butea monosperma* (Palash) were collected from four districts of Uttar Pradesh, India - Lalitpur, Banda, Hamirpur, and Jhansi during the peak flowering season (February to April, 2024). To ensure representative sampling, a multi-stage random sampling approach was adopted. In each district, five different locations were randomly selected, and from each location, five individual trees were chosen, resulting in a total of 25 trees per district and 100 trees overall. The selected trees served as biological replicates. Sampling was carried out by selecting trees from different diameter classes, representing various age groups, and only healthy, disease-free, and actively flowering trees were included to maintain consistency. Flowers were collected separately from each selected tree to preserve biological variation. The samples were then shade-dried and ground into a fine powder using a laboratory mill. The powdered samples were stored in airtight containers at 4 °C until further

analysis. For analytical precision, each biological sample was analyzed in triplicate, thereby incorporating both biological and analytical replication into the study design. The environmental data used in the study were obtained from NASA POWER (Anonymous, 2026).

2.2 Methods

2.2.1 Ash content

The total ash content of *Butea monosperma* flowers was determined following the standard method (Ranganna, 2001). A known weight of powdered flower sample (2 g) was placed in a pre-weighed silica crucible and incinerated in a muffle furnace at 550 ± 5 °C until a light grey or white residue was obtained, indicating complete combustion of organic matter. The crucibles were then cooled in a desiccator and reweighed. Ash content was expressed as a percentage of dry weight using the formula:

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

2.2.2 Determination of crude protein

Crude protein content of the flower samples was estimated by the Kjeldahl method (Ranganna, 2001). Approximately 1 g of powdered sample was digested with concentrated sulfuric acid (H_2SO_4) in the presence of a catalyst mixture (K_2SO_4: $CuSO_4$) until the solution became clear. The digest was then cooled, diluted with distilled water, and made alkaline with excess sodium hydroxide (NaOH). The released ammonia was distilled and absorbed in boric acid solution (4%) using a Kjeldahl distillation unit. The trapped ammonia was titrated against standardized 0.1 N hydrochloric acid (HCl) to determine the nitrogen content.

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

$$\text{Total nitrogen}\,(\%) = \frac{(V_1 - V_2) \times N \times 1.4007}{W}$$

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 (%) × 6.25.

2.2.3 Crude fat

The crude fat content of *Butea monosperma* flowers was determined using the Soxhlet extraction method (Ranganna, 2001). About 2–5 g of finely powdered dried flower sample was placed in a thimble and extracted with petroleum ether (boiling range 40–60 °C) as solvent for 7 h in a Soxhlet apparatus. The extraction was continued until the solvent in the siphon tube became clear, indicating complete removal of fat.

The solvent was then evaporated from the extraction flask on a water bath, and the flask was dried in an oven at 105 °C to a constant weight. The difference in weight of the flask before and after extraction was recorded as the fat content.

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

2.2.4 Determination of titratable acidity

Titratable acidity (TA) was determined by alkali titration and expressed as percentage citric acid (Ranganna, 2001). Accurately weigh W = 1.0 g of finely ground *Butea monosperma* flower into a 250 mL beaker, add Df = 100 mL CO_2 free distilled water, stir 10 min, and filter (Whatman No. 1). Pipette Va = 10.0 mL of the clear filtrate into a conical flask, add 2–3 drops phenolphthalein (or titrate potentiometrically) and titrate with standardized 0.1 N NaOH to a persistent light pink (≈30 s). Record the NaOH volume V (mL).

2.2.5 Total phenolics

The total phenolics content of the product was determined with the Folin–Ciocalteu reagent according to the method (Singleton and Rossi, 1965) using gallic acid as a standard. A 0.1 g sample was taken and ground with 10 mL of 80% ethanol in a pestle and mortar, centrifuged for 20 min at 10,000 rpm, and filtered. The filtrate was evaporated in an oven up to dryness, and the dried extract was dissolved in 5 mL of distilled water. 0.2–2.0 mL aliquot was taken in separate test tubes, and volume was made up to 3 mL. Then, 0.5 mL Folin–Ciocalteu reagent was added. After 3 min, 2 mL of Na_2CO_3 (20%) was added and mixed. Test tubes were placed in a boiling water bath for one min and then cooled. The optical density of the sample was recorded at 650 nm using a spectrophotometer. The quantification of total phenolics was performed using a standard calibration curve prepared with gallic acid.

2.2.6 Antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity of the samples was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method as described by Brand-Williams et al. (1995) with slight modifications. A 1 g sample was homogenized with 10 mL of 80% ethanol and centrifuged at 3000 rpm for 15 min. The supernatant was collected and used for analysis. An aliquot of 0.1 mL extract was mixed with 3.9 mL of freshly prepared DPPH solution (0.1 mM in methanol). The mixture was incubated in the dark at room temperature for 30 min.

The absorbance of the reaction mixture was recorded at 517 nm using a UV–Visible spectrophotometer. Methanol was used as the blank, and a control was prepared by mixing DPPH solution with methanol without the extract.

The percentage inhibition of DPPH radicals was calculated using the formula:

$$\text{DPPH Inhibition}(\%) = \frac{A_C - A_S}{A_C} \times 100$$

Where,

- A_C = Absorbance of control
- A_S = Absorbance of sample

2.2.7 Trace and essential elements analysis

Elemental analysis, including the necessary and trace element ions, was performed following the guidelines of the United States Pharmacopeia (USP232–233). An automatic microwave sample digester (Microwave 130,000 Multiwave GO Plus 50 Hz) was used to prepare the samples. The Anton Paar Multiwave GO Plus Microwave Digestion System, in short, digested a mixture of 0.5 g powdered sample and 10 mL nitric acid. The digestion program included a 20-min hold period followed by a 25-min ramp to 190 °C. After diluting the resulting solution with deionized water to a volume of 25 mL, the solutions were filtered to remove any remaining insoluble impurities. The resulting solutions were used to analyze trace elements, and for the analysis of essential elements, they were diluted with a 2% aqueous solution of nitric acid. The concentrations of trace and essential elements in the samples were then determined using an Agilent Technologies 5,800 Series inductively coupled plasma-optical emission spectrometry (ICP OES) instrument (Gandhi et al., 2023). This process was repeated for a set of results that were replicated four times.

2.2.8 Fourier transform infrared spectroscopy (FTIR) analysis

The mid-IR (MIR) is an important tool for identifying functional groups in the 4,000–600 cm^{-1} region of the spectrum. The region is divided into a 4,000–1,500 cm^{-1} functional group region and 1,500 to 600 cm^{-1} as a fingerprint region. To identify the different compounds in the sample, a FTIR spectrometer (Shimadzu IRSpirit-X) is used. The spectral resolution was 4 $cm^{-1,}$ and 24 sample scans were performed for each spectrum. The samples were measured without any preparation, directly on the ZnSe Diamond Crystal ATR crystal, and analyses were made. Between measurements, the ATR crystal was carefully cleaned using distilled water and dried. The spectra were taken against transmittance.

2.2.9 Gas chromatography–mass spectrometry (GC–MS)

GC analyzed samples extracts–MS/MS (Gas Chromatography Tandem Mass Spectrometry) using two combined Agilent HP-5MS UI columns ((5%-phenyl)-methylpolysiloxane nonpolar, 15 m x 250 µm x 0.25 µm) with an Agilent 8,890 GC System coupled with 7000D GC/TQ (Gandhi et al., 2024). Helium was used as the carrier gas during the phytochemical separation, with a constant flow rate of 1.0 mL/min in Column I and 1.1 mL min^{-1} in Column II. An autosampler (ALS) was used to inject the methanolic extract (10 mg mL^{-1}) into the device with a split ratio of 5:1 and an injection volume of 1 µL. The initial oven temperature during the chromatographic run was 60 °C. It then increased to 280 °C at 5 °C min^{-1} (hold time of 5 min), then to 10 °C min^{-1}. The mass detector was operated under the following conditions: source temperature of 230 °C, mass detector (MSD) transfer line temperature of 280 °C, ionization mode Electron Impact (EI) at 70 eV, scan time 300 ms (0.3 s), in positive and MS2 scan mode with the solvent delay of 2 min. He quenches gas, and N_2 collision gas pressures were set at 2.25 mL min^{-1} and 1.5 mL min^{-1}, respectively. The database in the GC–MS NIST library was compared with the mass spectra of the principal peaks, or major phytochemicals, in the GC–MS chromatograms.

2.3 Statistical analysis

The geographical location was prepared using RStudio 1 + 401 software. All measurements were carried out five times and expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) using SPSS v.25.0 to evaluate the effect of geographical location on the measured parameters. Where significant differences were observed, means were further separated using Tukey's Honest Significant Difference (HSD) post-hoc test at a significance level of $p < 0.05$. Correlations and regression between environmental factors and phytochemical composition were analyzed using Minitab 18.

3 Results and discussion

3.1 Agro-climatic influence on phytochemical composition

3.1.1 Temperature regime and secondary metabolite biosynthesis

The Bundelkhand region of central India exhibits a semi-arid to sub-humid climate with distinct district-level variation in average annual temperature, precipitation patterns, and relative humidity (Figure 1). These agro-climatic parameters critically influence the biosynthesis and accumulation of primary and secondary metabolites in *Butea monosperma* flowers, demonstrating that environmental stress is a fundamental determinant of phytochemical composition. The average annual temperatures across the four districts ranged from 25.4 °C (Lalitpur) to 26.7 °C (Banda), establishing a warm growing environment conducive to metabolic processes. Moderate temperature regimes have been shown to optimize flavonoid and carotenoid synthesis in flowering plants by coordinating the regulation of biosynthetic enzyme complexes (Pola et al., 2020; Qaderi et al., 2023). Conversely, thermal stress (both excessively high and low temperatures) can either promote or suppress secondary metabolite accumulation, depending on the plant species and stress intensity (Ashraf et al., 2018). The findings in this study reveal that *B. monosperma* flowers maintained adequate carotenoid levels across all locations (2.01–2.65 mg/100 g), suggesting that the temperature range in Bundelkhand (25.4–26.7 °C) provides optimal conditions for carotenoid biosynthesis and accumulation in floral tissues (Figure 2).

3.1.2 Precipitation patterns and abiotic stress-induced phenolic accumulation

Precipitation gradients across the Bundelkhand districts demonstrate marked variation, ranging from 773.41 mm/year (Banda) to

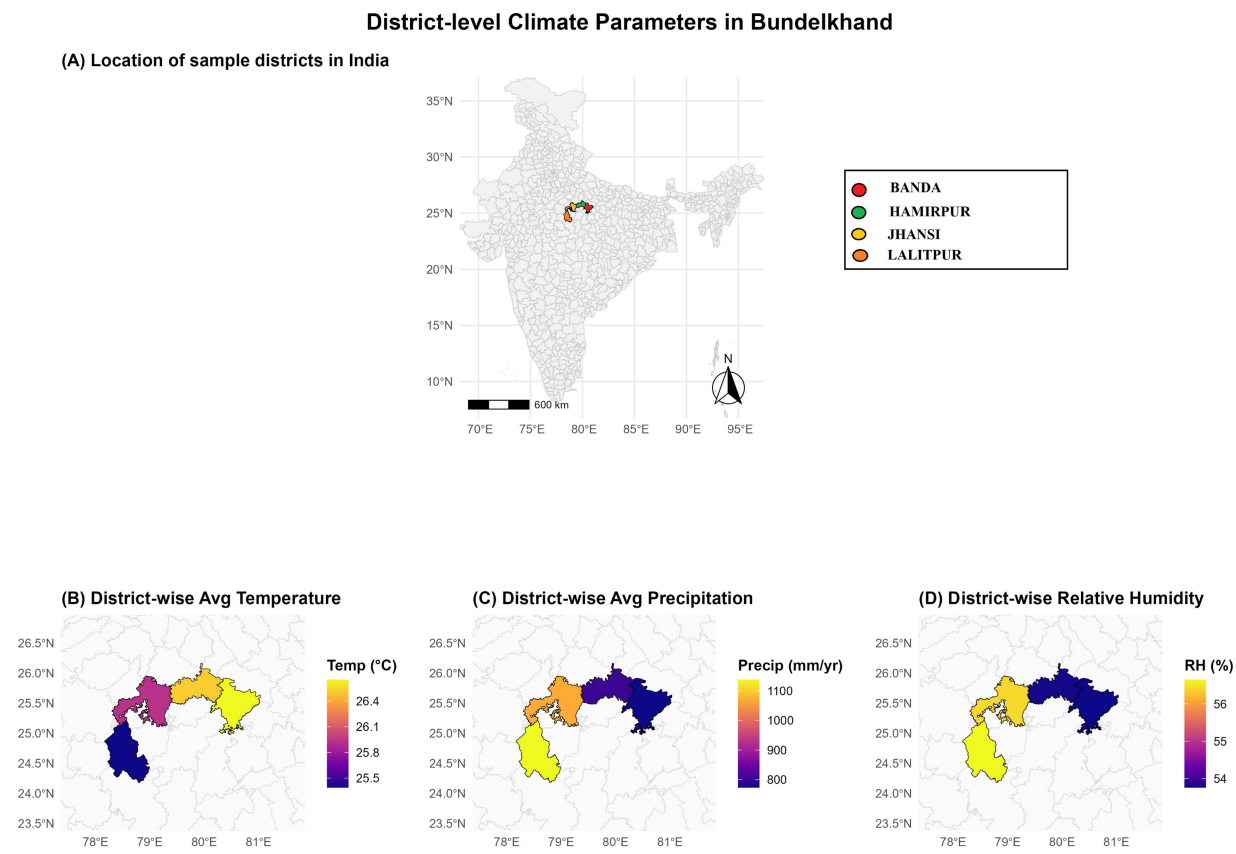


FIGURE 1
Annual climatic conditions of the Bundelkhand region **(A)** selected four districts of study- Banda, Hamirpur, Jhansi and Lalitpur, **(B)** district-wise average temperature, **(C)** district-wise average precipitation and **(D)** district-wise average relative humidity.

1137.65 mm/year (Lalitpur) (Figure 1), establishing differential drought stress regimes that profoundly influence phenolic biosynthesis. Water availability represents one of the most critical environmental factors regulating secondary metabolism in plants, as drought stress triggers adaptive biochemical responses including the accumulation of reactive oxygen species (ROS) and the compensatory synthesis of phenolic and flavonoid compounds (Impa et al., 2011; Hasanuzzaman et al., 2013). Plant responses to water deficit involve osmotic adjustment through the accumulation of organic and inorganic solutes, modification of hormone signaling cascades, and upregulation of antioxidant defense systems, all of which are linked to enhanced phenolic biosynthesis (Silva et al., 2010).

The district-level variation in total phenolic content directly correlates with precipitation patterns, *viz.* Hamirpur (802.48 mm/year; 39.9 mg/100 g phenolics) and Jhansi (1070.03 mm/year; 20.9 mg/100 g phenolics) demonstrated the highest and relatively lower phenolic content, respectively (Figure 2). This inverse relationship between water availability and phenolic accumulation reflects a well-documented physiological principle in which drought-stressed plants redirect metabolic resources toward secondary metabolite production as an adaptive strategy to mitigate oxidative damage and maintain cellular homeostasis (Kapoor et al., 2020). Conversely, plants growing under more favorable moisture conditions (Banda and Lalitpur) allocate metabolic resources to growth and nutrient acquisition rather than phenolic synthesis, resulting in lower phenolic content but potentially higher mineral concentrations (Cohen and Kennedy, 2010; Naikoo et al., 2019). The antioxidant activity values (13.8–16.8% DPPH inhibition) were similarly influenced by precipitation patterns, with the driest location (Hamirpur) showing the highest antioxidant capacity (16.8%), further substantiating that water-deficit stress enhances phenolic accumulation and associated free radical scavenging potential.

3.1.3 Influence of agro-climatic factors on mineral composition

Districts with moderate to high precipitation (Banda 773.41 mm; Lalitpur 1137.65 mm) demonstrated elevated iron concentrations (419.49 ppm in Banda; 207.15 ppm in Lalitpur), suggesting that adequate soil moisture enhanced iron mobilization and plant uptake (Figure 2). Conversely, zinc concentrations showed an inverse pattern, with the lowest precipitation district (Hamirpur, 802.48 mm) displaying moderate zinc levels (169.17 ppm) compared with higher levels in Banda (293.14 ppm), suggesting complex interactions among moisture availability, soil chemistry, and selective mineral uptake mechanisms specific to each micronutrient. Soil moisture content critically influences the bioavailability and plant uptake of mineral nutrients through its effects on nutrient diffusion rates, root-to-shoot translocation efficiency, and the activity of nutrient-uptake enzymes (Comerford, 2005). Plant roots growing in moist soil intercept and absorb higher concentrations of divalent cations such as calcium and magnesium than roots in drier soils, as water films in soil increase effective diffusion rates and facilitate ion transport along osmotic potential gradients (Karthika et al., 2018). The observed variation in mineral composition across districts appears partially influenced by these moisture-dependent bioavailability mechanisms.

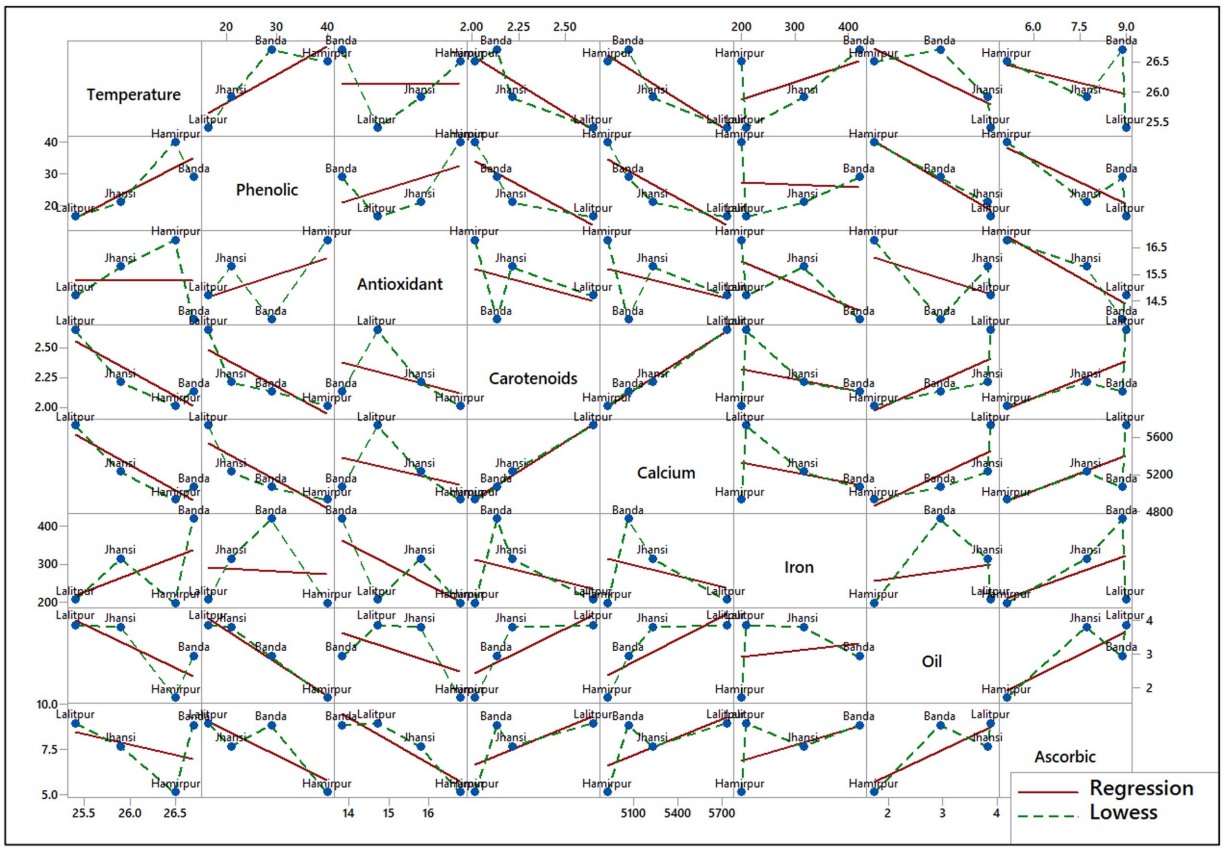


FIGURE 2
Correlations and regression between environmental factors and phytochemical composition.

The pronounced variation in molybdenum concentrations (915.12–3015.78 ppm) across districts reflects the strong influence of soil moisture and pH on molybdenum bioavailability (Shi et al., 2023). Molybdenum availability increases substantially in alkaline or neutral pH soils with adequate moisture, as MoO_4^{2-} ion solubility and plant uptake efficiency are enhanced under these conditions. The exceptionally high molybdenum concentrations, particularly in Banda (2689.58 ppm) and Hamirpur (3015.78 ppm), warrant careful consideration of potential phytotoxicity and food safety implications, as molybdenum can accumulate to levels that exceed nutritionally beneficial thresholds.

3.2 Physicochemical characteristics of *Butea monosperma* flowers

3.2.1 Proximate composition and nutritional profile

The proximate analysis of *Butea monosperma* flowers revealed moderate ash content (5.1–9.2%), crude protein (1.16–1.36%), crude fat (1.72–3.89%), and substantial titratable acidity (1.02–1.38%), indicating a composition profile characteristic of edible flowers with potential functional food applications (Table 1). Ash content represents the mineral matter essential for bone health, enzyme cofactor function, and electrolyte balance, with the highest values recorded in Banda (9.2%) and Hamirpur (8.7%), reflecting elevated bioavailability of minerals in these districts. The relatively low crude protein content (1.16–1.36%) is consistent with other edible flower species and reflects their role as nutritional supplements rather than primary protein sources. However, the presence of protein in *B. monosperma* flowers, combined with their high mineral content, positions them as valuable micronutrient-dense functional ingredients suitable for dietary fortification applications.

Crude fat content varied significantly across locations (1.72–3.89%), with the highest values in Lalitpur (3.89%) and Banda (2.95%), suggesting that warmer temperatures and specific soil conditions favor lipid biosynthesis and accumulation in floral tissues (Singer et al., 2016). These lipids serve multiple functions in flowers, including as structural membrane components, energy storage compounds, and precursors to volatile signaling compounds (Borghi et al., 2019). Importantly, the presence of oil content in palash flowers supports the potential extraction and utilization of lipid-soluble bioactive compounds, including carotenoids and fat-soluble vitamins (Fernandes et al., 2018). The titratable acidity values (1.024–1.38%) indicate the presence of organic acids, likely including citric acid, malic acid, and other weak acids that contribute to flavor attributes and participate in free radical scavenging through their reducing properties, thus contributing to the overall antioxidant potential of the flowers (Kandylis, 2022).

The ascorbic acid content (5.12–8.96 mg/100 g) represents a modest but nutritionally relevant contribution to vitamin C intake. While the concentrations are lower than those in dedicated vitamin C-rich sources such as citrus fruits, the presence of ascorbic acid in *B. monosperma* flowers, in combination with their rich phenolic content, suggests complementary antioxidant defense mechanisms (Gholivand and Piryaei, 2014). The highest ascorbic acid levels were recorded in Lalitpur (8.96 mg/100 g) and Banda (8.86 mg/100 g), environments characterized by moderate to high water availability and relatively warm temperatures that support vitamin C synthesis. The total carotenoid content (2.01–2.65 mg/100 g) identifies *Butea monosperma* flowers as a source of pro-vitamin A compounds, which the body converts to retinol and other

TABLE 1 Physicochemical characteristics of palash (*Butea monosperma*) flowers from different locations.

Physicochemical properties of flower	Lalitpur	Banda	Hamirpur	Jhansi	*F*-value	*p*-value
Ash content (%)	5.1 ± 0.2^{a}	9.2 ± 0.2^{c}	8.7 ± 0.3^{b}	6.9 ± 0.3^{a}	152.4	<0.001
Ascorbic acid (mg/100 g)	8.96 ± 0.15^{b}	8.86 ± 0.16^{b}	5.12 ± 0.24^{a}	7.68 ± 0.12^{c}	310.6	<0.001
Titratable acidity (%)	1.28 ± 0.02^{b}	1.02 ± 0.02^{a}	1.38 ± 0.03^{c}	1.15 ± 0.02^{a}	420.2	<0.001
Total phenolic content (mg/100 g)	16.4 ± 0.1^{a}	28.9 ± 0.1^{b}	39.9 ± 0.1^{c}	20.9 ± 0.1^{a}	1850+	<0.001
Crude protein (%)	1.26 ± 0.256^{a}	1.36 ± 0.286^{a}	1.16 ± 0.244^{a}	1.18 ± 0.301^{a}	0.84	NS
Antioxidant activity (%)	14.7 ± 0.09^{b}	13.8 ± 0.08^{a}	16.8 ± 0.08^{c}	15.8 ± 0.07^{b}	512.3	<0.001
Oil content (%)	3.89 ± 0.15^{c}	2.95 ± 0.26^{b}	1.72 ± 0.44^{a}	3.83 ± 0.11^{c}	96.7	<0.001
Total carotenoids (mg/100 g)	2.65 ± 0.21^{c}	2.13 ± 0.21^{a}	2.01 ± 0.20^{a}	2.21 ± 0.20^{b}	8.92	<0.01

*Values are expressed as mean ± SD ($n = 5$). Different superscript letters within rows indicate significant differences at $p < 0.05$ using Tukey's HSD test. NS, non-significant.

active forms of vitamin A essential for vision, immune function, and epithelial tissue maintenance (Blaner, 2020).

3.2.2 Antioxidant activity

The quantification of total phenolic content (16.4–39.9 mg/100 g gallic acid equivalents) and corresponding antioxidant activity measurements (13.8–16.8% DPPH inhibition) establish a strong positive correlation between phenolic abundance and free radical scavenging capacity in *Butea monosperma* flowers (Table 1). This relationship is mechanistically explained by the structural properties of phenolic compounds, which contain hydroxyl groups (-OH) capable of donating hydrogen atoms to stabilize reactive free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Xie and Schaich, 2014). The DPPH assay measures the ability of plant extract antioxidants to reduce the colored DPPH radical to the colorless DPPH-H molecule, with the reduction in absorbance at 517 nm directly reflecting antioxidant concentration and potency (Gulcin and Alwasel, 2023).

The highest phenolic content (39.9 mg/100 g) and corresponding elevated antioxidant activity (16.8% DPPH inhibition) detected in Hamirpur samples reflect the pronounced drought stress experienced in this district (802.48 mm annual precipitation, 26.5 °C average temperature), which stimulates phenolic biosynthesis as an adaptive response to ROS accumulation. Conversely, Jhansi, despite receiving higher precipitation (1070.03 mm), demonstrated lower phenolic content (20.9 mg/100 g) and antioxidant activity (15.8% DPPH inhibition), supporting the hypothesis that water-stressed plants prioritize secondary metabolite synthesis over growth-related primary metabolism. The antioxidant activity values recorded in *B. monosperma* flowers (13.8–16.8%) are comparable to those reported for commercially utilized medicinal plants and edible flowers, positioning palash flowers as competitive sources of natural antioxidants for functional food formulations and nutraceutical applications (Janarny et al., 2022).

3.3 Mineral profile

3.3.1 Macrominerals: calcium and magnesium

The mineral analysis of *Butea monosperma* flowers (Figure 3) revealed exceptionally high concentrations of calcium (4924–5,729 ppm) and magnesium (2338–2,846 ppm), establishing palash flowers as a potentially rich dietary source of these essential macrominerals critical for bone mineralization, neuromuscular function, and enzymatic reactions. Calcium concentrations were highest in Lalitpur (5728.82 ppm) and lowest in Hamirpur (4924.35 ppm).

Magnesium concentrations similarly ranged from 2337.51 ppm (Banda) to 2846.48 ppm (Lalitpur), indicating that *B. monosperma* flowers are a significant source of this essential cofactor for photosynthetic electron transport, ATP hydrolysis, protein synthesis, and antioxidant enzyme activation (Tränkner et al., 2018). The Ca: Mg ratio in the analyzed samples ranged from approximately 1.7:1 to 2.4:1, which falls within ranges associated with optimal plant and animal physiology (Jacobson et al., 1972; Janyszek-Sołtysiak et al., 2025). These findings suggest that *Butea monosperma* flowers could serve as a balanced source of both calcium and magnesium in functional food formulations.

3.3.2 Micronutrients: iron, zinc, and manganese

Iron concentrations in *Butea monosperma* flowers ranged substantially from 198.05 ppm (Hamirpur) to 419.49 ppm (Banda) (Figure 3). The exceptionally high iron concentration in Banda (419.49 ppm) correlates with this district's moderate precipitation (773.41 mm) and relatively high temperature (26.7 °C). Iron serves as an essential cofactor involved in oxygen transport (hemoglobin, myoglobin), electron transport (cytochrome complexes), and catalase-mediated hydrogen peroxide degradation (Gupta, 2014). The iron content in palash flowers (198–419 ppm) exceeds that of many commonly consumed vegetable sources, suggesting significant potential for dietary iron supplementation, particularly when combined with vitamin C (ascorbic acid, 5.12–8.96 mg/100 g) to enhance iron bioavailability (Monsen, 2000; García-Closas et al., 2004).

Zinc concentrations demonstrated considerable variation (37.14–293.14 ppm), with the highest values in Banda (293.14 ppm) and the lowest in Jhansi (37.14 ppm) (Figure 3). Zinc functions as an essential cofactor for over 100 enzymes involved in protein synthesis, immune function, wound healing, and nucleic acid metabolism (Maret, 2013; Lin et al., 2017). Manganese concentrations (26.86–41.02 ppm) similarly contribute to antioxidant defense through manganese-dependent

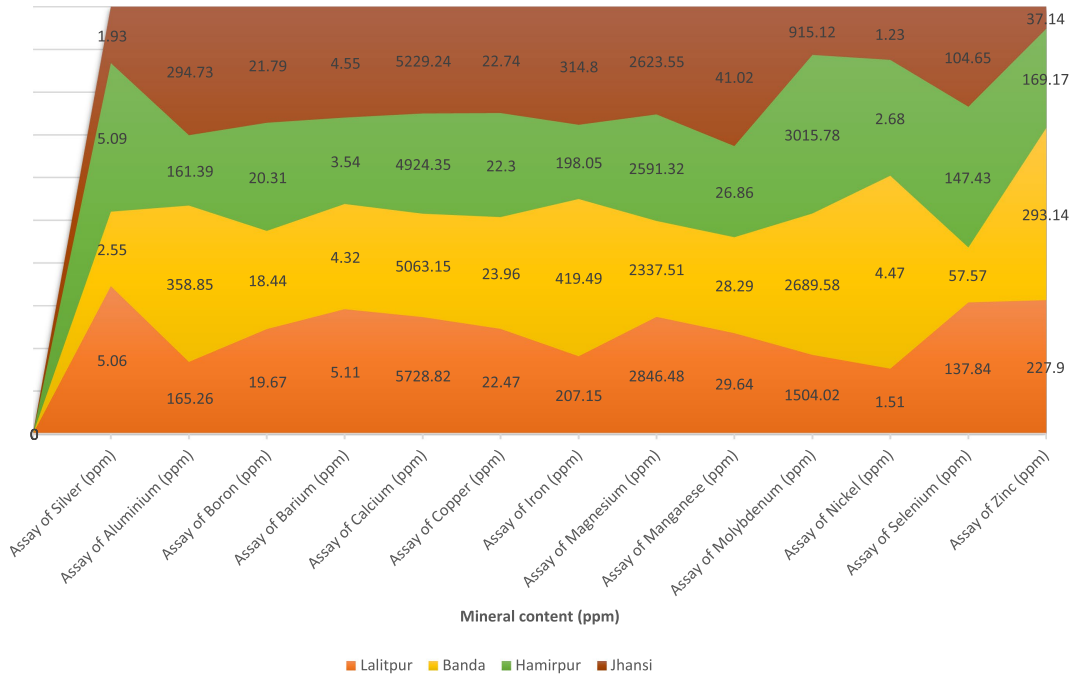


FIGURE 3
Mineral profile of palash (*Butea monosperma*) flowers from different locations.

superoxide dismutase enzyme and participate in bone development, carbohydrate metabolism, and wound healing processes (Taskozhina et al., 2024). The highest manganese content (41.02 ppm) was recorded in Jhansi, the district with the highest precipitation.

3.3.3 Trace elements: selenium and molybdenum

Selenium concentrations in *Butea monosperma* flowers ranged from 57.57 ppm (Banda) to 147.43 ppm (Hamirpur) (Figure 3), establishing these flowers as a potentially valuable dietary source of this essential trace element critical for selenoprotein synthesis, thyroid hormone metabolism, and antioxidant defense through glutathione peroxidase activity (Köhrle, 2005). Selenium exists in plants in both inorganic forms (selenite, selenate) and organic forms (selenomethionine, selenocysteine), with the organic forms generally demonstrating superior bioavailability in the human gastrointestinal tract (Kikkert and Berkelaar, 2013). The selenium concentrations documented in palash flowers (57.57–147.43 ppm) fall within a safe dietary range, with an upper intake level of 400 µg/day (Ren et al., 2024). The highest selenium concentration in Hamirpur (147.43 ppm) correlates with the semi-arid climate and drought stress, which may stimulate selenium uptake as a component of adaptive antioxidant defenses (Aissa et al., 2018).

The molybdenum concentrations revealed dramatic district-level variation (915.12–3015.78 ppm), with particularly elevated levels in Banda (2689.58 ppm) and Hamirpur (3015.78 ppm). This striking accumulation of molybdenum warrants careful consideration regarding food safety and toxicological implications, as molybdenum functions as a cofactor for essential molybdoenzymes (nitrate reductase, sulfite oxidase, xanthine oxidase) while excessive intake may interfere with copper metabolism and cause a pseudogout syndrome characterized by joint pain and inflammation (Ghasemzadeh et al., 2019; Thorndyke et al., 2022).

3.4 Gas chromatography–mass spectrometry

3.4.1 Bioactive phytochemical profile

The GC–MS analysis revealed substantial geographic variation in phytochemical composition across the four districts, with Banda demonstrating the greatest phytochemical diversity and complexity (Table 2; Figure 4). The samples from Banda district exhibited elevated concentrations of gallic acid (0.31%), turmeronol B (0.83%), rutamarin (11.83%), colchicine (9.5%), demecolcine (4.64%), vitamin A palmitate (4.71%), and melezitose (69.7%), suggesting that the specific agro-climatic conditions, soil properties, and microclimate of Banda create an environment optimally conducive to diverse secondary metabolite biosynthesis. This geographic heterogeneity in phytochemical composition has profound implications for functional food development and targeted harvesting strategies, as palash flowers from different districts' may exhibit distinct functional properties suitable for specific health applications (e.g., Hamirpur flowers with elevated phenolic content for antioxidant formulations; Banda flowers with balanced phytochemical diversity for broad-spectrum functional foods).

3.4.2 Carbohydrate constituents and prebiotic potential

Gas chromatography–mass spectrometry analysis identified melezitose as the predominant carbohydrate constituent, representing 69.7–81.58% of quantified compounds across all districts (Table 2; Figure 4). Melezitose, a trisaccharide with the structure α-D-Glcp-(1 → 3)-β-D-Fruf-(2 → 1)-α-D-Glcp, is an α-glucose-containing oligosaccharide naturally occurring in honeydew and manna secretions and various plant tissues (Behera and Balaji, 2022). The exceptional abundance of melezitose in *B. monosperma* flowers positions

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TABLE 2 Gas Chromatography–Mass Spectrometry (GC–MS) analysis of major bioactive compounds in *Butea monosperma* flowers.

S. No.	RT (min)	Phytochemicals	Lalitpur	Banda	Hamirpur	Jhansi
1.	6.238	Fumaric acid, pent-4-en-2-yl tridecyl ester	7.31	31.14	6.67	11.29
2.	6.769	Thymol, TBDMS derivative	1.25	0.54	1.04	1.67
3.	7.528	Variabilin, methyl ether	1.40	1.17	0.73	1.42
4.	7.876	Stearic acid, 3-(octadecyloxy) propyl ester	1.66	9.23	2.46	3.08
5.	7.953	Lycopene, 1,1′,2,2′-tetrahydro-1,1′-dimethoxy-, all-trans	1.14	-	-	-
6.	8.948	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, TMS	-	-	-	0.51
7.	9.059	Desogestrel	1.94	4.25	1.72	3.4
8.	10.064	Gallic acid, 4TMS derivative	-	0.31	-	0.62
9.	11.91	Turmeronol B, TMS	-	0.83	-	0.69
10.	12.905	p-Cymen-7-ol	0.46	5.95	0.72	2.75
11.	13.827	Rutamarin	0.62	11.83	-	1.19
12.	15.605	Etretinate	0.42	1.19	-	-
13.	15.837	Turmeronol B, TMS	-	0.78	-	0.94
14.	16.166	Colchicine	1.91	9.5	1.92	3.49
15.	17.591	Demecolcine	1.54	4.64	1.27	2.83
16.	18.22	Thiocolchicine	-	5.35	-	-
17.	18.692	cis-Retinoic acid, trimethylsilyl ester	-	1.1	0.51	0.86
18.	20.108	Gliricidol	-	-	-	0.41
19.	20.644	Cyperanic acid, 2TMS	-	-	-	0.37
20.	22.46	Melezitose	77.86	69.7	81.09	81.58
21.	23.19	Megestrol acetate	-	-	-	0.37
22.	25.306	Norethindrone, TMS	-	3.53	-	-
23.	27.306	Carvacrol, TMS derivative	-	1.66	-	0.35
24.	27.968	Vitamin A palmitate	1.70	4.71	1.29	3.88
25.	39.688	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, TMS	0.78	2.29	0.59	0.68

these flowers as a concentrated dietary source of this prebiotic oligosaccharide, which is selectively fermented by beneficial *Bifidobacterium* spp. and other commensal bacteria in the human colon (Behera and Balaji, 2022; Zhang et al., 2025). Prebiotics are non-digestible food components that selectively stimulate the growth and metabolic activity of health-promoting gut microbiota, thereby improving colonic pH, enhancing short-chain fatty acid production, reducing pathogenic bacterial proliferation, and supporting gastrointestinal barrier function and immune homeostasis (Ashaolu et al., 2022).

3.4.3 Phenolic acids and polyphenolic profile

Gas chromatography–mass spectrometry analysis identified gallic acid, a trihydroxybenzoic acid phenolic compound, at trace levels across samples (0.31–0.62% in Banda and Jhansi), confirming its presence in *Butea monosperma* flowers (Table 2; Figure 4). Gallic acid serves as a building block for hydrolyzable tannins and gallotannins, which are known for their potent free radical scavenging properties, ability to chelate metal ions, and antimicrobial activity against pathogenic bacteria and fungi (Dhiman and Mukherjee, 2022; Molnar et al., 2024). The identification of gallic acid, in conjunction with the quantified total phenolic content (16.4–39.9 mg/100 g), suggests the presence of diverse polyphenolic structures including catechins, epicatechins, and additional phenolic acids contributing to the documented antioxidant activity (13.8–16.8% DPPH inhibition).

3.4.4 Terpenoid profile

Thymol, a monoterpenoid compound, was identified in *B. monosperma* flowers (Table 2; Figure 4) at low levels (0.54–1.67% across samples), representing a bioactive phytochemical

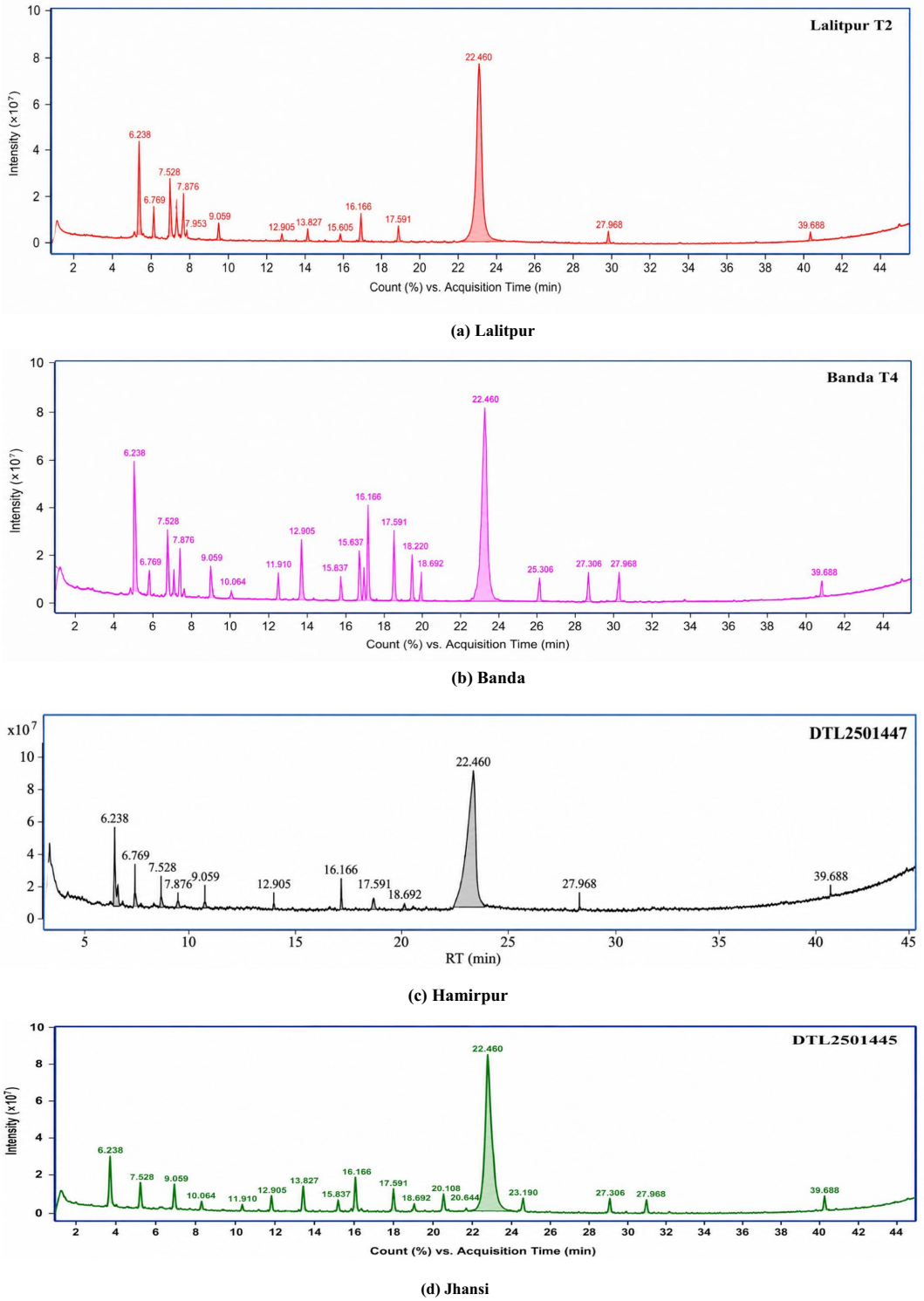


FIGURE 4
(a–d) Representative GC–MS spectrum of *Butea monosperma*, highlighting peaks corresponding to identified compounds (see Table 2).

with well-documented antimicrobial, anti-inflammatory, and antioxidant properties (Gago et al., 2025). Thymol exhibits efficacy against pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, as well as fungal pathogens such as *Candida albicans*, through mechanisms that involve disruption of bacterial cell membrane integrity and inhibition of microbial enzyme systems (Gallucci et al., 2009; Salehi et al., 2018). Additionally, GC–MS analysis identified other terpenoid constituents, including turmeronol B (detected in Banda and Jhansi at 0.69–0.83%) and p-cymen-7-ol (0.46–5.95%), both of which are monoterpenes recognized for their anti-inflammatory and antioxidant properties (de Cássia da Silveira e Sá et al., 2013). These terpenoid compounds contribute to the overall antioxidant capacity of palash flowers and support their traditional use in Ayurvedic medicine for inflammatory and infectious conditions (Joshi et al., 2024).

3.4.5 Carotenoid and vitamin constituents

The GC–MS analysis identified vitamin A palmitate (retinyl palmitate) in all four district samples (1.29–4.71% relative abundance), confirming the presence of preformed vitamin A in *Butea monosperma* flowers (Table 2; Figure 4). Vitamin A palmitate represents the esterified form of retinol (preformed vitamin A). It is highly bioavailable upon absorption in the small intestine, where ester hydrolysis releases retinol for hepatic uptake and storage. The vitamin A palmitate content in palash flowers, combined with the quantified total carotenoid content (2.01–2.65 mg/100 g), establishes these flowers as a multifaceted source of vitamin A nutritional support through both preformed retinoid and provitamin A carotenoid pathways. Vitamin A functions as an essential cofactor for vision (retinal in rhodopsin and cone opsins), immune cell differentiation and function, and maintenance of epithelial tissue barrier integrity (Saari, 2016). The highest vitamin A palmitate content was recorded in Banda (4.71%), consistent with this district's favorable growing conditions supporting lipid and carotenoid biosynthesis.

Notably, GC–MS analysis also identified lycopene, a non-provitamin A carotenoid, in the Lalitpur sample (1.14% relative abundance). Lycopene is known for its potent antioxidant properties and for epidemiological associations with reduced risk of cardiovascular disease and prostate cancer (Imran et al., 2020). While lycopene is not converted to vitamin A in the body, it functions as an independent antioxidant by scavenging singlet oxygen and other reactive oxygen species (Islamian and Mehrali, 2015), thereby contributing complementary antioxidant mechanisms to those provided by vitamin A and other phenolic compounds.

3.4.6 Alkaloid profile and toxicological considerations

Gas chromatography–mass spectrometry analysis identified colchicine alkaloids, including colchicine (1.91–9.5% relative abundance) and demecolcine (1.27–4.64%), as significant constituents of *Butea monosperma* flowers, particularly in the Banda district (colchicine 9.5%, demecolcine 4.64%) (Table 2; Figure 4). This identification is particularly significant from both functional and toxicological perspectives. Colchicine, a tropolone alkaloid, exhibits potent anti-inflammatory and antimitotic properties by disrupting microtubule polymerization and arresting cells in metaphase, mechanisms that underlie its therapeutic use in acute gout (Kurek, 2018). The detected colchicine concentrations in palash flowers (1.91–9.5% on a dry weight basis) highlight the need for careful quantitative assessment to ensure safety before food product development and commercialization (Finkelstein et al., 2010). Although colchicine and demecolcine were detected in trace amounts through GC–MS analysis, their concentrations were not quantified; therefore, no direct inference regarding toxicological safety or acceptable intake levels can be made. Further quantitative and toxicological studies are required to establish safe consumption limits.

Furthermore, GC–MS identified other alkaloid-like compounds, including desogestrel (1.72–4.25%) and thiocolchicine (5.35%),

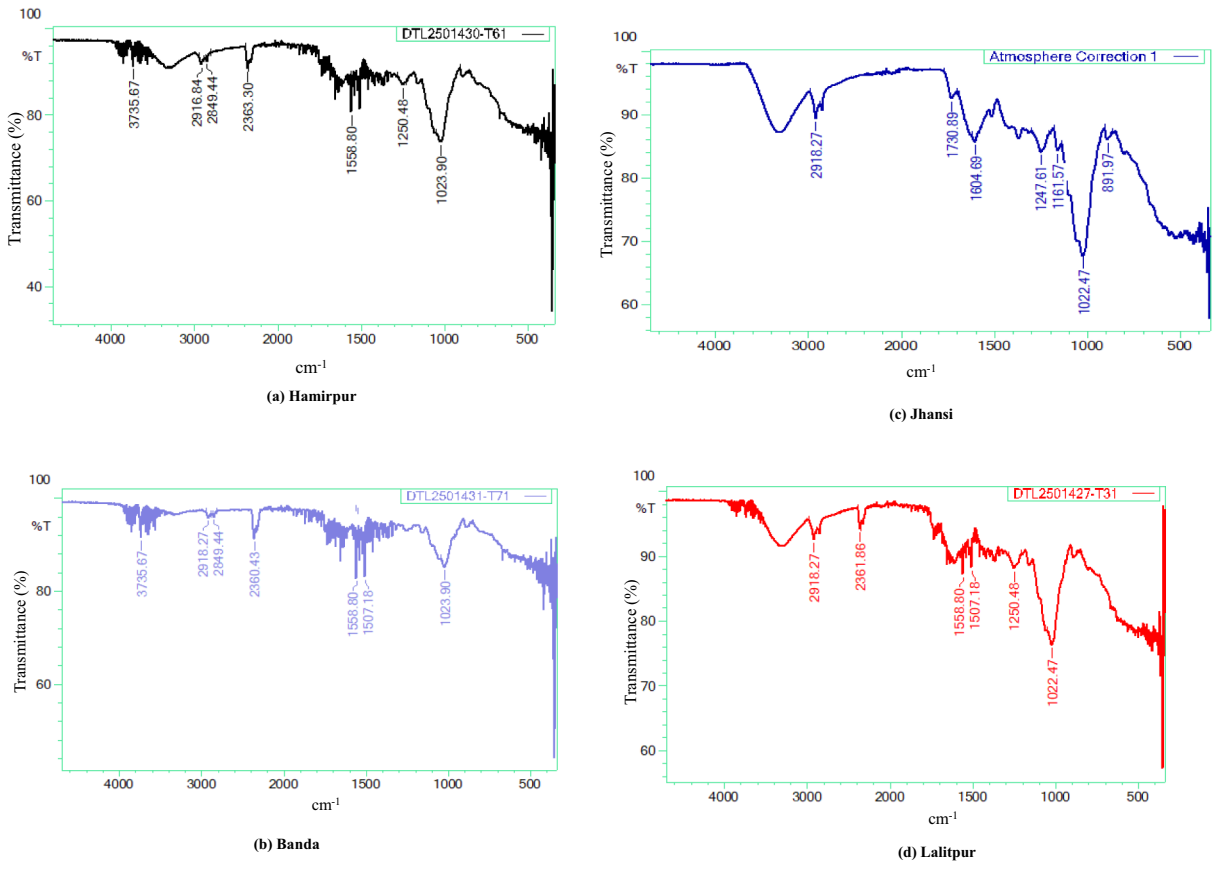


FIGURE 5
(a–d) FTIR characterization of functional groups in *Butea monosperma* flowers of different locations.

further underscoring the complex pharmacological profile of *B. monosperma* flowers. These alkaloid constituents, while present in lower quantities than colchicine, may contribute additional bioactivity but also need toxicological characterization.

3.5 FTIR spectroscopy: functional group characterization and structural validation

Fourier-transform infrared spectroscopy provided complementary molecular characterization, identifying major functional groups associated with bioactive compound classes present in *Butea monosperma* flowers (Figures 5a–d). The characteristic FTIR spectral features observed across samples included absorption bands consistent with polyphenols, carotenoids, carbohydrates, and various secondary metabolites identified by GC–MS analysis. FTIR absorption in the 3,400–3,500 cm^{-1} region indicates O-H stretching vibrations characteristic of hydroxyl groups in phenolic compounds, alcohols, and carbohydrates (Ricci et al., 2015). The intense absorption bands in this region correlate with the high phenolic content quantified by Folin–Ciocalteu analysis and support the mechanistic understanding that abundant hydroxyl-bearing structures drive the documented antioxidant activity through hydrogen atom donation to radical scavenging (Kainat et al., 2022).

Absorption bands in the 1,600–1,680 cm^{-1} region characteristic of aromatic C=C stretching and carbonyl groups (C=O) associated with ketones, aldehydes, esters, and conjugated carboxylic acids support the presence of diverse organic compounds, including terpenoids, flavonoids, and phenolic acids, as identified by GC–MS (Shurvell, 2006). The consistent presence of these spectral features across all four district samples confirms the fundamental structural similarity of *B. monosperma* flowers despite geographic variation in quantitative phytochemical composition. FTIR bands in the 1,200–1,300 cm^{-1} region correspond to C-O stretching vibrations of esters, ethers, and carbohydrate ring structures, providing spectroscopic confirmation of the substantial carbohydrate content (primarily melezitose) and lipid ester structures identified through GC–MS analysis (Oladunmoye et al., 2018; Ahmad and Ayub, 2022). These functional group identifications provide structural validation of the bioactive compound classes quantified through quantitative phytochemical analysis and support the interpretation of the antioxidant and functional food potential of *B. monosperma* flowers.

4 Conclusion

The present study provides a comprehensive evaluation of *Butea monosperma* flowers, demonstrating their potential as a nutritionally rich and bioactive ingredient for functional food applications. The flowers exhibited appreciable mineral levels, particularly calcium, magnesium, and iron, along with moderate protein and lipid content, supporting their use as micronutrient-enriching components in food formulations. The total phenolic content and corresponding antioxidant activity confirm their potential as natural antioxidant sources, suitable for replacing or reducing synthetic additives in food systems. Importantly, the study establishes that agro-climatic variation significantly influences phytochemical composition, with drought-prone regions showing enhanced phenolic accumulation and antioxidant activity. This highlights the need for region-specific raw material selection and standardization to ensure consistent functional quality in product development. From a formulation perspective, the presence of bioactive compounds such as phenolics, terpenoids, carotenoids, and prebiotic carbohydrates (e.g., melezitose) suggests multiple application pathways. Palash flower powder or extracts can be incorporated into functional beverages, herbal teas, fortified bakery products, nutraceutical powders, and natural colorant systems, contributing both nutritional and functional properties. The identified lipid-soluble compounds (e.g., carotenoids and vitamin A derivatives) further support their use in fat-based or emulsion-based formulations, while phenolic compounds enhance oxidative stability. However, detecting alkaloid compounds such as colchicine derivatives requires quantitative standardization and safety evaluation before large-scale food applications, underscoring the importance of dose optimization and regulatory compliance. Future research should focus on toxicological validation, bioavailability studies, and pilot-scale product development, along with sensory and shelf-life evaluation, to facilitate the successful commercialization of palash-based functional foods.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

PT: Conceptualization, Investigation, Project administration, Resources, Writing – original draft. GA: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. PS: Data curation, Investigation, Validation, Writing – original draft. RK: Investigation, Methodology, Software, Writing – original draft. MD: Formal analysis, Visualization, Writing – review & editing. MS: Resources, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AY declared a past co-authorship with the author MD to the handling editor.

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