Morpho-anatomical variation and secondary metabolism associated with floral polymorphism in Cosmos sulphureus Cav

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Research Article

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Abstract

Cosmos sulphureus is widely recognized for its medicinal value, yet the relationship between its floral polymorphism and internal structural and phytochemical variation remains insufficiently understood. In this study, five natural varieties of *C. sulphureus* collected from southern Vietnam were examined using an integrated approach combining morphology, anatomy, and phytochemical analysis. Comparative assessments of vegetative and reproductive traits, stem and leaf anatomy, and lignin distribution revealed substantial variation,primarily associated with floral form. Multivariate analysis separated the varieties into three groups, largely distinguishing single-flowered from semi-double forms. Single-flowered varieties exhibited greater plant height and structural reinforcement, characterized by enlarged vascular tissues and continuous sclerenchyma in the stem. In contrast, semi-double varieties showed reduced lignification but markedly higher accumulation of secondary metabolites, including phenolics, flavonoids, and alkaloids. Histological observations further indicated a higher abundance of secretory structures in semi-double flowers, suggesting an anatomical basis for enhanced metabolite storage. Among the examined varieties, the orange semi-double type consistently displayed the highest phytochemical levels across tissues. These results suggest a coordinated shift in resource allocation from structural support toward secondary metabolism associated with floral architectural modification. By linking floral form with internal anatomy and chemical investment, this study advances current understanding of trait integration in *C. sulphureus*and provides a framework for the selection of germplasm with enhanced phytochemical potential.

Introduction

Cosmos sulphureus Cav. is an annual herbaceous species of the Asteraceae family, native to Mexico and South America and now widely cultivated in tropical and subtropical regions due to its broad ecological adaptability (Himanshu and Sohal 2024). The species is readily recognized by its capitulum-type inflorescences, which display a remarkable degree of intraspecific variation. While the typical wild form bears eight ray florets, cultivated and natural populations frequently exhibit pronounced differences in ray floret number, floral architecture, and coloration (Araujo et al. 2021). Such floral polymorphism provides an accessible model for examining functional divergence within a single species.

Beyond its ornamental value, *C. sulphureus* is a well-documented source of bioactive secondary metabolites. Leaf and flower extracts contain diverse classes of compounds, including flavonoids, phenolic acids, tannins, and terpenoids, which contribute to antioxidant, antimicrobial and enzyme-inhibitory activities (Ortega-Medrano et al. 2023). Previous studies have reported α-glucosidase inhibition by ethanolic extracts and antiproliferative effects of flower extracts against breast cancer cell lines (Olujimi 2025). In ecological contexts, *C. sulphureus* also serves as a continuous floral resource for meliponine and solitary bees, underscoring its functional relevance at both biochemical and ecosystem levels (Malerbo-Souza et al. 2026).

Accumulating evidence suggests that phenotypic variation within a species is often accompanied by quantitative and qualitative shifts in secondary metabolism. In *C. sulphureus*, genotypes differing in flower color have been shown to exhibit contrasting antioxidant capacities (Andrushchenko et al. 2022). Comparable morphology–phytochemistry relationships have been reported in several crop and medicinal species, including red clover, ginger, garlic and citrus, where variation in plant architecture or organ structure significantly influences metabolite accumulation (Johnson et al. 2022; Mikulić et al.; Akullo et al. 2023). Moreover, studies on *Cyclocarya paliurus* have demonstrated that leaf morpho-anatomical traits, such as thickness and stomatal density, are tightly linked to the accumulation of polyphenols, flavonoids and triterpenoids (Xu et al. 2025), highlighting the functional role of internal structure in shaping metabolic outcomes. Despite the extensive documentation of floral diversity and bioactivity in *C. sulphureus*, the structural basis underlying variation in secondary metabolite accumulation among different floral forms remains poorly understood. In particular, the extent to which external morphology, internal anatomical organization and lignification patterns are coordinated with phytochemical allocation within the species has not been systematically examined.

Therefore, the present study investigates the relationships between external morphological traits, internal leaf and stem anatomy, and phytochemical accumulation among natural varieties of *C. sulphureus*. By integrating morpho-anatomical analyses, lignin autofluorescence imaging and quantitative assessment of phenolic compounds, flavonoids and alkaloids, this work aims to clarify how structural differentiation within a species is linked to functional variation in secondary metabolism.

Materials and Methods

Plant Material

Plant material was collected from natural populations of *C. sulphureus* Cav. grown under open-field conditions in Ho Chi Minh City, Vietnam, during September–October 2025 (10°52'32.8"N, 106°47'56.9"E). Sampling focused on healthy, fully developed individuals representing the range of floral forms observed in the field.

Morpho-anatomical characterization and phenotypic clustering

Leaf anatomical structure was examined using fresh leaves collected from the third node below the shoot apex. Transverse sections were obtained from the terminal leaf segment, cleared in a sodium hypochlorite–water solution (1:1, v/v), rinsed thoroughly with distilled water, and subsequently treated with 20% acetic acid. Sections were then double-stained with iodine green and red carmine for 15 min to evaluate vascular bundle diameter, palisade tissue, and spongy mesophyll organization (Tran et al. 2022). Stem samples were transversely sectioned into thin slices and mounted on glass slides for autofluorescence observation. Images were acquired using a Carl Zeiss Apotome 3 microscope

(Germany) equipped with ZEN software, under UV-blue excitation (405 nm), based on the natural autofluorescence of lignin (Yu et al. 2023).

Phenotypic relationships among the five *C. sulphureus* varieties were evaluated by integrating morphological and anatomical traits into a binary data matrix (Win, 2016; Araujo et al. 2020). The binary matrix included characters related to ray floret color at anthesis (orange, yellow, light yellow), capitulum type (single or semi-double), apical ray floret lobing, secretory duct diameter in the leaf midrib (≥ 40 μm or < 40 μm), presence of a continuous sclerenchymatous ring in the stem, and the degree of stem lignification. Phenotypic similarity was calculated using the Jaccard similarity coefficient, and hierarchical clustering was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The dendrogram was generated using NTSYS-pc software version 2.10e.

Determination of alkaloid, total phenolic and flavonoid contents

Leaf samples were thoroughly washed with distilled water to remove surface contaminants and oven-dried at 60°C until constant weight, while ray florets were processed fresh. Briefly, 1.0 g of dried leaf powder or fresh ray florets was mixed with 10 mL of 50% (v/v) ethanol and subjected to ultrasonic treatment at 40 kHz and 50°C for 30 min, followed by incubation at room temperature for an additional 3 h. After extraction, the mixtures were filtered through Whatman No. 1 filter paper to remove plant residues, and the resulting filtrates were stored at − 4°C until further phytochemical analyses (Zhang et al. 2018).

For total alkaloid analysis, 1 mL of extract was mixed with 1 mL of 2 N HCl and centrifuged at 4,000 × g for 5 min. The supernatant was then reacted with bromocresol green solution (10^{-4} M), phosphate buffer (pH 4.7), and chloroform. After phase separation, the chloroform layer was collected, and absorbance was measured at 415 nm. Alkaloid concentration was calculated using a berberine calibration curve (Valieva and Akulov 2024). For total phenolic analysis, 1 mL of extract was mixed with 1 mL of 0.2 N Folin–Ciocalteu reagent, followed by the addition of 1 mL of 10% Na_2CO_3. The reaction mixture was incubated at 40°C for 30 min, and absorbance was measured at 765 nm. For total flavonoid analysis, 1 mL of extract was mixed with 0.2 mL of 5% $NaNO_2$. After 5 min, 0.2 mL of 10% $AlCl_3$ was added, and the mixture was incubated at room temperature for 10 min. Subsequently, 2 mL of 1 M NaOH and 1 mL of distilled water were added, and absorbance was measured at 510 nm. Total phenolic and flavonoid contents were calculated using gallic acid and quercetin calibration curves, respectively (Thang and Linh, 2023).

Statistical analysis and multivariate analysis

To explore the relationships between morphological traits and phytochemical accumulation among the five natural *C. sulphureus* varieties, an integrated heatmap was generated to visualize coordinated variation patterns between phenotypic and biochemical parameters. Raw quantitative data were

normalized to a 0–100% scale to enable direct comparison among variables with different units of measurement. The heatmap was constructed using GraphPad Prism software (version 9.0). Principal component analysis was performed using jamovi software (version 2.7.17) to reduce data dimensionality and identify the major traits contributing to varietal differentiation. Quantitative data were recorded in Microsoft Excel and subjected to statistical analysis using SPSS software (version 22.0). Results are presented as mean ± standard deviation. Differences among means were evaluated using Duncan's multiple range test, with statistical significance set at $p < 0.05$.

Results

Integrated morphological and anatomical differentiation among *Cosmos sulphureus* varieties

A comparative assessment of five *C. sulphureus* varieties derived from a natural population, namely OS (orange, single-flowered), YS (yellow, single-flowered), OSD (orange, semi-double), YSD (yellow, semi-double), and LYSD (light yellow, semi-double), revealed pronounced phenotypic variation at the inflorescence level, with differences most evident in capitulum traits. Variation was primarily expressed through ray floret pigmentation (orange, yellow, and light yellow) and the degree of floral complexity, ranging from simple, single-layer capitula bearing eight ray florets (OS, YS) to more elaborate semi-double forms characterized by a markedly increased number of ray florets (OSD, YSD, LYSD). Notably, the structural organization of disc florets remained highly conserved among all examined varieties (Fig. 1).

Morphological divergence between single and semi-double varieties was further reflected in ray floret apex morphology, with OS and YS exhibiting deeply lobed apices, whereas semi-double varieties showed shallow notches. Vegetative traits were comparatively stable: all varieties shared opposite phyllotaxy and bi- to tripinnately compound leaves with linear-lanceolate segments. However, quantitative growth traits distinguished variety groups, as OS and YS attained greater plant height, while LYSD consistently exhibited reduced stature. Leaf size parameters were maximized in YSD, whereas stem diameter did not contribute significantly to inter-variety separation (Table 1).

Anatomical observations largely supported the morphological grouping. In the leaf midrib, OS and YS showed a more robust and better-developed central vascular bundle than the other varieties (Fig. 2). Differences were also evident in secretory duct development: OSD was characterized by the largest duct diameter, whereas the remaining varieties exhibited noticeably smaller ducts. Mesophyll organization also varied, as OS exhibited enlarged accessory vascular bundles relative to the remaining varieties. Epidermal cell morphology was associated with floret color rather than capitulum type, with yellow-flowered varieties (YS, YSD, LYSD) characterized by larger, oval epidermal cells compared with the elongated cells observed in orange-flowered varieties (OS, OSD). Additionally, stem autofluorescence analysis revealed pronounced differences in lignin distribution among variety groups (Fig. 2). The single-flowered varieties OS and YS showed stronger lignin-associated autofluorescence and developed a continuous sclerenchymatous sheath in the pericyclic region, consistent with enhanced mechanical reinforcement. In contrast, semi-double varieties exhibited discontinuous sclerenchyma strands

localized at the phloem periphery, corresponding to a reduced lignification pattern. Although all varieties maintained a collateral vascular bundle arrangement, LYSD was distinguished by a more irregular stem outline and lower fluorescence intensity, indicating structural divergence within the semi-double group.

Table 1 Variation in agronomic and morphological traits among five *C. sulphureus* varieties

Variety	Plant height (cm)	Stem diameter (cm)	Leaf width (cm)	Leaf length (cm)	Flower diameter (cm)	Ray floret number (unit)
OS	125,33 a	0,47 ns	13,50 d	13,88 e	7,36 a	8,00 c
YS	123,67 a	0,45 ns	13,75 c	17,27 c	7,43 a	8,00 c
OSD	118,00 b	0,43 ns	13,82 c	14,89 d	6,84 b	19,67 b
YSD	110,33 c	0,48 ns	21,19 a	20,11 a	5,84 c	23,67 a
LYSD	103,00 d	0,40 ns	14,63 b	19,08 b	5,35 d	21,00 b

Different letters within a column indicate significant differences (Duncan's test, $p < 0.05$); ns, not significant; OS, orange-single; YS, yellow-single; OSD, orange semi-double; YSD, yellow semi-double; LYSD, light yellow semi-double.

Consistent with the morphological and anatomical differentiation described above, cluster analysis based on 11 quantitative descriptors resolved the five varieties into three major groups (Fig. 3). The single-flowered varieties OS and YS formed a tight cluster (similarity coefficient = 0.71), reflecting their shared capitulum architecture, deeply lobed ray florets, and greater plant height. In contrast, the orange semi-double variety OSD was clearly separated as a phenotypic outlier (similarity coefficient = 0.33), in agreement with its distinctive combination of semi-double capitulum structure and enlarged secretory ducts. The remaining semi-double varieties, YSD and LYSD, grouped together with moderate similarity (0.60), indicating partial convergence associated with increased ray floret number and reduced lignification patterns. Overall, the clustering pattern confirms that capitulum architecture, rather than vegetative traits alone, is the primary driver of phenotypic divergence among C. sulphureus varieties.

Varietal differences in phytochemical accumulation

The contents of alkaloids, polyphenols, and flavonoids varied significantly among varieties and between leaf and flower extracts (Fig. 4). Alkaloid accumulation showed a clear tissue-dependent pattern, with total alkaloid content consistently higher in leaves than in flowers across all varieties. Among the examined varieties, OSD exhibited the highest total alkaloid content in both tissues, clearly separating it from the remaining varieties. Notably, OSD maintained comparatively elevated alkaloid levels in flowers, whereas the other varieties showed substantially lower floral total alkaloid content. Differences in polyphenol accumulation were also evident among varieties. Total phenolic content in flowers was significantly higher in the semi-double varieties OSD, YSD, and LYSD than in the single-flowered varieties

OS and YS. The highest floral total phenolic content was recorded in OSD. Flavonoid content further distinguished OSD from the other varieties. Leaf extracts of OSD contained markedly higher flavonoid levels compared with OS and YS, as well as with YSD and LYSD.

Multivariate analysis of morphological traits and secondary metabolite profiles

The integrated heatmap revealed clear differences in the distribution of phenotypic and biochemical traits among the five *C. sulphureus* varieties (Fig. 5). The single-flowered varieties OS and YS clustered closely together and were associated with greater plant height and larger flower diameter, while exhibiting relatively low levels of total alkaloids, flavonoids, and phenolics, particularly in leaf tissues. In contrast, OSD formed a distinct cluster characterized by consistently high contents of secondary metabolites, especially flavonoids and phenolics in floral tissues. The semi-double varieties YSD and LYSD showed intermediate profiles across most measured traits. To further quantify and validate these patterns, principal component analysis was conducted (Fig. 5). The first three principal components had eigenvalues greater than 1 and together explained approximately 87% of the total variance, with PC1 accounting for about 54%, PC2 for 24%, and PC3 for 9%. The PCA score plot based on PC1 and PC2, which together explained nearly 78% of the total variance, revealed a clear separation among varieties. OS and YS were positioned in the negative region of PC1, reflecting their similar morphological characteristics and lower accumulation of secondary metabolites. In contrast, OSD was distinctly separated along the positive direction of PC2, indicating its strong association with elevated levels of flavonoids, phenolics, and antioxidant capacity. YSD and LYSD clustered closely in the positive region of PC1, suggesting comparable growth performance and intermediate biochemical profiles. Overall, the multivariate patterns revealed by PCA were highly consistent with the clustering observed in the heatmap, confirming the robustness of varietal differentiation based on combined phenotypic and biochemical traits.

Discussion

The present study reveals substantial variation in floral morphology among natural varieties of *C. sulphureus*, while vegetative leaf traits remain relatively conserved. Notably, the pronounced increase in ray floret number in semi-double varieties (OSD, YSD, and LYSD) indicates a modification in floral development rather than a broad reorganization of vegetative architecture. This pattern is consistent with observations in other Asteraceae species, where alterations in floral organ identity are frequently linked to homeotic regulation mediated by MADS-box genes (Tripathi et al. 2026). Reduced activity of the C-function within the ABC model of floral development can result in the partial transformation of reproductive organs into corolla-like structures, leading to an increased number of ray florets (Khojayori et al. 2024; Zhang et al. 2024). Accordingly, the transition from the typical single-flowered form with eight ray florets to the semi-double phenotype observed in this study likely reflects a comparable homeotic shift, in which disc florets partially acquire ray-floret identity. Such architectural changes expand floral tissue area and may enhance the capacity for secondary metabolite accumulation.

Anatomical characteristics closely corresponded with the phenotypic groupings obtained from UPGMA cluster analysis. Single-flowered varieties (OS and YS) exhibited larger central vascular bundles in the leaf midrib and a continuous sclerenchymatous ring in the stem, structural features commonly associated with improved mechanical strength and transport efficiency. These traits plausibly contribute to the greater plant height observed in these varieties, suggesting that vascular reinforcement and mechanical optimization underpin their superior vertical growth (Chang et al. 2022; Tiwary et al. 2025). Differences in lignin autofluorescence further underscored structural divergence among varieties. The higher fluorescence intensity detected in OS and YS indicates increased lignin deposition, whereas semi-double varieties showed reduced autofluorescence, consistent with lower lignification levels. Because lignin, phenolics, and flavonoids share common precursors within the phenylpropanoid pathway, reduced lignification in semi-double varieties may reflect a shift in carbon allocation toward the biosynthesis of soluble secondary metabolites (Wagay et al. 2023; Ninkuu et al. 2025). This trade-off provides a plausible metabolic basis for the elevated phenolic and flavonoid contents observed in these varieties, particularly in OSD. Overall phytochemical levels were comparable to previously reported ranges for *C. sulphureus*. Considerable variation in phenolic and flavonoid contents has been documented among populations, whereas alkaloids were not detected (Ortega-Medrano et al. 2023). In contrast, the present study identified measurable alkaloid contents in both leaves and flowers, with the highest levels recorded in OSD. Such discrepancies may arise from environmental differences among sampling sites or underlying genotypic variation, highlighting the influence of local ecological conditions on secondary metabolite expression.

Among the examined varieties, the orange semi-double OSD consistently accumulated the highest levels of phenolics, flavonoids, and alkaloids across tissues. Similar variety-dependent patterns have been reported in other Asteraceae species, where enhanced flavonoid accumulation in ray florets has been associated with differential regulation of flavonoid biosynthetic genes during floral development (Pu et al. 2023). Importantly, floral color intensity alone does not reliably predict antioxidant or phytochemical capacity, as lighter-colored genotypes may exhibit equal or higher bioactivity than darker ones. These results suggest that differences in phytochemical accumulation in *C. sulphureus* are driven mainly by floral form and developmental identity, rather than by pigmentation intensity.

Conclusions

Natural varieties of *C. sulphureus* exhibited clear morpho-anatomical and biochemical differentiation across vegetative and reproductive organs. Cluster analysis of 11 phenotypic traits separated the varieties into three distinct groups, mainly distinguishing single- and semi-double flower forms. Single-flowered varieties showed stronger vertical growth and higher lignification, whereas semi-double varieties displayed modified floral architecture and greater accumulation of phenolic and flavonoid compounds. Among the varieties, OSD exhibited the highest phytochemical content in both leaves and flowers.

Abbreviations

LYSD: Light yellow, semi-double

OS: Orange, single-flowered

OSD: Orange, semi-double

UPGMA: Unweighted Pair Group Method with Arithmetic Mean

YS: Yellow, single-flowered

YSD: Yellow, semi-double

Declarations

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Author contributions Conceptualization: TTT; Methodology: LTN and HTN; Formal analysis and investigation: LNN and TTN. Writing - original draft preparation: TTN; Writing - review and editing: TTT. All authors read and approved the manuscript.

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Data availability The authors declare that all data supporting the findings of this study are available within the manuscript or are available from the corresponding author upon request.

Conflict of interest The authors declare they have no competing interests.

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Figures

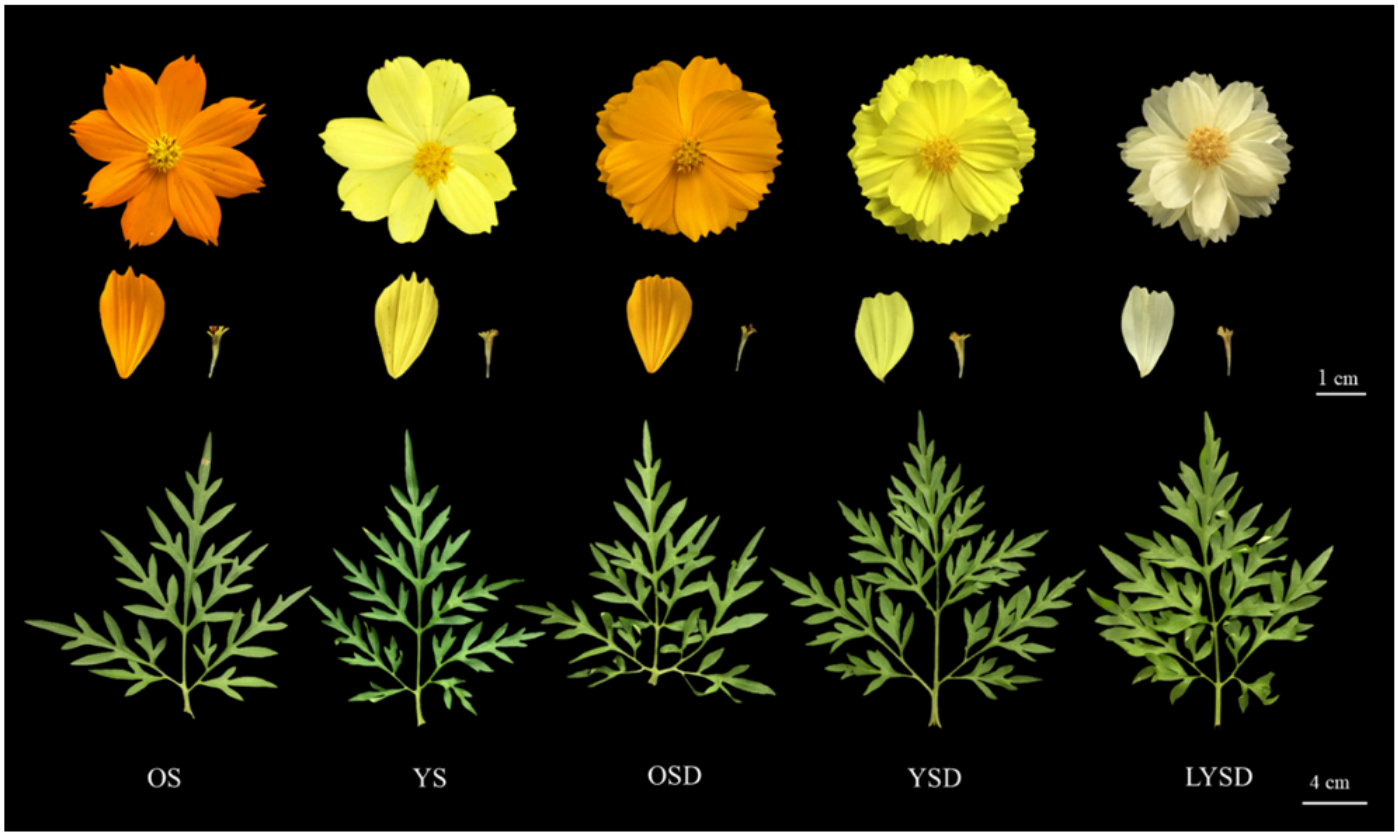

Figure 1

Variation in floral and leaf morphology among five *C. sulphureus* varieties

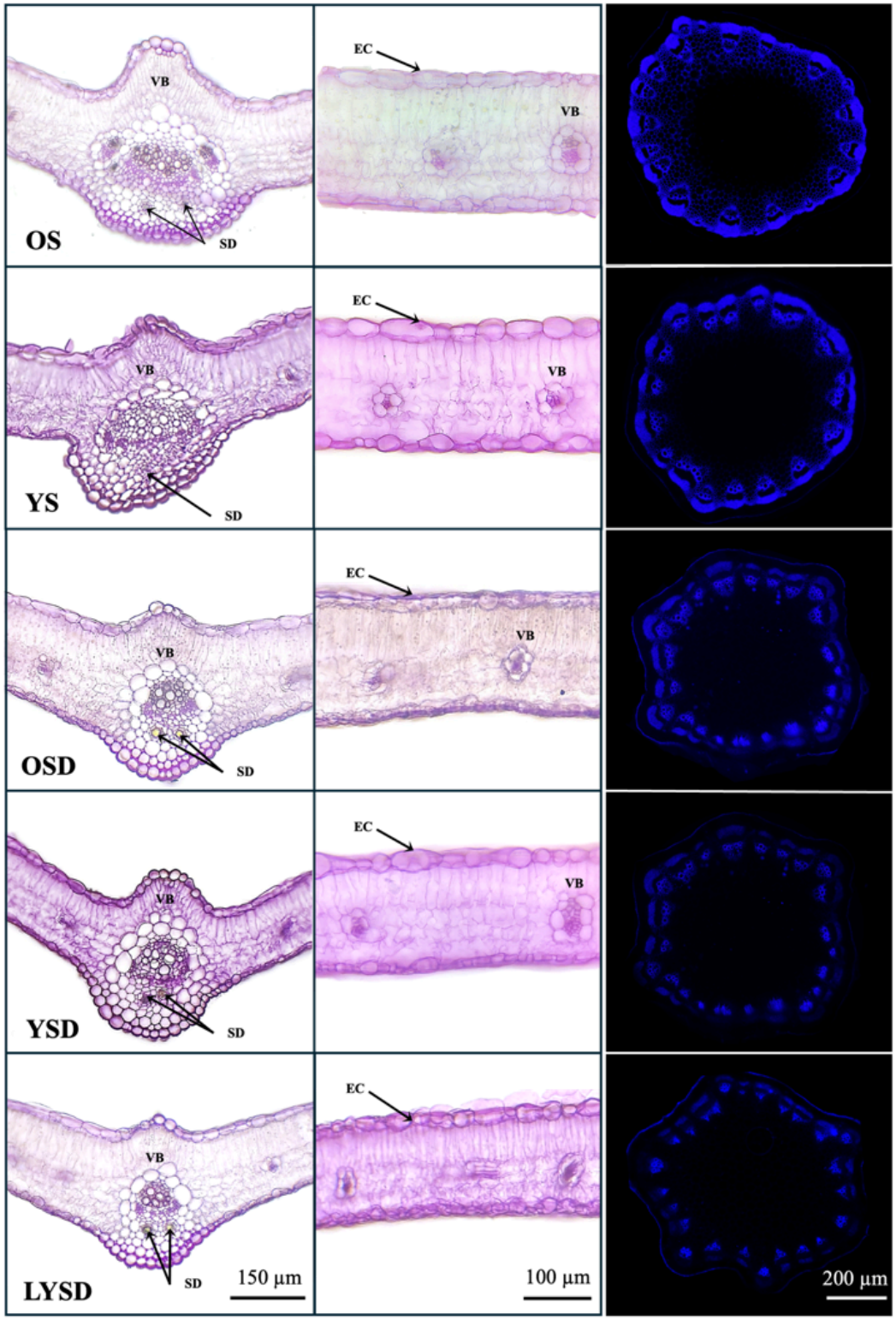

Figure 2

Midrib,mesophyll anatomical structures of the leaf and lignin autofluorescence patterns in stem cross-sections of five *C. sulphureus* varieties (SD: secretory duct; VB: Vascular bundle; EC: Epidermal cell).

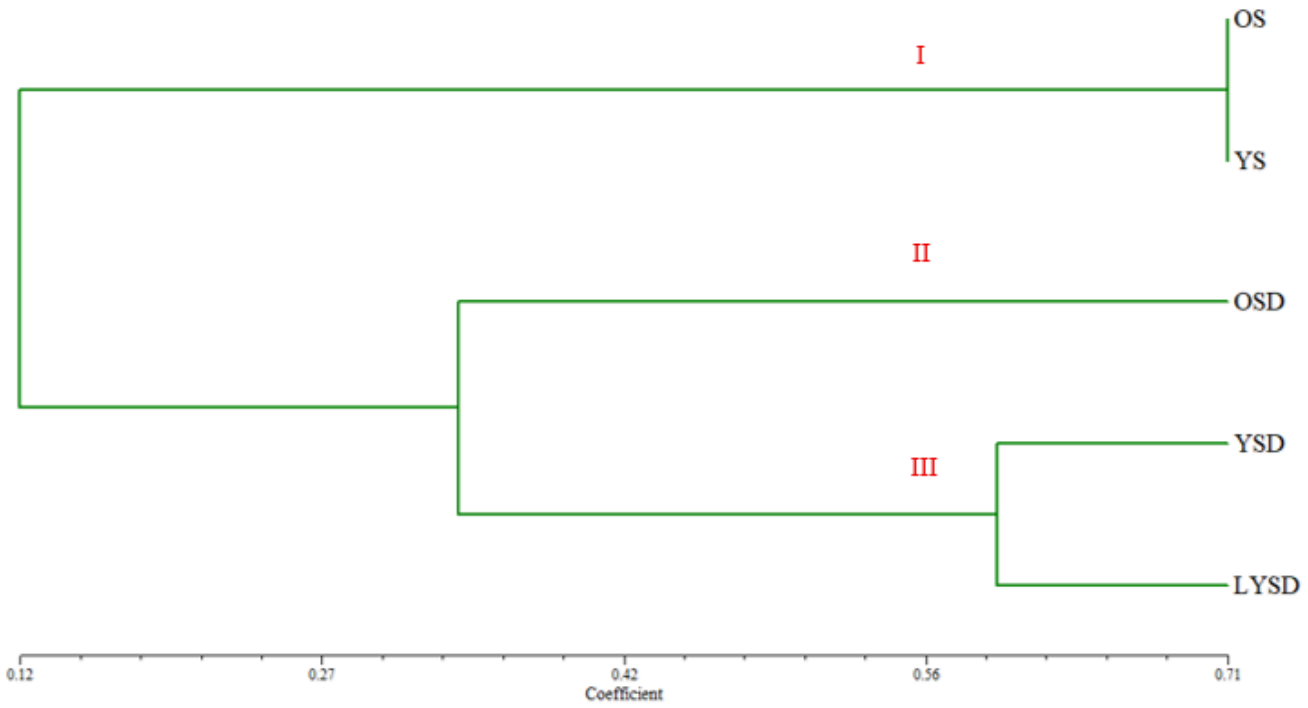

Figure 3

UPGMA dendrogram of five *C. sulphureus* varieties based on 11 morphological and anatomical traits

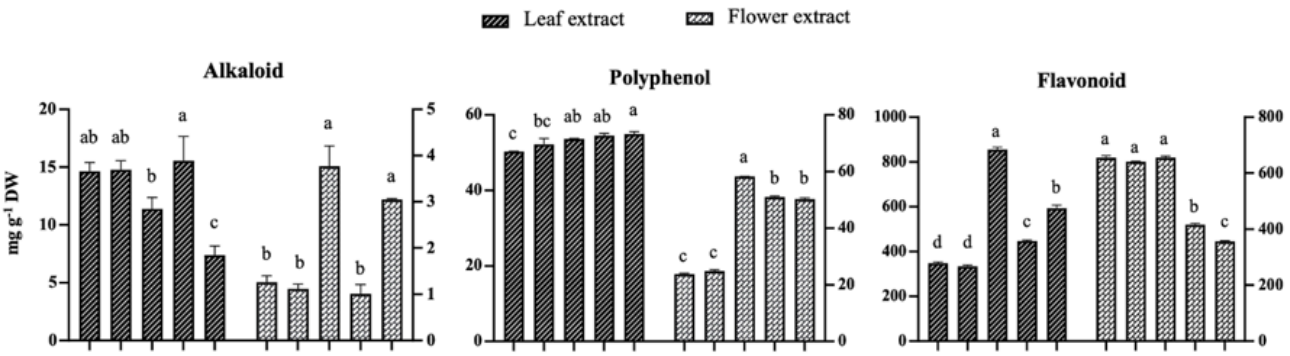

Figure 4

Total alkaloid, flavonoid, and polyphenol contents in leaf and flower extracts of five *C. sulphureus* varieties (from left to right: OS, YS, OSD, YSD, and LYSD). Different letters indicate significant differences ($p < 0.05$).

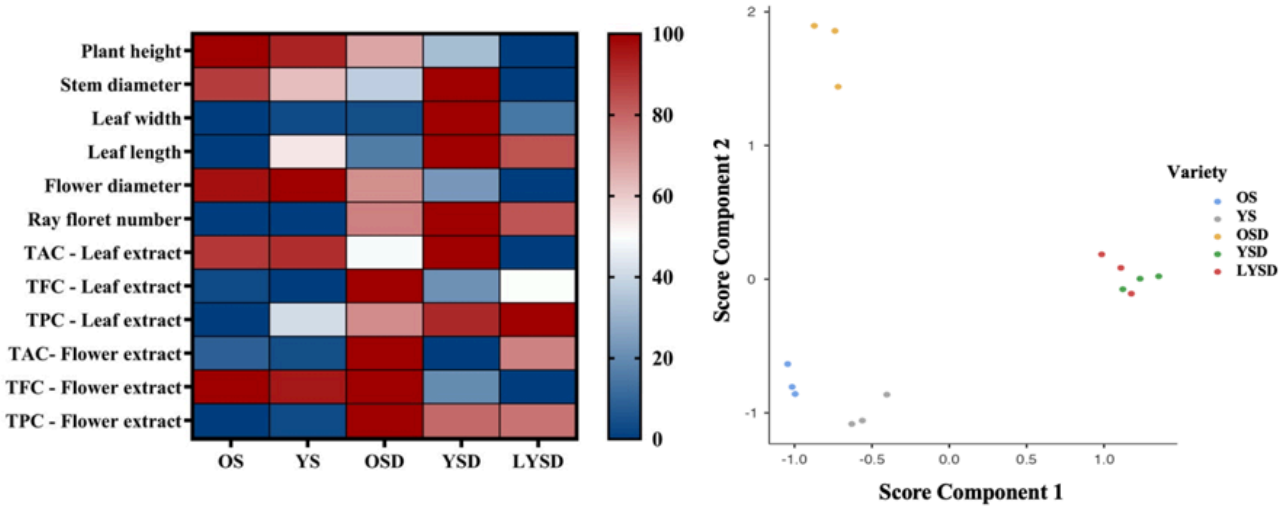

Figure 5

Integrated heatmap and principal component analysis score plot of morphological traits and secondary metabolite contents among five *C. sulphureus* varieties.