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Integrated Profiling of the Nutritional Composition, Phytochemical Attributes, and Antioxidant Capacity of Tropical *Nymphaea* Accessions

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Abstract:
The accumulation of primary metabolites and phytochemicals in *Nymphaea* flowers suggests considerable metabolic diversity within this genus. Nevertheless, while current horticultural selection primarily emphasizes ornamental aesthetics, systematic evaluations of the phytochemical composition across germplasm collections remain scarce. In this study, 20 parameters encompassing nutritional composition, secondary metabolite content, and *in vitro* antioxidant capacity were assessed across 13 tropical *Nymphaea* accessions. Significant inter-accession variability was observed for all the measured traits. Subsequent correlation and regression analyses identified crude protein, total free amino acids, total phenolics, and total flavonoids as the primary determinants significantly associated with antioxidant capacity. Furthermore, a comprehensive assessment using the entropy-weighted TOPSIS model revealed that nutritional metrics contributed more substantially to the overall variance than did antioxidant indices. Among the accessions, NY5 consistently exhibited the most favorable integrated profile. Collectively, these findings offer foundational insights into the phytochemical landscape of tropical *Nymphaea*, facilitating future germplasm characterization.
Keywords: *Nymphaea*; Floral biology; Primary metabolites; Phytochemical profiling; *In vitro* antioxidant capacity

1 Introduction

Nymphaea Linn., a genus within the Nymphaeaceae family, comprises perennial aquatic macrophytes characterized by their distinct ecological and phytochemical attributes. Distributed widely across freshwater ecosystems globally (excluding Antarctica), these plants play an irreplaceable key role in maintaining wetland structural integrity, regulating hydrological cycles, and mediating aquatic biogeochemical processes (Alemu et al., 2024; Zhang et al., 2014). Beyond their limnological significance, *Nymphaea* species exhibit remarkable metabolic diversity and accumulate primary metabolites such as proteins, dietary fibers, amino acids, and minerals within their floral, foliar, and rhizomatous tissues (Alemu et al., 2023). This metabolic profile has long

intersected with human ethnobotanical practices; historical records document the utilization of specific accessions in certain regions of Asia and Africa, often as supplementary resources in traditional subsistence systems (Chrissie et al., 2001; Liu et al., 2016; Alemu et al., 2024). Furthermore, contemporary phytochemical investigations have identified more than 150 secondary metabolites (e.g., flavonoids, phenols, and alkaloids) from various *Nymphaea* organs (Boniface et al., 2022; Zhang et al., 2023). *In vitro* assays and model organism studies suggest that these compounds possess diverse biochemical properties, including antioxidant and immunomodulatory capacities (Alemu et al., 2023). Despite the growing body of literature on the chemical constituents of *Nymphaea*, systematic comparative analyses of the nutritional and phytochemical landscapes across diverse tropical accessions remain limited. Addressing this knowledge gap is crucial for establishing baseline data for germplasm characterization and understanding the full extent of metabolic variation within this genus.

In China, ethnobotanical records attest to the traditional use of certain Nymphaea species in regional dietary and wellness practices (Jirapong et al., 2012; Zhu et al., 2012). Contemporary phytochemical analyses have characterized the infusion profiles of these flowers, identifying the accumulation of diverse secondary metabolites. Correlative studies have linked these compositional features to in vitro antioxidant indices, including DPPH, ABTS, and FRAP scavenging capacities (Yin et al. 2015; Song et al., 2024; Mao et al., 2025). Furthermore, investigations on *Nymphaea pubescens* Willd. have isolated phenolic, flavonoid, and saponin compounds that demonstrate notable free radical scavenging potency ($EC_{50} < 100$ μg/mL) and modulate biochemical pathways, such as the inhibition of iNOS activity in cellular models (Sukalyani et al., 2013). Despite these valuable insights, a comprehensive, multi-accession comparative framework integrating nutritional, secondary metabolic, and functional data remains lacking.

The genus *Nymphaea* encompasses more than 50 recognized species and over 1,000 registered horticultural accessions, exhibiting extensive phenotypic diversity across global collections (Perry et al., 2005; Li et al., 2019). Historically, selection priorities have heavily favored aesthetic traits—particularly floral architecture and chromatic variation—while systematic evaluations of nutritional and phytochemical profiles have remained conspicuously underrepresented (Asharani & Biseshwori, 2014). This imbalance underscores the urgent need to establish a robust, chemometric framework capable of capturing the full metabolic landscape of the genus. For instance, *Nymphaea* 'Eldorado' has been reported to exhibit a distinctive phytochemical profile compared with that of wild-type relatives (Zhou et al., 2024), further reinforcing that morphological descriptors alone are insufficient for comprehensive germplasm characterization. Accordingly, this study aims to address this gap by providing a comprehensive assessment of nutritional and phytochemical landscapes across tropical *Nymphaea* accessions, thereby facilitating more informed germplasm characterization.

Given that floral tissues serve as both the primary reproductive organs and the principal sites for specialized metabolite accumulation in this genus (Lakshman, 2016), we conducted comprehensive phytochemical profiling of flowers from 13 tropical *Nymphaea* accessions, including the reference accession *N.* 'Eldorado'. A suite of 20 parameters, including proximate nutritional composition, secondary metabolite content, and *in vitro* antioxidant capacity, was quantitatively assessed. Multivariate statistical analyses, specifically an entropy-weighted TOPSIS model, were employed to elucidate patterns of metabolic divergence among the accessions. This work provides foundational chemometric data that enhance the characterization of metabolic diversity within tropical *Nymphaea* germplasms.

2. Materials and Methods

2.1 Experimental materials

Thirteen tropical *Nymphaea* accessions (Table 1) were obtained from the germplasm collection of the Pingguo Hualian Science & Technology Institute (Pingguo, China). For each accession, six replicate plants were cultivated in individual pots under controlled conditions, with a spacing of 1.5 m × 2.0 m and a water depth of 60 cm. Flower samples were harvested on sunny mornings in August 2022. Twelve fully developed and undamaged flowers were collected from each cultivar. After being harvested, the samples were transported to the laboratory. Flower samples were rinsed with distilled water, subjected to enzyme inactivation at 105 °C, and subsequently dried at 70 °C to a constant weight. The dried samples were ground, passed through a 40-mesh sieve, and stored at -80 °C for subsequent analysis.

2.2 Determination of nutritional components

The crude protein (CP) content in *Nymphaea* samples was determined by the Kjeldahl method (Nayab et al., 2022). The total dietary fiber (TDF) content was measured by the enzymatic gravimetric method (Katherine et al., 2019). Mineral element concentrations (K, P, Ca, Fe, Zn) were analyzed via inductively coupled plasma–mass spectrometry (ICP–MS) following the protocol of Yang et al. (2025). The content of vitamin B_3 (VB_3) was assessed by high-performance liquid chromatography (HPLC) (Jale et al., 2019), with a standard calibration curve of $y = 21305.3379x + 599.9580$ ($R^2 = 1.0000$). The total free amino acid (TFAA) content was determined by the ninhydrin colorimetric method (Amelie et al., 2024), using the curve $y = 0.3804x + 0.0069$ ($R^2 = 0.9979$).

2.3 Determination of bioactive components

The total phenolic content (TP) was assessed by the Folin–Ciocalteu method (Jaykumar et al., 2013), and the total flavonoid content (TF) was determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method (Luksamee et al., 2023). Gallic acid (GA), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), and gallocatechin (GC) concentrations were analyzed via high-performance liquid chromatography (HPLC) (Song et al., 2012).

The standard curve for TP was $y = 0.012x + 0.0335$ ($R^2 = 0.9994$); for TF, it was $y = 0.0024x–0.0037$ ($R^2 = 0.9997$). For individual bioactive components, the standard curves were as follows: GA: $y = 14805.4360x–197.5510$ ($R^2 = 0.9998$); C: $y = 3161.5978x–2.6201$ ($R^2 = 0.9992$); EC: $y = 3905.3638x–1883.4881$ ($R^2 = 0.9998$); EGCG: $y = 6605.5456x + 1383.1871$ ($R^2 = 0.9999$); and GC: $y = 8783.5907x–283.2449$ ($R^2 = 0.9993$).

2.4 Assessment of *In Vitro* Antioxidant Capacity

The *in vitro* antioxidant capacity of the 13 *Nymphaea* accessions was assessed using four complementary assays: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging rate, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging rate, hydroxyl radical (·OH) scavenging rate, and ferric reducing antioxidant power (FRAP). Sample pretreatment was performed as follows: 0.5 g of sample was extracted with 50 mL of deionized water at 90 °C for 30 min, followed by reduced-pressure filtration. The filtrate was centrifuged at 6000 r/min for 5 min, and the supernatant was diluted to 50 mL with deionized water to obtain the test solution for subsequent analyses.

2.4.1 ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay

The ABTS scavenging rate was determined according to the method of Netiya et al. (2024) with minor modifications. Briefly, the ABTS working solution was prepared by mixing 7 mmol/L ABTS and 2.5 mmol/L $K_2S_2O_8$ at a 1:1 volume ratio, followed by incubation in the dark for 16 hours. The mixture was subsequently diluted with ddH_2O such that the absorbance stabilized at 734 nm within

0.70 ± 0.02. A total of 3 mL of ABTS working solution was added to 1 mL of sample test solution, and the mixture was incubated in the dark for 30 min. The absorbance (A_1) was measured at 734 nm. Moreover, the absorbance values of two control groups were determined: A_2 (1 mL sample test solution + 3 mL ddH_2O) and A_3 (1 mL ddH_2O + 3 mL ABTS working solution). The ABTS scavenging rate was calculated using Formula (1):

$$\text{ABTS scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_3}\right) \times 100\% \quad (1)$$

2.4.2 DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The DPPH scavenging rate was assessed following the method of Su et al. (2024) with minor adjustments. Briefly, 8.0 mg of DPPH was dissolved in 100 mL of anhydrous ethanol to prepare a 0.2 mmol/L DPPH working solution. Afterward, 4 mL of the 0.2 mmol/L DPPH working solution was mixed with 1 mL of sample test solution, and the mixture was incubated in the dark at 37 °C for 20 min. The absorbance (A_1) was measured at a wavelength of 517 nm. To eliminate the interference of the inherent color of *Nymphaea* samples on the absorbance values, a background control (A_2) was prepared by replacing DPPH with 2 mL of anhydrous ethanol in the reaction system. Moreover, A blank control (A_3) was simultaneously determined using 1 mL of deionized water instead of the sample test solution. A vitamin C standard was used as the positive control. The DPPH scavenging rate was calculated using Formula (2):

$$\text{DPPH scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_3}\right) \times 100\% \quad (2)$$

2.4.3 Hydroxyl radical (•OH) assay

The •OH scavenging rate was determined based on the method of Chen et al. (2012) with appropriate modifications. Briefly, 1 mL of sample test solution was sequentially added to 1 mL of 9.0 mmol/L $FeSO_4$, 1 mL of 9.0 mmol/L salicylic acid, and 1 mL of 8.8 mmol/L H_2O_2. The mixture was incubated at room temperature for 20 minutes, after which the absorbance (A_1) was measured at a wavelength of 510 nm. A blank control (A_0) was prepared using ddH_2O in place of the sample solution, and a background control (A_2) was established by replacing H_2O_2 with deionized water in the reaction system. The •OH scavenging rate was calculated using Formula (3):

$$\text{Hydroxyl radical scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (3)$$

2.4.4 FRAP assay

The FRAP value was determined according to the method of Oliver et al. (2025) with minor modifications. Briefly, the FRAP working solution was prepared by mixing 0.3 mol/L acetate buffer (pH 3.6), 0.01 mol/L TPTZ, and 0.02 mol/L $FeCl_3$ at a volume ratio of 10:1:1 (v:v:v) and preheated at 37 °C for 20 min prior to use. Subsequently, 20 μL of the sample test solution was added to 180 μL of the preheated working solution, and the mixture was incubated at 37 °C for 10 min. The absorbance was measured at a wavelength of 593 nm. A ferrous sulfate standard curve was constructed, and the FRAP value was calculated using Formula (4):

$$\text{FRAP(mmol/mL)} = C \times N \quad (4)$$

where C refers to the Fe^{2+} equivalent (mmol/mL) obtained by substituting the sample absorbance minus the blank absorbance into the regression equation, and N denotes the dilution factor.

2.5 Data processing and analysis

The data were summarized and preliminarily processed using Microsoft Excel 2021. One-way analysis of variance (ANOVA) and Pearson correlation analyses were conducted using the SPSSPro

platform (https://www.spsspro.com). The entropy-weighted TOPSIS model was employed to evaluate the multidimensional phytochemical profiles of the accessions. Correlation matrices were visualized as heatmaps using the corrplot package in R (version 4.4.2).

3 Results

3.1 Floral Morphology Characterization of *Nymphaea* Accessions

In this study, twelve hybrid *Nymphaea* genotypes alongside the reference accession *Nymphaea* 'Eldorado' (NY13) were examined, all of which were taxonomically ascribed to the section *Brachyceras* Caspary (Péter, 2011). As delineated in Table 1, all 13 accessions were propagated vegetatively via tubers. Morphometric analysis revealed that the flowers consistently exhibited a diameter ⩾12 cm and a petal number ⩾29. Based on the Royal Horticultural Society's RHS Color Chart (RHSCC, 6th edition, 2015), the accessions were divided into four distinct chromatic groups: NY1, NY4, NY5, NY6, and NY7 to the Violet–red Group; NY2, NY3, NY8, and NY11 to the Purple–blue Group; NY9, NY10, and NY12 to the Rose-red Group; and the control cultivar NY13 to the Yellow Group. These phenotypic descriptors, encompassing floral architecture and pigmentation, establish a baseline morphological dataset for the studied germplasm. This characterization contextualizes the subsequent phytochemical analyses by defining the morphological boundaries of the plant material investigated.

Table 1 Details of the thirteen tropical *Nymphaea* accessions

No.	Plant Material	Ecotype	Subgenera	Flowering type	Propagation Method	Flower Color (RHS Number)	Color Group	Petal Number	Flower Diameter (cm)	Floral Image
NY1	*Nymphaea* 'Zi Yu'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Deep Violet-red (70B)	Violet-red	40-45	16-18	
NY2	*Nymphaea* 'Blue Jewel'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Intense Purple-blue (94A)	Purple-blue	35-38	15-18	
NY3	*Nymphaea* 'Yu Lin Lan'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Vivid Purple-blue (93B)	Purple-blue	32-35	16-18	
NY4	*Nymphaea* 'Chi Zi Xin'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Deep Violet-red (70B)	Violet-red	29-35	17-19	

NY5	*Nymphaea* 'Lan Kong Que'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Dark Purple（88B）	Violet-red	38-40	17-18	
NY6	*Nymphaea* 'Red Jewel'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Violet-red（72C）	Violet-red	46-55	13-15	
NY7	*Nymphaea* 'Xu Ri'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Extra Deep Violet-red（70A）	Violet-red	35-39	15-17	
NY8	*Nymphaea* 'Lan Xiang'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Bright Purple-blue（90A）	Purple-blue	36-40	17-18	
NY9	*Nymphaea* 'Gui Hong'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Rose-red（64C）	Rose-red	31-34	17-19	
NY10	*Nymphaea* 'Zi Yun'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Vivid Rose-red（64B）	Rose-red	31-34	15-17	
NY11	*Nymphaea* 'Tian ZhiLan'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Light Purple-blue（93C）	Purple-blue	32-35	15-18	
NY12	*Nymphaea* 'Tian Gang'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Vivid Rose-red（64B）	Rose-red	35-38	15-17	

NY13	*Nymphaea* 'Eldorado'	Tropical	*Brachyceras* Caspary	Day-blooming	Tuberization	Bright Golden Yellow (12A)	Yellow	40-45	12-15



Note: Due to the large size variation among accessions, representative images are displayed without scale bars; actual flower diameters are provided quantitatively in the table.

3.2 Nutritional profiles of *Nymphaea* accessions

To profile the nutritional composition of *Nymphaea* flowers, the concentrations of CP, TDF, VB_3, and TFAA were quantified across the 13 accessions (Figure 1, Table S1). The statistical significance of inter-accession variations was determined using Duncan's multiple range test ($P < 0.05$).

The CP content ranged from 170.30 to 273.18 mg/g DW, with a mean of 194.95 mg/g DW. Accession NY5 accumulated the greatest amount of CP (273.18 mg/g DW), which differed significantly from that of all the other genotypes. Conversely, NY12 displayed the lowest CP content (170.30 mg/g DW). The TDF content varied significantly among the accessions, ranging from 40.28% to 60.93%, with an average of 48.01%. The highest TDF accumulation was observed in NY6 (60.93%), whereas that in NY13 was the lowest (40.28%).

Further analysis focused on the VB_3 and TFAA profiles. The VB_3 content ranged from 3.03 to 131.11 μg/g DW, with an average of 49.73 μg/g DW. Like the CP distribution, the concentration of VB3 was greatest in NY5 (131.11 μg/g DW), whereas it was lowest in NY13 (3.03 μg/g DW). The TFAA content ranged from 19.90 to 61.97 mg/g DW, with an average of 34.69 mg/g DW. NY5 outperformed the other accessions, displaying the greatest TFAA accumulation (61.97 mg/g DW), while NY9 showed the lowest level (19.90 mg/g DW). Notably, the TFAA values for NY9 were statistically comparable to those of NY6 and NY7, yet remained significantly inferior to those of the remaining accessions.

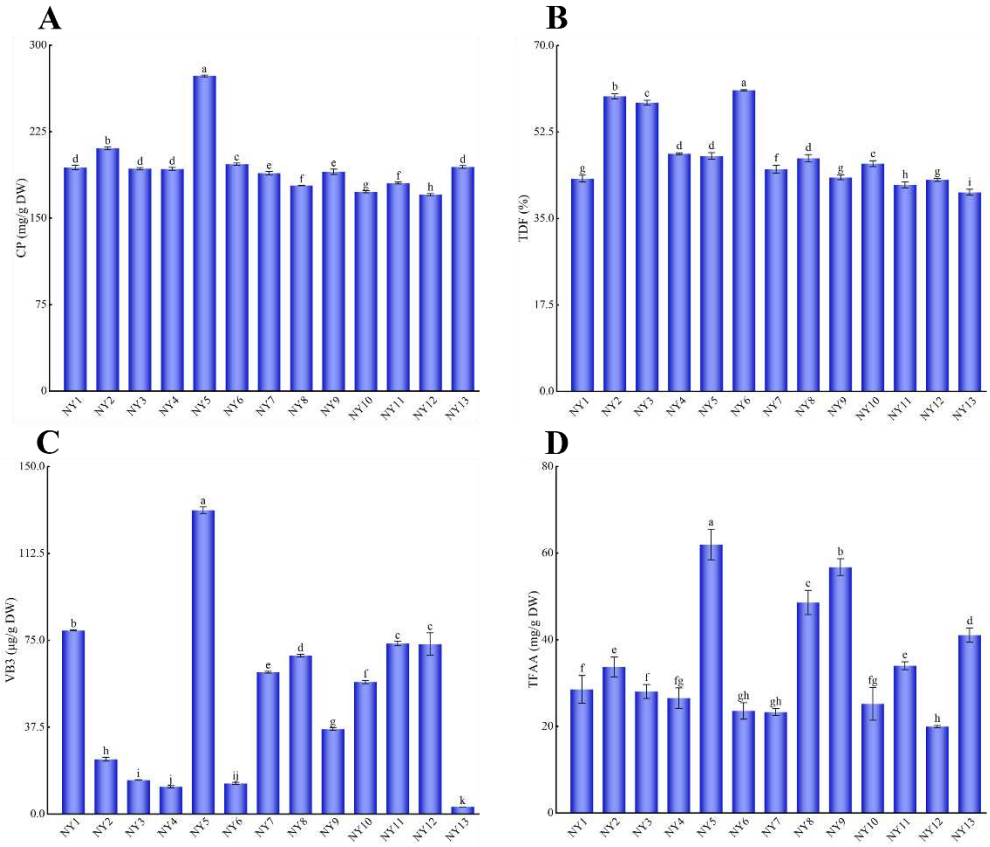


Figure 1. Nutritional profiles of *Nymphaea* accessions. (A) Crude protein. (B) Total dietary fiber. (C) Vitamin B_3. (D) Total free amino acids. The data are presented as the mean ± standard deviation (SD; $n = 3$). The distinct lowercase letters above the bars denote statistically significant differences among the accessions according to Duncan's multiple range test ($P < 0.05$). Same as below.

3.3 Mineral element profiles of *Nymphaea* accessions

To characterize the mineral nutrient profiles, five essential elements (K, P, Ca, Fe, and Zn) were quantified across the 13 accessions (Figure 2 and Table S2). Significant inter-accession variation was observed for all the elements ($P < 0.05$). Among the macroelements, K was the most abundant, ranging from 7.31 to 22.53 g/kg DW, with an average of 12.41 g/kg DW. NY4 exhibited the greatest K accumulation (22.53 g/kg DW), which was significantly greater than that in all the other genotypes, whereas NY7 presented the lowest concentration (7.31 g/kg DW). The P levels were relatively homogeneous across the accessions, varying from 3.83 to 5.17 g/kg DW, with an average of 4.46 g/kg DW. The highest P content was recorded in NY3 (5.17 g/kg DW), whereas that in NY12 was the lowest (3.83 g/kg DW), with no significant difference relative to NY1. The Ca content ranged from 1.71 to 3.15 g/kg DW (mean: 2.18 g/kg DW), peaking in NY2 and reaching a minimum in NY13. Consistent with the trends observed in animal nutrition studies, the Ca/P ratio in all the accessions was less than 1.0, peaking at 0.71 in NY2.

In terms of microelements, the Fe content varied substantially, ranging from 26.78 to 279.14 mg/kg DW (mean: 88.27 mg/kg DW). NY12 demonstrated a distinctively high Fe concentration (279.14 mg/kg DW), significantly exceeding that of all the other accessions. Zinc concentrations ranged from 30.93 to 56.10 mg/kg DW (mean: 46.91 mg/kg DW). NY11 presented the highest Zn content (56.10 mg/kg DW), which was statistically comparable to that of NY5 and NY13 but significantly greater than that of the remaining genotypes. The lowest Zn accumulation was observed in NY2 (30.93 mg/kg DW).

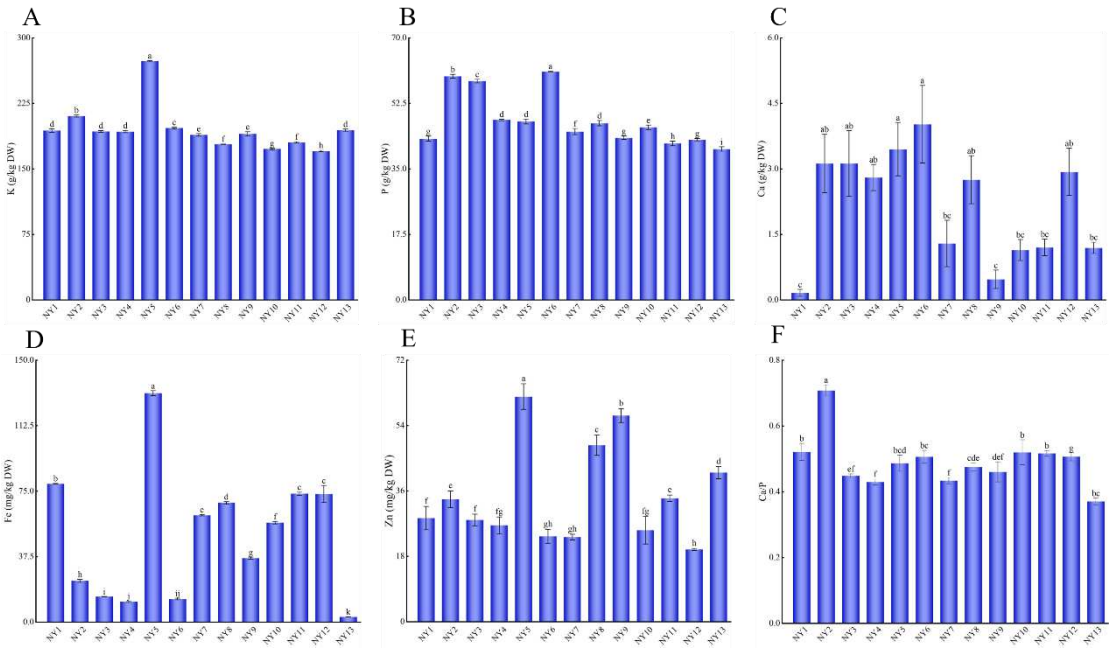


Figure 2. Mineral element profiles across *Nymphaea* accessions. (A) Potassium. (B) Phosphorus. (C) Calcium. (D) Iron. (E) Zinc. (F) Calcium-to-phosphorus ratio.

3.4 Phytochemical Profiling of Phenolic Compounds

To characterize the phytochemical profiles, TP and TF contents were quantified across the 13 accessions (Figure 3, Table S3). The TP content ranged from 15.40 to 89.58 mg/g DW, with a mean of 46.57 mg/g DW. Similarly, the TF content varied from 8.54 to 44.94 mg/g DW, with an average of 26.74 mg/g DW. Accession NY5 resulted in the greatest accumulation of both TP (89.58 mg/g DW) and TF (44.94 mg/g DW), significantly outperforming all the other genotypes. Conversely, NY9 contained the lowest concentrations of these metabolites (15.40 and 8.54 mg/g DW, respectively).

Subsequent analysis focused on specific phenolic subclasses, including GA and catechin derivatives (C, EC, EGCG, and ECG), which are frequently reported constituents in *Nymphaea* species (Ewa & Maria, 2019). The GA content ranged from 436.11 to 1186.65 μg/g DW (mean: 954.30 μg/g DW). NY10 presented the highest GA levels (1186.65 μg/g DW), which were statistically comparable to those of NY2, NY8, and NY12 but significantly greater than those of the remaining accessions. NY13 possessed the lowest GA concentration (436.11 μg/g DW).

C levels ranged from 27.22 to 340.71 μg/g DW (mean: 122.50 μg/g DW), peaking in NY10 (340.71 μg/g DW). NY13 contained the minimum C content (27.22 μg/g DW), which was not significantly different from that of NY2, NY3, or NY8. EC emerged as the predominant phenolic constituent, averaging 3385.93 μg/g DW and ranging from 2629.90 to 4681.23 μg/g DW. The highest EC accumulation occurred in NY3 (4681.23 μg/g DW), whereas that in NY7 was the lowest (2629.90 μg/g DW), comparable to that in NY6, NY9, and NY12.

The EGCG content varied, ranging from 51.05 to 870.41 μg/g DW (mean: 612.07 μg/g DW). NY2 contained the highest EGCG concentration (870.41 μg/g DW), whereas NY13 had the lowest (51.05 μg/g DW). Similarly, the epicatechin gallate (ECG) content varied from 13.02 to 228.85 μg/g DW (mean: 135.20 μg/g DW). NY2 presented the highest ECG concentration (228.85 μg/g DW), which was statistically similar to that of NY3 but significantly greater than that of the other genotypes. Consistent with the trend observed for the other phenolics, the ECG content of NY13 was the lowest

(13.02 μg/g DW).

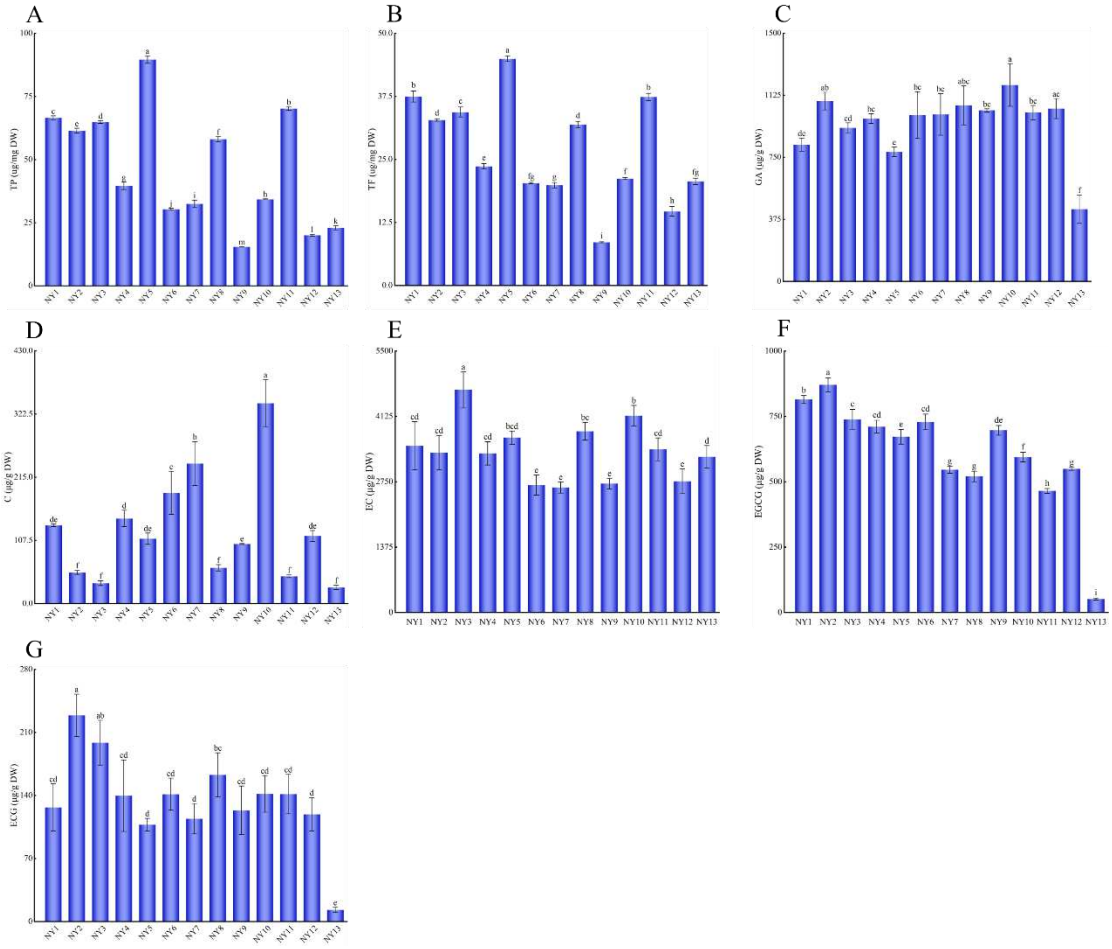


Figure 3. Phytochemical profiles of *Nymphaea* accessions. (A) Total phenol content. (B) Total flavonoids. (C) Gallic acid. (D) Catechin. (E) Epicatechin. (F) Epigallocatechin gallate. (G) Epicatechin gallate.

3.5 *In vitro* Antioxidant Capacity

To assess the oxidant scavenging capacity and reductive potential of the floral extracts, four complementary *in vitro* assays were employed (Figure 4, Table S4). The ABTS scavenging rate ranged from 17.41% to 71.40% (mean: 42.67%), with NY5 exhibiting the highest activity (71.40%) and NY12 the lowest (17.41%).

Similarly, the DPPH scavenging rate ranged from 32.92% to 81.87% (mean: 63.89%). The highest scavenging rate was observed in NY3 (81.87%), a value statistically comparable to that of NY9 but significantly greater than that of the remaining accessions. Conversely, NY12 exhibited the minimum scavenging rate (32.92%). The •OH scavenging rates ranged from 17.28% to 45.33% (mean: 29.90%), with NY5 again demonstrating the highest activity (45.33%) and NY12 the lowest (17.28%).

Moreover, the FRAP varied from 0.34 to 1.17 mmol/L (mean: 0.72 mmol/L). Consistent with the trends observed in the other assays, the reducing power of NY5 was the greatest (1.17 mmol/L), while that of NY12 was the lowest (0.34 mmol/L). This distinct phenotypic variation in antioxidant indices coincided with the differential accumulation of secondary metabolites observed in Section 3.4, particularly regarding total phenolic and flavonoid contents.

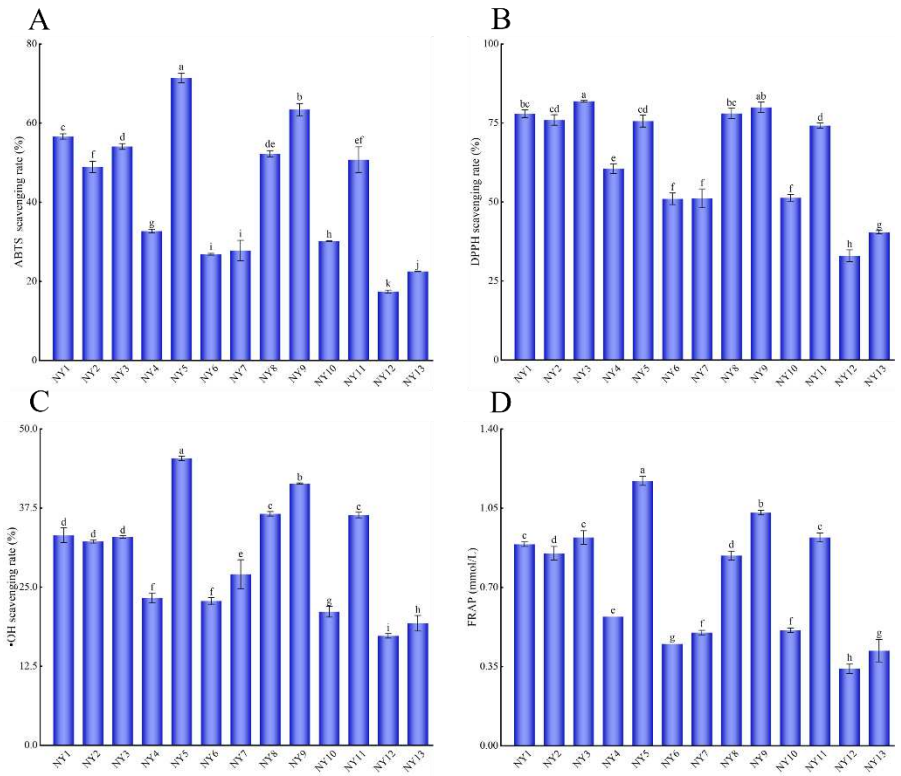


Figure 4 *In vitro* antioxidant capacity of *Nymphaea* accessions. (A) ABTS scavenging rate. (B) DPPH scavenging rate. (C) ·OH scavenging rate. (D) FRAP value.

3.6 Correlation Analysis

To further explore the metabolic relationships between the 20 quantified quality traits, a Pearson correlation analysis was performed on the 13 accessions (Figure 5). Strong positive correlations were observed between CP and TFAA. Both nitrogenous metabolites covaried significantly with phenolic profiles (TP and TF) and *in vitro* antioxidant indices (ABTS, DPPH, •OH, and FRAP). This covariation suggests a coordinated metabolic network in which elevated levels of primary metabolites are associated with increased phytochemical accumulation and redox capacity.

In terms of mineral elements, Ca content was significantly positively correlated with TP, TF, and all the other antioxidant metrics. This relationship implies a potential physiological link between ion homeostasis and the biosynthesis or stability of secondary metabolites. Furthermore, robust positive correlations were detected between TP, TF, and *in vitro* antioxidant capacity. This observation aligns with the prevalent hypothesis in plant biochemistry that polyphenolic compounds constitute a major fraction of the electron-donating capacity measured in these assays.

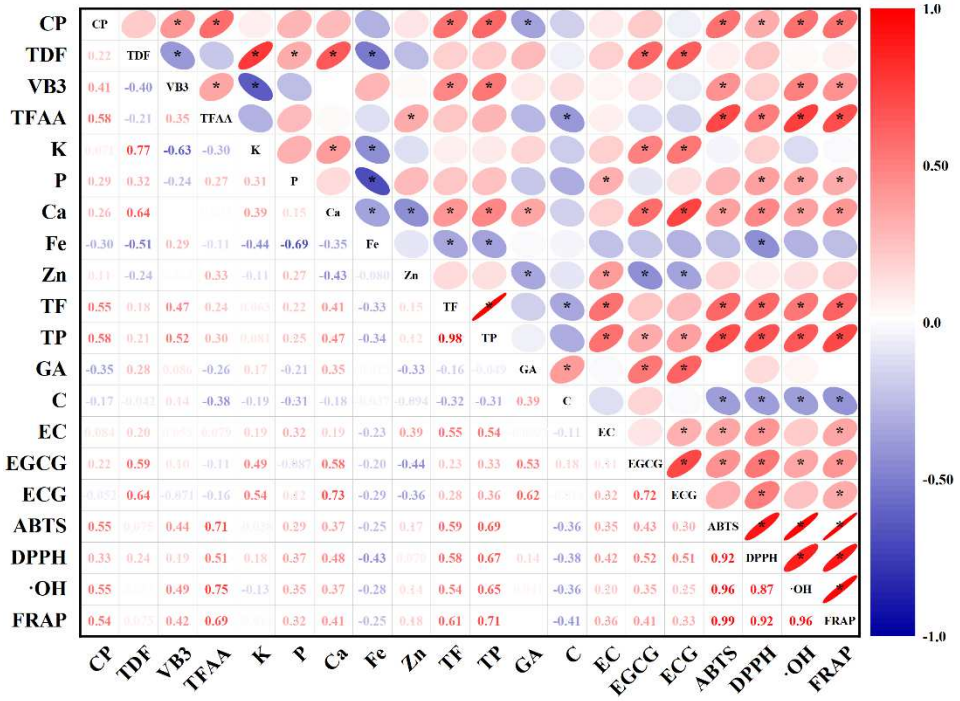


Figure 5 Pearson correlation matrix of biochemical parameters across *Nymphaea* accessions. The color intensity and hue represent the strength and direction of the correlations, respectively. * Denotes statistical significance ($P < 0.05$).

3.7 Multivariate Discrimination of Metabolic Phenotypes Using Entropy-Weighted TOPSIS

To integrate the multidimensional biochemical data and elucidate metabolic divergence among the accessions, an entropy-weighted TOPSIS model was employed. Twenty quantitative parameters, including nutritional composition, phytochemical profiles, and *in vitro* antioxidant indices, were incorporated as evaluators (Table 2). K exhibited the highest information weight (12.063%) and the lowest entropy value (0.733), indicating substantial inter-accession variability and strong discriminative power. Conversely, GA yielded the lowest weight (1.734%) and an entropy approaching unity (0.962), reflecting minimal variance across samples and limited discriminatory capacity. Notably, compared with secondary metabolites (e.g., GA and EGCG), primary metabolites (e.g., K, Fe, CP, TFAA, and TDF) generally possessed higher entropy weights, suggesting that nutritional parameters contributed more substantially to the mathematical variance within this dataset.

Table 2 Entropy weights assigned to biochemical parameters across *Nymphaea* accessions.

Measured Indicators	e-Value	d-Value	Weight (%)	Rank
CP	0.843	0.157	7.085	4
TDF	0.854	0.146	6.577	6
VB_3	0.876	0.124	5.576	8
TFAA	0.854	0.146	6.599	5
K	0.733	0.267	12.063	1
P	0.93	0.07	3.176	15
Ca	0.914	0.086	3.868	13
Fe	0.822	0.178	8.03	2
Zn	0.94	0.06	2.72	17
TF	0.927	0.073	3.281	14

TP	0.886	0.114	5.123	9
GA	0.962	0.038	1.734	20
C	0.841	0.159	7.166	3
EC	0.864	0.136	6.144	7
EGCG	0.961	0.039	1.748	19
ECG	0.957	0.043	1.952	18
ABTS	0.901	0.099	4.484	12
DPPH	0.93	0.07	3.165	16
•OH	0.896	0.104	4.695	11
FRAP	0.893	0.107	4.815	10

The relative closeness coefficients (C values) revealed distinct metabolic phenotypes among the 13 accessions (Table 3). Accession NY5 achieved the highest coefficient (0.584), characterized by the shortest Euclidean distance to the positive ideal solution ($D^+ = 0.519$) and the greatest distance from the negative ideal solution ($D^- = 0.729$). This finding indicates that the metabolic profile of NY5 most closely approximated the mathematically defined optimal parameter space within this cohort. In contrast, NY13 had the lowest coefficient (0.245), corresponding to the highest D^+ (0.850) and lowest D^- (0.277), indicating that the metabolic profile was most divergent from the positive ideal reference. The C values ranged from 0.245 to 0.584 without extreme outliers, affirming the robustness and discriminatory resolution of the multivariate model in characterizing *Nymphaea* germplasm.

Table 3 Relative C values of *Nymphaea* accessions derived from the entropy-weighted TOPSIS model.

Cultivar	D^+	D^-	C-values	Rank
NY1	0.655	0.472	0.419	7
NY2	0.573	0.605	0.514	3
NY3	0.562	0.666	0.542	2
NY4	0.673	0.483	0.418	8
NY5	0.519	0.729	0.584	1
NY6	0.706	0.454	0.391	9
NY7	0.786	0.335	0.299	12
NY8	0.636	0.494	0.437	4
NY9	0.688	0.506	0.424	5
NY10	0.719	0.451	0.386	10
NY11	0.666	0.485	0.421	6
NY12	0.838	0.365	0.304	11
NY13	0.850	0.277	0.245	13

D^+: Euclidean distance to the positive ideal solution; D^-: Euclidean distance to the negative ideal solution; C-values: Relative closeness coefficient, representing the proximity of each accession's profile to the mathematically defined ideal vector.

4 Discussion

Nymphaea flowers accumulate a diverse array of primary and secondary metabolites, a characteristic that has been extensively documented in the phytochemical and ethnobotanical literature (Yin et al., 2015). Biochemical composition varies substantially among accessions, and such inter-accession divergence is governed primarily by genetic background (Alemu et al., 2023; Aliyu et al.,2017). Consequently, multidimensional biochemical profiling of *Nymphaea* floral tissues necessitates the integration of multiple analytical parameters, including nutrient constituents,

mineral elements, phytochemical contents, and *in vitro* antioxidant indices.

Protein and dietary fiber are fundamental biochemical constituents frequently quantified in floral metabolomic surveys (Jakubczyk et al., 2022). Previous studies have indicated that CP in angiosperm flowers typically ranges from 2% to 23%, whereas TDF varies from 17.2% to 75.9% (Sahil et al., 2025). In this study, the CP and TDF levels observed across the 13 accessions occupied these reported ranges, reflecting a distinct metabolic allocation strategy within the genus.

Floral amino acid profiles exhibit pronounced interspecific variation, indicating the influence of both genetic architecture and environmental factors (Han et al., 2025; Juan et al., 2012). Comparative phytochemical surveys revealed a wide spectrum of TFAA accumulation across angiosperms, including *Trevesia palmata* (116.72 mg/g DW), *Broussonetia kurzii* (224.59 mg/g DW), *Dregea volubilis* (128.54 mg/g DW), *Oroxylum indicum* (47.41 mg/g DW), and *Musa* × *paradisiaca* (66.12 mg/g DW) (Sarana et al. 2025). Within our panel of *Nymphaea* accessions, TFAA levels in NY5 (61.97 mg/g DW) were 3.11-fold greater than those in NY9 (19.90 mg/g DW), underscoring substantial intraspecific metabolic divergence within this genus.

Vitamin B_3, also known as niacin, is an essential micronutrient involved in fundamental redox reactions within plant cells (Afuape et al., 2022). Reported niacin concentrations in floral tissues exhibit considerable interspecific variation, with values ranging broadly across botanical families—218.90 mg/100 g DW in *Cucurbita moschata*, 169.69 mg/100 g DW in *Dianthus chinensis*, 107.70 mg/100 g DW in *Fuchsia regia*, and 128.89 mg/100 g DW in *Viola cornuta* (Ilaria et al., 2024). In the present study, the maximum VB_3 accumulation recorded among the accessions was 131.11 μg/g DW, a magnitude potentially reflective of phylogenetic constraints within the genus. Notably, the VB_3 level in accession NY5 (131.11 μg/g DW) increased 43-fold over that observed in NY13 (3.03 μg/g DW). This substantial intraspecific divergence highlights the significant genotypic variation in NAD^+ biosynthesis pathways among *Nymphaea* germplasms, warranting further genetic investigation.

Nymphaea taxa, including the roots, stems, leaves, flowers, and seeds of *N. alba* and *N. nouchali*, accumulate a broad spectrum of mineral nutrients (Wasagu et al., 2015; Dias et al., 2021). Notably, the Ca/P ratio is a well-documented metric for evaluating mineral partitioning in floral tissues (Angela et al., 2025). While Ca/P ratios in the range of 1:1 to 2:1 are widely reported across angiosperm lineages with elevated calcium allocation demands (Zhang et al., 2023; Zhang et al., 2017), all tested *Nymphaea* accessions in the present study presented Ca/P ratios less than 1.0. This deviation from the broader taxonomic trend likely reflects phylogenetic constraints or adaptive strategies for nutrient acquisition in freshwater aquatic environments.

Phenolic compounds represent a principal class of secondary metabolites in the genus *Nymphaea* and are frequently utilized as biochemical markers for germplasm characterization (Shi et al., 2022). Quantitative analyses indicated that the TP content in *Nymphaea* flowers varies considerably, with reports ranging from 0.77 to 43.01 mg/g DW across 33 cultivars (Yin et al., 2015), whereas specific assessments record 42.12 mg/g DW in *N. rubra* (Marufa et al., 2024) and 194.87 mg/g DW in *N. pubescens* (Pokhrel et al., 2022). Consistent with these findings, the present study revealed significant genotypic variation in TP accumulation across the tested accessions. Comparative phytochemical profiling further revealed a distinct tissue-specific distribution pattern within the genus. The TP concentrations in reproductive structures (flowers) are markedly greater than those in vegetative storage organs, such as those in seeds (0.54 mg/g DW in *N. micrantha*) and tubers (0.188 mg/g DW in *N. nouchali*) (Fatou et al., 2022; Adrita et al., 2024), and the concentrations also

exceed the foliar concentrations (Alam et al., 2021; Marufa et al., 2024). Collectively, these observations imply that floral tissues constitute the primary site for phenolic biosynthesis and sequestration in *Nymphaea* species.

C and their derived flavonoids constitute a major subclass of polyphenolic secondary metabolites (Maria et al., 2021). These compounds are ubiquitous in angiosperm floral tissues and contribute to diverse biochemical properties, including redox modulation and anti-inflammatory responses (Li et al., 2014; Liang et al., 2018; Pires et al., 2021). In the present study, the 13 accessions displayed a spectrum of floral pigmentation, which coincided with significant inter-accession variation in the accumulation of C and their derivatives. Analogous to the genotype-dependent catechin accumulation reported in *Tulipa gesneriana* (Agnieszka et al., 2020), the observed metabolic divergence in *Nymphaea* is likely governed by underlying genetic determinants. Furthermore, the covariation between floral anthocyanin profiles and flavonoid biosynthesis, particularly with respect to catechin derivatives, underscores the intricate linkage between visual phenotypes and phytochemical portfolios in these accessions (Yang et al., 2008).

The entropy-weighted TOPSIS algorithm represents a robust multivariate statistical tool that has been widely adopted for discriminating biochemical phenotypes in floricultural research, including studies on *Viola verecunda*, *Hemerocallis citrina*, *Handroanthus chrysanthus*, and *Prunus serrulata* (Jia et al., 2014; Feng et al., 2022; Xu et al., 2020). In this investigation, this model was utilized to resolve multidimensional metabolic variations across *Nymphaea* accessions. This approach mitigates the biases inherent in subjective weighting methods by quantifying the informational entropy of each parameter, lending mathematical rigor to comparative analysis (Chen et al., 2025; Guo et al., 2025). The efficacy of this method lies in its ability to rank accessions based on their Euclidean distance from a mathematically defined positive ideal solution. Unlike univariate screening approaches, this multivariate framework excels at identifying genotypes that exhibit balanced metabolic profiles across numerous biochemical parameters. Our analysis revealed that most of the tested accessions, including NY1, NY2, and NY3, presented higher relative closeness coefficients than the reference accession NY13. These findings indicate that these novel genotypes possess a broader metabolic amplitude, effectively circumventing the phenotypic limitations of established cultivars. Consequently, these findings provide a robust chemometric framework and expand the germplasm base for future metabolic studies in *Nymphaea*.

5 Conclusion

In this study, multidimensional phytochemical and nutritional profiles were characterized across 12 hybrid *Nymphaea* accessions, with *Nymphaea* 'Eldorado' (NY13) serving as the reference accession. A comparative assessment of 20 biochemical parameters revealed substantial intraspecific metabolic divergence in floral tissues. CP, TFAA, TP, and TF emerged as key discriminant parameters for resolving metabolic variation among *Nymphaea* germplasms. Notably, all the accessions exhibited Ca/P ratios less than 1.0, a pattern consistent with the phylogenetic trends observed in aquatic monocots, potentially reflecting adaptations to freshwater nutrient cycling regimes. The application of the entropy-weighted TOPSIS model to these parameters revealed that) contributed the most information weight, whereas G) contributed the least. Nutritional parameters collectively explained a greater proportion of the total dataset variance than antioxidant indices did, reflecting higher inter-accession variability in primary metabolic traits. Relative closeness coefficient calculations positioned NY5 as the accession with the metabolic profile most proximal to the mathematically defined positive ideal solution, followed by NY3, NY2, NY8, and NY9. In

contrast, the reference accession NY13 presented the lowest coefficient, indicating maximal divergence from this idealized metabolic parameter space. Collectively, these findings expand the chemometric framework for *Nymphaea* germplasm characterization, clarify the covariance structure between primary metabolic constituents and *in vitro* antioxidant indices, and provide a robust baseline dataset to guide future metabolic and phytochemical investigations in this genus.

Abbreviations

CP	Crude protein
TDF	Total dietary fiber
TFAA	Total free amino acid
VB_3	Vitamin B_3 (niacin)
K	Potassium
P	Phosphorus
Ca	Calcium
Fe	Iron
Zn	Zinc
Ca/P	Calcium-to-phosphorus ratio
TP	Total phenolic
TF	Total flavonoid
GA	Gallic acid
C	Catechin
EC	Epicatechin
EGCG	Epigallocatechin gallate
GC	Gallocatechin
ABTS	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	1,1-Diphenyl-2-picrylhydrazyl
•OH	Hydroxyl radical
FRAP	Ferric reducing antioxidant power
ICP-MS	Inductively coupled plasma mass spectrometry
HPLC	High-performance liquid chromatography
TOPSIS	Technique for Order Preference by Similarity to an Ideal Solution

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The raw data supporting the findings of this article are available from the authors without unreasonable reservation.

Competing interest

The authors declare that they have no known competing financial interests, personal relationships,

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