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Flower color-associated variation in phenolic content and antioxidant capacity in *Portulaca grandiflora*

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Abstract. *Hardiany L, Zahra A, Nafisah AZ, Santika S, Aisyah SI, Dinarty D, Nurcholis W. 2026. Flower color-associated variation in phenolic content and antioxidant capacity in* Portulaca grandiflora*. Biodiversitas 27 (4): d270413. https://doi.org/10.13057/biodiv/d270413.* Phenolic and flavonoid compounds are important plant secondary metabolites contributing to antioxidant defense through radical scavenging and redox-related mechanisms. *Portulaca grandiflora* is an ornamental species with diverse flower colors, yet information on the relationship between flower color, phytochemical composition, and antioxidant activity is limited. This study evaluated four defined flower color variants—Light Reddish Purple bicolor (LRP-bi), Strong Reddish Purple (SRP), Light Orange Yellow (LOY), and Brilliant Yellow (BY)—to determine Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant capacity using CUPRAC, FRAP, ABTS, and DPPH assays. Significant differences were observed among variants. SRP exhibited the highest TPC (3.98 mg GAE g^{-1} FW) and the strongest reducing capacity, with CUPRAC values approximately 6.8-fold higher than LOY. In contrast, LOY and BY showed the highest TFC (1.15 and 1.09 mg QE g^{-1} FW, respectively), while LRP-bi demonstrated the highest radical scavenging activity (ABTS and DPPH). Correlation analysis indicated strong positive associations between TPC and reducing power assays ($r \approx 0.87$), whereas TFC showed negative relationships with antioxidant responses. These findings indicate that flower color is associated with variation in phenolic composition and antioxidant capacity, with red-purple variants exhibiting stronger reducing potential. Although based on a limited number of variants and in vitro assays, this study highlights flower color as a practical phenotypic indicator of phytochemical variation. This approach may support rapid screening, germplasm evaluation, and selection of ornamental plants with enhanced antioxidant-related traits.

Keywords: Antioxidant activity, intraspecific variation, ornamental germplasm, phenolic compounds, phenotypic diversity

INTRODUCTION

Phenolic compounds and flavonoids are important secondary metabolites that contribute to plant defense, adaptation, and ecological interactions (Zagoskina et al. 2023). These compounds also play a major role in antioxidant activity through free-radical scavenging and redox reactions. Because oxidative stress is closely associated with cellular damage and chronic degenerative diseases, plant-derived antioxidants are increasingly valued as safer natural alternatives (Kozlov et al. 2024). Accordingly, phenolic-rich plant extracts continue to attract attention for food, pharmaceutical, cosmetic, and nutraceutical applications due to their multifunctional bioactivity and potential safety (Sun and Shahrajabian 2023).

In ornamental plants, flower color variation is an important component of biodiversity and may also reflect differences in phytochemical traits (Zhao and Tao 2015; Verdonk et al. 2022). This idea is especially relevant for *Portulaca grandiflora* Hook., an ornamental species widely cultivated because of its attractive and diverse flower colors. Beyond its ornamental value, the genus *Portulaca* has also been traditionally used for inflammatory and skin-related conditions. These uses are associated with the presence of bioactive secondary metabolites, particularly phenolics and flavonoids, which have been linked to antioxidant, antibacterial, and wound-healing activities (Mane et al. 2022; Nurcholis et al. 2022; Yulianto et al. 2023; Setyawan et al. 2025; Wisdawati et al. 2025; Heriansyah et al. 2026; Komalasari et al. 2026).

Previous phytochemical studies have shown that *P. grandiflora* contains various bioactive constituents, including alkaloids, phenolic compounds, flavonoids, saponins, tannins, triterpenoids, and steroids. Extracts from different plant parts also exhibit antioxidant activity, with reported values of 18.55-26.93 µmol TE/g FW in CUPRAC and ABTS assays (Aisyah et al. 2023). At the same time, substantial variation has been reported in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), ranging from 4.72-90.68 mg GAE/g and 1.11-61.58 mg QE/g, respectively, depending on plant part, extraction method, and solvent system (Husnawati et al. 2020; Nurcholis et al. 2023). These findings indicate that *P. grandiflora* is phytochemically rich, although antioxidant-related traits vary considerably across study conditions.

Flower pigmentation in *Portulaca* is associated with several bioactive pigments, including betalains and phenolic-related antioxidants. Betacyanins generally contribute to red-purple coloration, whereas betaxanthins are responsible for yellow-orange hues. Because these pigment groups differ in chemical structure, polarity, and redox behavior, they may contribute differently to antioxidant capacity measured by in vitro assays. Red and purple flower colors are often associated with phenolic-derived pigments, while yellow to orange coloration may involve other pigment classes with distinct chemical properties. Since many floral pigments are functionally related to antioxidant compounds, flower color may provide a visible indication of variation in phenolic composition and antioxidant behavior. In addition, higher phenolic content is often associated with stronger reducing capacity because of the electron-donating properties of phenolic compounds (Mattioli et al. 2020).

From a biodiversity perspective, intraspecific variation in visible characters may represent functional trait diversity. In ornamental plants, flower color is one of the most obvious phenotypic traits and may serve as a practical indicator of biochemical variation. Therefore, characterizing phytochemical and antioxidant differences among flower color variants may improve understanding of how ornamental germplasm diversity relates to bioactive compound accumulation. Such information is relevant not only for the conservation of horticultural genetic resources but also for evaluating ornamental plants as potential sources of antioxidant-related traits.

Despite growing interest in phytochemical variation in *Portulaca*, previous studies have mostly emphasized differences among plant parts, extraction conditions, or solvent systems rather than systematically defined flower color variants. As a result, comparative information linking specific flower color variants of *P. grandiflora* with TPC, TFC, and antioxidant capacity remains limited. The functional significance of color-associated phytochemical variation in this species is therefore still insufficiently understood. To the best of our knowledge, previous studies have not specifically evaluated defined flower color variants of *P. grandiflora* in relation to TPC, TFC, and multiple antioxidant assays.

This study was conducted to investigate whether flower color variation in *P. grandiflora* is associated with differences in phytochemical composition and antioxidant capacity. It was hypothesized that red-purple flower variants would show higher total phenolic content and stronger antioxidant activity than yellow-orange variants. In addition, this study examined whether total phenolic content is more closely associated with antioxidant responses than total flavonoid content among the evaluated variants.

MATERIALS AND METHODS

Study area

This study was conducted from October to December 2024 at the Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor, Indonesia. The Strong Reddish Purple (SRP) and Brilliant Yellow (BY) variants were collected from the Cikabayan Experimental Field, Bogor, whereas the Light Reddish Purple bicolor (LRP-bi) and Light Orange Yellow (LOY) variants were obtained from the plant collection of the Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. The two sampling locations were located approximately 1.7 km apart within the same campus area and therefore experienced broadly comparable environmental conditions, with all plants cultivated under open-field conditions. Both sites are situated within the IPB Dramaga Campus area (6°30′-6°45′ S, 106°30′-106°45′ E) at an elevation ranging from 182 to 210 m above sea level. Environmental data recorded by the Automatic Weather Station (AWS) of Institut Pertanian Bogor during the sampling period indicated an average air temperature of 26.8°C, relative humidity of 84.1%, and mean daily rainfall of 11.0 mm day^{-1}. Climate parameters were recorded automatically at 5-minute intervals and averaged on a daily basis during the study period. Because the flower color variants were collected from two locations based on availability, the experimental design does not represent a fully crossed variant by location design. Therefore, the effects of flower color variants and environmental conditions cannot be completely separated and should be interpreted with this limitation in mind.

Plant materials

Four flower color variants of *P. grandiflora* were examined and classified according to the Royal Horticultural Society Color Chart (RHSCC), as presented in Table 1. Flower color classification using the RHS Color Chart was performed by a single observer under natural daylight conditions during morning observations. Several flowers from multiple plants within each variant were examined to ensure consistency of the assigned color code. These variants were defined based on floral phenotype and RHSCC codes. They do not represent registered cultivars but rather phenotypically defined color variants. Flower color stability was consistently observed from July 2024 until the time of sampling. Representative floral morphologies corresponding to the variants described in Table 1 are shown in Figure 1.

Plants were approximately 12 weeks old and at the flowering stage at the time of sampling. For each flower color variant, three independent biological replicates (n = 3) were prepared. Each biological replicate consisted of pooled flowers collected from five individual plants of the same color variant, with approximately equal numbers of flowers taken from each plant. From each plant, 10-15 flowers were available, and five fully opened flowers were selected. Pooling was applied to obtain a representative composite sample and to reduce potential variability among individual plants while providing sufficient material for extraction. Approximately 4 g of fresh flower material per replicate was obtained through pooling. In this study, the pooled flower sample was treated as the biological replicate for statistical analysis. However, this pooling approach reduces the ability to estimate within-variant biological variation among individual plants. All variants were harvested on the same day between 09:00 and 10:00 a.m.

Flowers were separated from the stems immediately after harvesting and directly used for extraction.

Extraction procedures

Microwave-Assisted Extraction (MAE) was conducted according to Haryoto et al. (2024), with a modification in the extraction solvent. The extraction conditions (135 W for 3 min) were selected based on the similarity of plant material and microwave system, as the same instrument model (Sharp R21D0(S)-IN, Indonesia) was used for *P. grandiflora* flower samples in the referenced study. In the present study, however, 96% methanol served as the extraction solvent. Methanol is a polar solvent widely used for extracting phenolic compounds and other polar phytochemicals from plant tissues. The use of 96% methanol may favor the extraction of polar phenolics and pigment-related compounds, which should be considered when comparing results with studies employing different solvents. Fresh flower samples (4 g fresh weight) were homogenized to obtain a uniform paste and extracted with 30 mL of 96% methanol using MAE at 135 W for 3 min. Each extract was prepared from an independent pooled flower sample representing one biological replicate (n = 3) per color variant. Samples were handled under reduced light conditions, and extracts were permitted to cool to room temperature after microwave treatment to minimize pigment degradation. The extracts were then filtered using Whatman No. 1 filter paper. The resulting extract corresponded to a concentration of 0.133 g FW mL^{-1}, calculated from 4 g FW extracted in 30 mL of solvent. The filtrate was used for subsequent analyses.

Determination of total phenolic content

The Total Phenolic Content (TPC) was measured by the Folin-Ciocalteu colorimetric method following Asyhar et al. (2023), with minor adjustments for microplate analysis. Briefly, 20 µL of flower extract (0.133 g FW mL^{-1}) was mixed with 120 µL of 10% (v/v) Folin-Ciocalteu reagent in a 96-well microplate (BiologiX) and incubated for 5 min at room temperature. Subsequently, 80 µL of 10% (w/v) Na_2CO_3 solution was added. The mixture was then kept under dark conditions at room temperature for 30 min before absorbance was recorded at 750 nm using a nano-spectrophotometer. (SPECTROstar^{Nano}, BMG LABTECH, Germany). Gallic acid standards (25-300 ppm) were used to construct the calibration curve ($y = 0.0046x - 0.0037$, $R^2 = 0.9985$). The high coefficient of determination ($R^2>0.99$) indicates excellent linearity across the standard concentration range. The obtained values were presented as mg gallic acid equivalent per g fresh weight (mg GAE g^{-1} FW). Each value represents the mean of three biological replicates, and absorbance readings were performed in triplicate as technical measurements.

Determination of total flavonoid content

The Total Flavonoid Content (TFC) was measured by the Aluminum Chloride ($AlCl_3$) colorimetric method according to Calvindi et al. (2020). This assay primarily detects flavonoids capable of forming stable complexes with $AlCl_3$, particularly flavonols and flavones, and may underestimate other flavonoid subclasses, which should be considered when interpreting the reported TFC values. In a 96-well microplate, 10 µL of flower extract (0.133 g FW mL^{-1}) was sequentially mixed with 50 µL methanol (pro analysis grade), 10 µL of 10% (w/v) $AlCl_3$, 10 µL of glacial acetic acid (CH_3COOH), and 120 µL of distilled water. After incubation under dark conditions for 30 min at room temperature, absorbance was determined at 415 nm using a nano-spectrophotometer (SPECTROstar^{Nano}, BMG LABTECH, Germany). Quercetin standards (25-250 ppm) were used to construct the calibration curve ($y = 0.0020x - 0.0407$, $R^2 = 0.9977$). The high coefficient of determination ($R^2>0.99$) indicates excellent linearity across the standard concentration range. Flavonoid content was reported as mg quercetin equivalent per g fresh weight. (mg QE/g FW). Each value represents the mean of three biological replicates, with absorbance measured in technical triplicate.

Table 1. Flower color variants of *Portulaca grandiflora*

Flower color	RHSCC*	Sample code
Light reddish purple, bicolor form	NN74C	LRP-bi
Strong reddish purple	NN78A	SRP
Light orange yellow	23C	LOY
Brilliant yellow	14C	BY

Note: *RHSCC: Color classification is based on the Royal Horticultural Society Color Chart

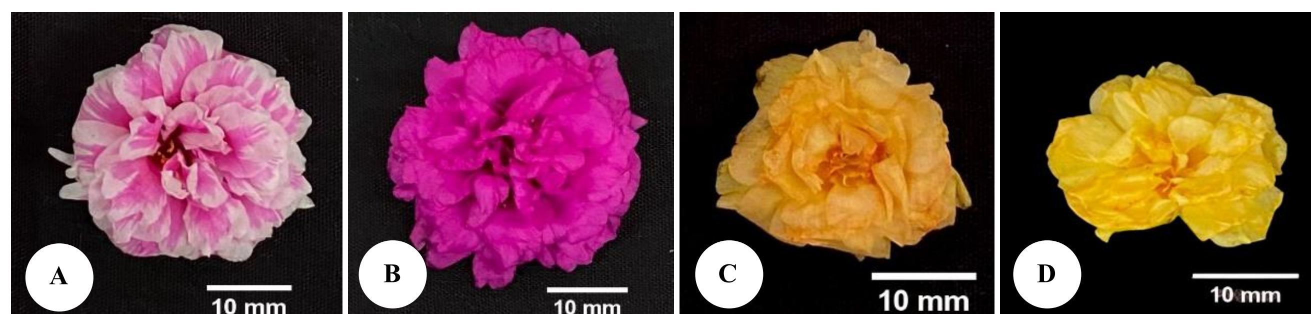

Figure 1. Flower morphology of *Portulaca grandiflora*, showing different petal color variants: A. LRP-bi, B. SRP, C. LOY, and D. BY. Flowers had an average diameter of approximately 25-30 mm across the four color variants

Antioxidant assays

Four in vitro assays were employed to evaluate the antioxidant capacity of *P. grandiflora* flower extracts: two reducing power-based assays, Cupric ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP), and two radical scavenging assays, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). All assays were achieved by a 96-well microplate format using a fixed extract concentration (0.133 g FW mL^{-1}) and measured with a nano-spectrophotometer (SPECTROstar^{Nano}, BMG LABTECH, Germany). Methanol or reagent blanks were included in each assay. Each reported value represents the mean of three independent biological replicates, with absorbance measured in technical triplicate. For all antioxidant assays, the coefficients of determination for the Trolox calibration curves (R^2>0.99) indicated excellent linearity across the respective standard concentration ranges. Antioxidant activity was reported as μmol Trolox equivalent per g fresh weight (μmol TE/g FW). Because measurements were conducted at a single concentration, antioxidant activity was evaluated as Trolox-equivalent capacity rather than as dose-response parameters; Inhibitory Concentration values (IC_{50}) and kinetic analyses were not determined in this study, and all reported antioxidant values therefore reflect activity at the tested extract concentration only.

The CUPRAC assay was conducted according to Arista et al. (2023) with minor modifications. Briefly, 50 μL of flower extract was sequentially mixed with 50 μL of 0.01 M $CuCl_2 \cdot 6H_2O$, 50 μL of 0.0075 M neocuproine, and 50 μL of ammonium acetate buffer (pH 7.0) in a 96-well microplate (BiologiX). The prepared mixture was thoroughly mixed and maintained in dark conditions at room temperature for 30 min. Absorbance was measured at 452 nm using a nano-spectrophotometer. Trolox standards (100-700 μM) were used to construct the calibration curve (y = 0.0010x + 0.0115, R^2 = 0.9932), and the results were expressed as μmol TE/g FW.

FRAP activity was measured following Makkiyah et al. (2023) with slight modifications. Fresh FRAP solution was prepared from acetate buffer, TPTZ solution, and $FeCl_3$ solution in a 10:1:1 ratio. In a 96-well microplate (BiologiX), 10 μL of flower extract was mixed with 300 μL of FRAP reagent, homogenized, and incubated for 30 min at room temperature in the dark. Absorbance was recorded at 597 nm using a nano-spectrophotometer. Trolox standards (25-600 μM) were used for calibration (y = 0.0019x - 0.0229, R^2 = 0.9979), and FRAP values were expressed as μmol TE/g FW.

ABTS radical scavenging activity was evaluated following the method of Aisyah et al. (2023) with slight modifications. The $ABTS^{\bullet+}$ working solution was prepared by mixing 7.7 mM ABTS and 2.4 mM potassium persulfate ($K_2S_2O_8$) at a ratio of 2:1 (v/v), incubated in the dark, and stored at 4°C until use. For the assay, 20 μL of flower extract was added to 180 μL of $ABTS^{\bullet+}$ solution in a 96-well microplate (BiologiX), homogenized, followed by incubation under dark conditions for 6 min at room temperature. Absorbance was measured at 734 nm. Trolox standards (25-400 μM) were used to generate the calibration curve (y = 0.2296x - 1.1766, R^2 = 0.9963). Radical scavenging activity was first calculated as the percentage of inhibition relative to the blank. The inhibition values were then interpolated on the Trolox standard curve to obtain antioxidant capacity in μmol TE/L. These values were subsequently normalized based on extract concentration and fresh weight to express antioxidant capacity as μmol TE/g FW.

DPPH radical scavenging activity was determined based on the procedure described by Nurcholis et al. (2023) to Nurcholis et al. (2023). A total of 100 μL of flower extract was mixed with 100 μL of 125 μM DPPH solution prepared in methanol (pro analysis) in a 96-well microplate (BiologiX). The reaction solution was mixed thoroughly and left under dark conditions at room temperature for 30 min prior to absorbance measurement at 515 nm. Trolox standards (10-55 μM) were used for calibration (y = 1.5868x + 0.6399, R^2 = 0.9952). Radical scavenging activity was first calculated as the percentage of inhibition relative to the blank. The inhibition values were then interpolated on the Trolox standard curve to obtain antioxidant capacity in μmol TE/L. These values were subsequently normalized based on extract concentration and fresh weight to express antioxidant capacity as μmol TE/g FW.

Data analysis

Quantitative datasets were subjected to one-way ANOVA using flower color variant as the tested factor (α = 0.05). The value n = 3 refers to three independent biological replicates per color variant. ANOVA assumptions of normality and homogeneity of variances were evaluated through residual plot inspection. Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. When significant differences were detected, Tukey's post-hoc test was applied. Statistical analyses for ANOVA and post-hoc comparisons were performed using IBM SPSS Statistics Version 25 (IBM Corp., Armonk, NY, USA). Results are presented as mean±SD, and 95% confidence intervals were calculated. Pearson correlation analysis between phytochemical contents and antioxidant capacity was conducted using mean values of the four flower color variants (n = 4) in GraphPad Prism Version 8 (GraphPad Software, San Diego, CA, USA), which was also used for all graphical visualizations. Given the limited number of data points, correlation coefficients are presented for descriptive and exploratory purposes only and should not be interpreted as inferential evidence. Principal Component Analysis (PCA) and correlation heatmap were generated using Python (Version 3.10). Analyses were performed using mean values of the four flower color variants (n = 4).

RESULTS AND DISCUSSION

Total phenolic content and total flavonoid content of *Portulaca grandiflora* flowers

The dataset satisfied normality and variance homogeneity assumptions prior to analysis.TPC and TFC differed significantly among the four flower color variants of *P. grandiflora*. SRP exhibited the highest TPC, whereas LOY and BY showed lower values (Table 2). The TPC of SRP was approximately 19.2% higher than BY and 18.8% higher than LOY. LOY and BY were not significantly different from each other ($p<0.05$). In contrast, the highest TFC values were observed in LOY and BY, followed by SRP, whereas LRP-bi showed the lowest TFC. LOY exhibited approximately 88.5% higher TFC than LRP-bi. Statistical groupings based on Tukey's test are presented in Figure 2 and summarized in Table 2.

Antioxidant capacity of *Portulaca grandiflora* flowers

Antioxidant capacity differed significantly among the four flower color variants across all assays evaluated (Figure 3, Table 2). In reducing power-based assays, SRP showed the highest activity in both CUPRAC and FRAP, whereas LOY exhibited the lowest CUPRAC values and LOY and BY showed the lowest FRAP values. The CUPRAC value of SRP was approximately 6.8-fold higher than LOY. For radical scavenging assays, LRP-bi exhibited the highest ABTS and DPPH activities, whereas BY showed the lowest values. The DPPH activity of LRP-bi was approximately 65.2% higher than BY at the single concentration tested.

Correlation between phenolics, flavonoids, and antioxidant capacities of *Portulaca grandiflora* flowers

Pearson correlation analysis was conducted using mean values from the four color variants (n = 4). The results are presented descriptively in Figure 4. TPC showed strong positive correlations with CUPRAC (r = 0.87) and FRAP (r = 0.87), a strong positive correlation with ABTS (r = 0.75), and a weak positive correlation with DPPH (r = 0.27). In contrast, TFC showed strong negative correlations with ABTS (r = -0.89) and DPPH (r = -0.92), and moderate negative correlations with CUPRAC (r = -0.60) and FRAP (r = -0.58). Because the analysis was based on only four aggregated data points, these coefficients are presented for descriptive purposes only.

Table 2. Total phenolic content, total flavonoid content, and antioxidant capacities of four *Portulaca grandiflora* flower color variants

Flower color variants	TPC (mg GAE/g FW)	TFC (mg QE/g FW)	CUPRAC (µmol TE/g FW)	FRAP (µmol TE/g FW)	ABTS (µmol TE/g FW)	DPPH (µmol TE/g FW)
LRP-bi	3.64 ± 0.04^{ab}	0.61 ± 0.01^{d}	12.28 ± 0.92^{b}	10.41 ± 0.08^{b}	2.99 ± 0.01^{a}	4.23 ± 0.07^{a}
SRP	3.98 ± 0.23^{a}	0.87 ± 0.02^{c}	20.83 ± 1.02^{a}	12.31 ± 0.21^{a}	2.94 ± 0.01^{b}	2.98 ± 0.15^{b}
LOY	3.35 ± 0.14^{b}	1.15 ± 0.01^{a}	3.08 ± 0.79^{d}	8.65 ± 0.36^{c}	2.54 ± 0.02^{c}	2.78 ± 0.07^{bc}
BY	3.34 ± 0.21^{b}	1.09 ± 0.03^{b}	6.13 ± 0.62^{c}	8.89 ± 0.24^{c}	2.45 ± 0.04^{d}	2.56 ± 0.06^{c}

Note: Values are expressed as mean±SD (n = 3). Different letters within the same column indicate significant differences at $p<0.05$ according to Tukey's test

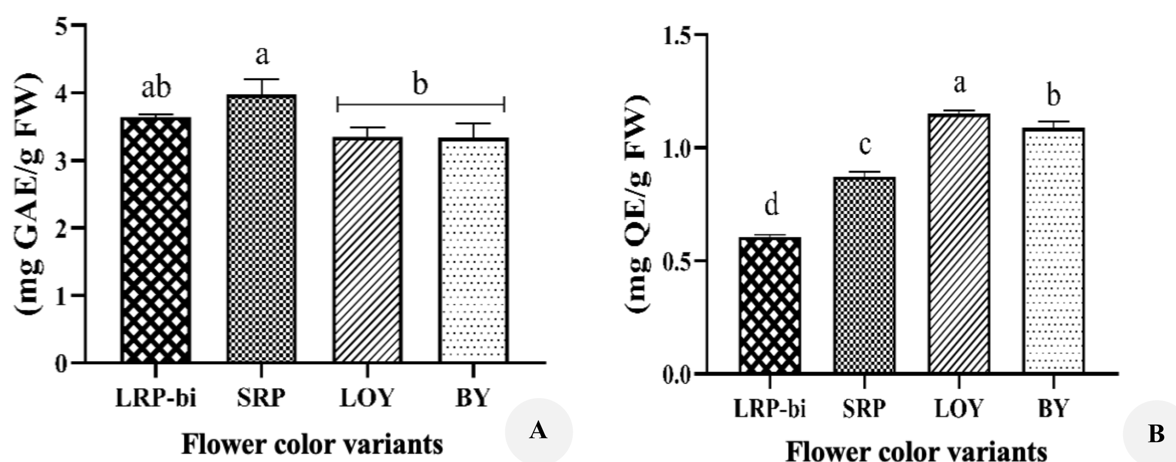

Figure 2. A. TPC, and B. TFC of methanolic extracts of *Portulaca grandiflora* flowers. Data are presented as mean±SD (n = 3). Bars with different letters indicate significant differences at $p<0.05$ according to Tukey's test. Abbreviations: LRP-bi: Light Reddish Purple (bicolor form), SRP: Strong Reddish Purple, LOY: Light Orange Yellow, BY: Brilliant Yellow

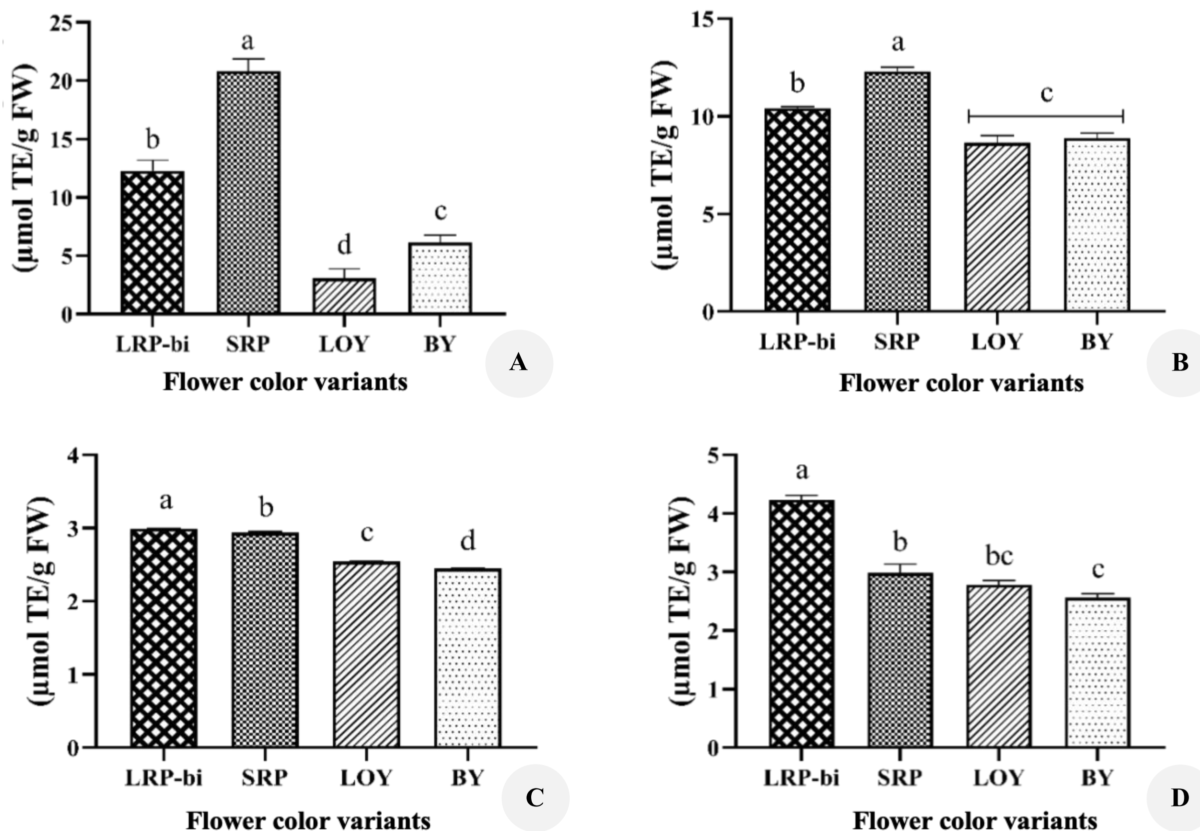

Figure 3. Antioxidant capacity of methanolic extracts of *Portulaca grandiflora* flowers: A. CUPRAC, B. FRAP, C. ABTS, and D. DPPH. Data are presented as mean±SD (n = 3). Bars with different letters indicate significant differences at $p<0.05$ according to Tukey's test. Among the four color variants examined, SRP exhibited the highest reducing power in CUPRAC and FRAP assays, whereas LRP-bi showed the highest radical scavenging activity in ABTS and DPPH assays based on methanolic extracts evaluated at a single tested concentration

Multivariate analysis of phytochemical and antioxidant variation

Principal component analysis explained 99.1% of the total variance, with PC1 and PC2 accounting for 77.8% and 21.3%, respectively. PCA revealed separation among flower color variants along PC1 and PC2. Red-purple variants (SRP and LRP-bi) tended to be associated with higher TPC and reducing power (CUPRAC and FRAP), while yellow variants (LOY and BY) were linked to higher TFC and lower antioxidant capacity. LRP-bi was further separated along PC2, corresponding to higher ABTS and DPPH activity. Loading vectors indicated positive associations among TPC, CUPRAC, and FRAP, whereas TFC displayed an opposite trend (Figure 5.A). The correlation heatmap suggested positive relationships between TPC and reducing power assays (CUPRAC and FRAP), while TFC showed negative correlations, particularly with ABTS and DPPH. Heatmap analysis was based on mean values in Table 2 (Figure 5.B).

Discussion

The results revealed clear differences in phytochemical content and antioxidant capacity among the four flower color variants of *P. grandiflora*. The contrasting distribution of TPC and TFC among the four flower color variants suggests differences in phenolic subclass composition rather than uniform changes in total phenolic accumulation. The red-purple variants (SRP and LRP-bi) were characterized by higher overall phenolic levels, whereas the yellow variants (LOY and BY) exhibited relatively higher TFC values. Because individual phenolic compounds were not identified or quantified in the present study, these differences should be interpreted as variation in overall phenolic distribution rather than confirmed differences in specific subclasses.

Previous studies in *Portulaca* species have reported associations between flower color and pigment composition, including betalains, anthocyanins, and carotenoids (Mattioli et al. 2020; Spórna-Kucab et al. 2022). In *P. grandiflora*, red-purple pigmentation is generally linked to betacyanins, whereas yellow-orange coloration is associated with betaxanthins. Molecular investigations in related species (*Portulaca umbraticola* Kunth) have identified genes involved in betacyanin and betaxanthin biosynthesis (Sumi et al. 2025). These literature-based findings provide a plausible biochemical context; however, because pigment classes were not directly quantified in this study, any pigment-related interpretation should be regarded as hypothesis-generating and supported by external literature rather than derived from the current dataset.

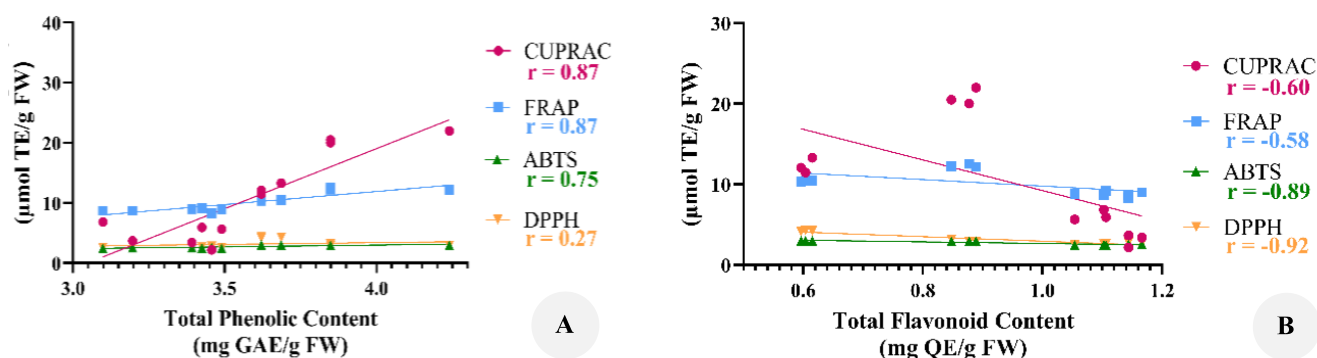

Figure 4. Exploratory Pearson correlation plots showing relationships between: A. TPC and antioxidant capacities, and B. TFC and antioxidant capacities of *Portulaca grandiflora* flower extracts (n = 4). Each panel displays individual data points with fitted linear regression lines. Correlation coefficients (r) are provided for descriptive purposes only. These plots represent exploratory visual summaries and should not be interpreted as inferential models, as the analysis was based on four aggregated mean values. No inferential significance testing is emphasized due to the limited sample size

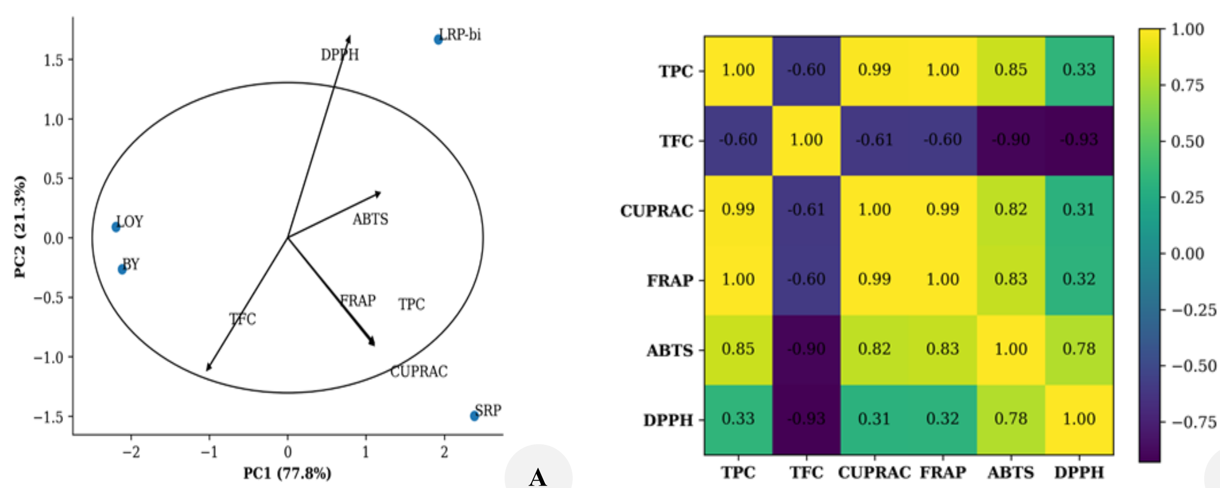

Figure 5. Multivariate analysis of phytochemical and antioxidant variation in *Portulaca grandiflora* flower color variants. A. Principal component analysis (PCA) showing separation among variants along PC1 and PC2, B. Correlation heatmap illustrating relationships among phytochemical parameters and antioxidant assays

Methodological considerations may also contribute to the observed TPC-TFC contrast. The Folin-Ciocalteu assay detects a broad range of reducing substances, including diverse phenolic compounds (Pérez et al. 2023), and may also respond to other reducing metabolites present in plant extracts, whereas the $AlCl_3$-based method selectively quantifies flavonoids capable of forming aluminum complexes (Sultana et al. 2024). This difference in assay chemistry may explain why TPC and TFC did not follow the same directional pattern among flower color variants. Consequently, TFC values represent a specific flavonoid fraction rather than the total flavonoid pool, and direct comparisons between TPC and TFC should be interpreted cautiously.

The antioxidant patterns observed among color variants are generally consistent with their phenolic profiles. Yellow variants (LOY and BY) generally displayed lower antioxidant values across assays. The higher reducing capacity detected in the SRP variant may be related to its elevated total phenolic level. Previous studies in *P. grandiflora* have reported similar associations between phenolic content and antioxidant activity (Aisyah et al. 2023), with further variation observed across mutant lines and extraction conditions (Nurcholis et al. 2023). However, interpretation of total flavonoid content should be approached cautiously, as the $AlCl_3$ colorimetric assay may underestimate certain flavonoid subclasses depending on their structural characteristics. This methodological limitation may partly explain the weaker association between TFC and antioxidant responses. Across variants evaluated at the tested extract concentration, reducing power-based assays (CUPRAC and FRAP) produced stronger responses than radical scavenging assays (ABTS and DPPH). Across the four variants evaluated, antioxidant capacity at the single concentration tested followed the overall order: CUPRAC>FRAP>DPPH>ABTS. This pattern may indicate

a greater contribution of single-electron transfer mechanisms under the applied in vitro conditions (Sun and Shahrajabian 2023). However, mechanistic pathways were not directly examined and therefore cannot be confirmed from the present data.

Differences in antioxidant responses among color variants may reflect variation in phenolic subclass distribution or pigment composition. Nevertheless, because pigment profiling was not performed, these explanations remain literature-informed hypotheses rather than experimentally verified conclusions.

The positive associations between TPC and reducing power assays are consistent with the established role of phenolic compounds as effective electron donors and metal chelators (Kuljarusnont et al. 2024). The comparatively weaker association with DPPH may reflect the structural selectivity of this radical toward particular phenolic configurations (Asyhar et al. 2023). In contrast, TFC showed negative correlations with antioxidant assays within this dataset. Because the $AlCl_3$ assay predominantly detects flavonols and flavones (Sultana et al. 2024), TFC values do not necessarily represent the complete flavonoid fraction. Moreover, the correlation analysis was conducted using four aggregated mean values ($n = 4$); therefore, these relationships should be considered exploratory and interpreted with caution rather than as definitive quantitative associations.

The PCA results provided an exploratory summary of variation among phytochemical and antioxidant parameters. Phenolic compounds appeared to contribute to differentiation among flower color variants, as shown by the association between TPC and reducing power assays (CUPRAC and FRAP). The correlation heatmap supported these findings, showing positive relationships among TPC, CUPRAC, and FRAP, while TFC showed an opposing pattern. This consistency suggests that phenolic compounds may contribute to antioxidant capacity. The observed separation among color variants may reflect underlying phytochemical variation and antioxidant potential.

Beyond antioxidant characterization, the findings highlight measurable intraspecific phenotypic and biochemical variation within *P. grandiflora*. Flower color, a visible morphological trait, was associated with differences in phenolic composition and antioxidant-related functional responses under standardized conditions. Such visible floral polymorphism represents intraspecific biodiversity that may reflect functional trait variation within ornamental plant germplasm and may be useful for distinguishing germplasm within ornamental plant collections. From a biodiversity perspective, this intraspecific variation represents functional trait diversity within a single ornamental species. Characterizing this variation may support germplasm evaluation, bioresource identification, and conservation-oriented characterization of ornamental plant diversity. Such diversity may serve as a valuable resource for germplasm characterization, ornamental selection, and plant bioresource evaluation.

Visible phenotypic traits, such as flower color, may function as preliminary indicators of underlying biochemical differentiation within the evaluated variants. This linkage between morphology and phytochemical traits contributes to a broader understanding of how ornamental diversity can intersect with functional plant properties and supports the relevance of intraspecific diversity within plant bioresource studies.

However, broader ecological or genetic generalization requires caution. The present study evaluated four variants collected from two locations, and biological replicates consisted of pooled flowers. Although the two sites were located within the same campus area and experienced broadly comparable environmental conditions, genotype and environmental effects were not experimentally separated. Therefore, color-associated differences observed in this study should not be interpreted strictly as genetic or purely phenotypic effects independent of environmental conditions. In addition, the evaluated variants represent phenotypically defined color variants rather than registered cultivars, further supporting cautious interpretation of genotype-level differences. Furthermore, the results reflect methanol-extractable compounds obtained under the selected microwave-assisted extraction conditions and may not represent the complete phytochemical composition of each variant. Accordingly, the present findings represent an initial assessment of functional diversity rather than a comprehensive evaluation of genetic variation within the species.

Overall, the present study provides a comparative screening of four defined flower color variants of *P. grandiflora* under standardized extraction and assay conditions. The results indicate that certain variants, particularly SRP, exhibited higher phenolic content and stronger antioxidant responses at the tested extract concentration. These observations suggest potential value for further investigation in germplasm evaluation or breeding-oriented research, while providing preliminary information rather than immediate practical applications. Because the study was limited to two collection sites, pooled biological replicates, and overall phenolic estimation (TPC and TFC) without compound-level identification, further research is required. Future studies should include (i) targeted profiling of individual phenolic and pigment compounds, (ii) controlled cultivation experiments to distinguish genetic and environmental effects, and (iii) validation in biological or physiological systems beyond in vitro antioxidant assays.

In conclusion, this study demonstrated clear differences in phytochemical content and antioxidant capacity among the four flower color variants of *P. grandiflora* examined. The Strong Reddish Purple (SRP) variant exhibited the highest total phenolic content (3.98 ± 0.23 mg GAE g^{-1} FW) and the strongest reducing capacity, with CUPRAC (20.83 ± 1.02 μmol TE g^{-1} FW) and FRAP (12.31 ± 0.21 μmol TE g^{-1} FW) values exceeding those of other variants, including a ~6.8-fold higher CUPRAC compared to Light Orange Yellow (LOY). In contrast, the LOY and Brilliant Yellow (BY) variants showed the highest total flavonoid content (1.15 ± 0.01 and 1.09 ± 0.03 mg QE g^{-1} FW, respectively), while the Light Reddish Purple bicolor (LRP-bi) variant exhibited the strongest radical scavenging activity (ABTS: 2.99 ± 0.01 μmol TE g^{-1} FW; DPPH: 4.23 ± 0.07 μmol TE g^{-1} FW). Correlation analysis indicated

strong positive associations between TPC and reducing power assays (r ≈ 0.87), suggesting that phenolic compounds contribute substantially to electron transfer capacity, whereas TFC showed negative associations with antioxidant responses within the evaluated dataset. These findings indicate that flower color is associated with variation in phenolic composition and antioxidant-related functional traits, with red-purple variants exhibiting greater reducing potential. However, the results are limited by the small number of variants, the pooled sampling design, the absence of compound-level profiling, and the reliance on in vitro assays. Future studies should incorporate a broader range of genotypes, apply factorial experimental designs to separate genetic and environmental effects, and integrate compound-specific analyses (e.g., LC-MS/HPLC) and biological validation to clarify the mechanistic basis and functional relevance of these associations.

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