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RESEARCH PAPER

Quality characterization of flavonoids from flowers, stems and leaves of *Chrysanthemum morifolium* Ramat. 'Chuju'

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Abstract

Chrysanthemum morifolium Ramat. 'Chuju' is a plant with dual edible and medicinal values. While the bioactive constituents of Chuju flowers have been extensively investigated, the flavonoid composition and quality characteristics in its stems and leaves remain systematically uncharacterized. This study employed ultrasound-assisted ethanol extraction to obtain flavonoid extracts from flowers, stems, and leaves during the flowering period, followed by comprehensive analysis of total flavonoid content, antioxidant capacity, and stability. The results showed that the total flavonoid content in the flowers, stems and leaves of Chuju was relatively high. The total flavonoid content in the flowers was 73.99 mg/g DW, followed by leaves (51.95 mg/g DW) and stems (32.72 mg/g DW). HPLC analysis revealed that the flowers, stems and leaves of Chuju all contained four types of flavonoids and phenolic acids: chlorogenic acid, rutin, luteolin-7-O-glucoside, and 3,5-O-dicaffeoylquinic acid. The content of these components varied in different parts, with the highest content of chlorogenic acid being in the flowers (3.51 mg/g DW), followed by the stems (1.56 mg/g DW). The content of luteolin-7-O-glucoside was the highest in the leaves (0.69 mg/g DW), significantly higher than that of the other components. The extracts from flowers, stems, and leaves demonstrated strong DPPH and ABTS radical scavenging activities. Stability tests indicated flavonoids from flowers, stems, and leaves were sensitive to light, oxygen, high temperature, and extreme pH conditions. These findings have revealed the potential of the stems and leaves of Chuju as a new source of flavonoids.

Keywords

Antioxidant activity, *Chrysanthemum morifolium*, phenolic acids, quality characteristics, stems and leaves, total flavonoids

Introduction

Chrysanthemums have a two-thousand-year cultivation history in China, traditionally used for medicine and tea (Hou et al. 2020; Shao et al. 2020; Sun et al. 2021; Zheng et al. 2024). Modern studies show they are rich in bioactive components including flavonoids, phenolic acids, essential oils, polysaccharides and other natural active ingredients (Pandey et al. 2022; Chen et al. 2024). As one of China's four major medicinal chrysanthemums, *Chrysanthemum morifolium* Ramat. 'Chuju' is mainly cultivated in Chuzhou, Anhui Province. Its unique growing environment contributes to high levels of flavonoids and phenolic acids, enhancing its medicinal and culinary value (Tao et al. 2019; Wang et al. 2019b).

Flavonoids possess a characteristic C6-C3-C6 skeleton (Addi et al. 2021; Chu et al. 2024). They are classified into six subclasses, including flavones, flavonols, flavanones, flavanonols, flavanols or catechins,

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anthocyanins, and chalcones (Panche et al. 2016). Flavonoids are the main medicinal active components in chrysanthemum (Chen et al. 2021; Wang et al. 2021). They not only play a crucial role in the growth and physiological function of chrysanthemum, but also have strong antioxidant (Williams. 2000; Hu et al. 2017; Chen et al. 2023; Doan et al. 2024), anti-inflammatory and anti-tumor functions (Liu et al. 2018; Mohamad and Che Zahari 2024). Chen et al. (2021) identified and quantified chemicals of 7 caffeoylquinic acids, 21 flavones and flavonols, 4 carotenoids, and 13 other compounds in 27 cultivars and representative tea of *Chrysanthemum morifolium*. It was found that cynaroside and apigenin were the main components of *Imperial Chrysanthemum* flavonoids which could reduce postprandial blood glucose and improve antioxidant status (Chen et al. 2023). Yin et al. (2025) found Chuju flavonoids, particularly apigenin and luteolin derivatives, can regulate glycolipid metabolism. Similarly, Sun et al. (2021) reported that chrysanthemum flavonoids effectively improve blood lipids and hepatic steatosis, with strong antioxidant and lipid-regulating abilities. Thus, chrysanthemum is an excellent source of natural flavonoids for health-promoting products.

However, most of the existing research focuses on the study of bioactive compounds in chrysanthemum capitula (head flower) (Lin and Harnly 2010; Wang et al. 2019a; Yuan et al. 2020; Chen et al. 2024; Liu et al. 2024), with limited investigation into flavonoid composition, content, and bioactivity in stems and leaves. In China, chrysanthemum harvesting occurs from September to November before frost (Lee and Kim 2020; Wang et al. 2024), with residual stems and leaves often discarded as low-value byproducts due to their unappealing appearance. The stems and leaves of chrysanthemum also accumulate substantial secondary metabolites, particularly flavonoids and phenolic acids during growth (Pandey et al. 2022). Preliminary studies indicated the flavonoid content of leaves reached 30%–50% of capitula, containing bioactive phenolic acids like chlorogenic and caffeic acids with potential antioxidant and antimicrobial activities (Beninger et al. 2004; Xue et al. 2019; Shao et al. 2020; Doan et al. 2024).

To fill this gap, our study provides the first systematic comparison of flavonoid quality in Chuju flowers, stems, and leaves. We assess total flavonoid content, specific phenolic profiles, antioxidant properties, and stability across these parts. This approach highlights the potential of underutilized stems and leaves.

Therefore, this study aims to evaluate the quality characteristics of flavonoids from Chuju three anatomical parts (flowers, stems, and leaves) harvested during the same growth period. The main component composition, antioxidant activity, and storage stability of flavonoids were assessed. The research results can provide theoretical support for the efficient utilization and quality control of Chuju chrysanthemum stems and leaves, and also offer a reference for the development of other medicinal plant by-products.

Materials and methods

Source of raw material

The Chuju plants (*Chrysanthemum morifolium* Ramat. 'Chuju') used in this study were cultivated at the Chuzhou Chrysanthemum Planting Base in Daliu Town, Nanqiao District, Chuzhou, Anhui Province, China. The agronomic conditions for cultivating the *Chrysanthemum morifolium* Ramat. 'Chuju' cultivar were as follows. The soil is a yellow-brown earth characterized by a sandy loam texture, good drainage, and a pH of 5.5–7.0, which is ideal for root development and waterlogging prevention. A base dressing of fully decomposed organic fertilizer was applied before soil preparation to ensure balanced nutrition. Furthermore, topdressing was supplied during the seedling, branching/bud formation, and initial bud stages. Irrigation was carefully managed to maintain moist but unsaturated soil conditions, as this species requires consistent moisture but is sensitive to waterlogging.

The leaves, stems and flowers of Chuju were freshly harvested in November 2024. Then, they were dried in a constant-temperature drying oven (Model DHG-9070, Shanghai Yiheng Scientific Instruments Co., Ltd., China) at 60 ± 1 °C. Finally, the dried Chuju flowers, stems, and leaves were milled using a laboratory mill, passed through a 40-mesh sieve, and stored as aliquots for subsequent analysis.

Extraction of total flavonoids from Chuju flowers, stems, and leaves

Ultrasound-assisted extraction with aqueous ethanol was selected to obtain the flavonoid extracts (Lin and Harnly 2010; Yuan et al. 2015; Liu et al. 2025). A concentration of 60% (V/V) aqueous ethanol was used based on preliminary optimization experiments, which indicates that this concentration exhibits an optimized polarity for effectively extracting a wide range of flavonoids from Chuju plants.

Aliquots (2.00 ± 0.01 g) of pulverized Chuju flowers (40-mesh passed) were precisely weighed and extracted with 100 mL of 60% (V/V) aqueous ethanol at 40 °C under ultrasonic irradiation (28 kHz, 300 W) for 30 min. The mixture was vacuum-filtered with the residue subjected to two additional extraction cycles under identical conditions. The combined filtrate volumes from all three extractions were recorded, and total flavonoid concentrations were determined. Identical extraction and analytical procedures were applied to stem and leaf samples. The parallel tests were repeated three times (n = 3).

Determination of total flavonoid content of flowers, stems, and leaves

The total flavonoid content was determined according to the method described by Chen et al. (2023) with minor modifications. Rutin standard (7.5 mg) was

accurately weighed and dissolved in 60% (V/V) ethanol in a 25 mL volumetric flask to prepare a 0.3 mg/mL stock solution. Aliquots (0.5, 1.0, 1.5, 2.0, and 2.5 mL) of the stock solution were transferred to separate 10 mL volumetric flasks, each mixed with 2.4 mL of 60% (V/V) ethanol. Added 0.4 mL of 5% (W/V) $NaNO_2$, mixed thoroughly, and stood for 6 min. Added 0.4 mL of 10% (W/V) $Al(NO_3)_3$, mixed thoroughly, and stood for 6 min. Added 4 mL of 4% (W/V) NaOH with immediate mixing and brought to final volume with 60% (V/V) ethanol and the solutions were incubated for 15 min. Absorbance was measured at 510 nm using a UV-visible spectrophotometer (Model L6, Shanghai Yidian Analytical Instruments Co., Ltd.). The calibration curve (y = 1.05338x - 0.0208, R^2 = 0.9995) showed excellent linearity, where x = rutin mass (mg) and y = absorbance.

Total flavonoid content of the flowers, stems and leaves were determined as follows: The sample solution was appropriately diluted in a volumetric flask. Absorbance was measured at 510 nm against a reagent blank. The total flavonoid content was quantified by interpolation from the established calibration curve. The total flavonoid content was quantified for each extract, and the measurement was repeated three times (n = 3).

HPLC analysis

HPLC analysis was performed using an Agilent 1260 series HPLC system (Agilent Technologies, USA) equipped with a ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 μm). The mobile phase consisted of (A) 0.1% phosphoric acid in water and (B) acetonitrile, delivered at a flow rate of 1.0 mL/min with the following gradient program: 0 min (90% A), 11 min (88% A), 13 min (83% A), 45 min (83% A), 46 min (79% A), 55 min (76% A), 80 min (74% A), 85 min (55% A), 120 min (50% A), and 125 min (90% A). The column temperature was maintained at 35 °C, and the detection wavelength was set at 348 nm. The injection volume was 10 μL.

Four standard stock solutions including chlorogenic acid (0.33 mg/mL), luteoloside (0.18 mg/mL), 3,5-O-dicaffeoylquinic acid (0.42 mg/mL), and rutin (0.06 mg/mL) were individually prepared in 70% methanol. A mixed working standard solution was prepared in 70% methanol from each individual standard stock solution and diluted to the appropriate concentration range for the establishment of calibration curves. The experiments were repeated three times to ensure accuracy.

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated according to the method of Wang et al. (2019b) and Hou et al. (2020) with minor modifications. The flavonoid extracts from Chuju flowers, stems and leaves were prepared at concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL. L-ascorbic acid was used as positive control. 1.0 mL of each test sample was added to 2.0 mL of a 0.06 mmol/L solution of DPPH in ethanol. The mixture was mixed and incubated for 30 min in the dark at room temperature, and then the absorbance was determined at 517 nm. DPPH radical scavenging ability (%) = (Absorbance of control - Absorbance of sample)/Absorbance of control × 100. All measurements were performed in three independent replicates (n = 3).

ABTS radical scavenging activity

The ABTS radical scavenging activity was determined according to the method of Li et al. (2018) with modifications. 1.0 mL of Chuju sample solution was added to 4.0 mL of ABTS working solution, reacted in the dark for 10 min, and the absorbance was measured at 734 nm. L-ascorbic acid was used as positive control. ABTS radical scavenging ability (%) = (Absorbance of control-Absorbance of sample)/Absorbance of control × 100. All measurements were performed in three independent replicates (n = 3).

Flavonoid stability analysis

Effects of light and oxygen on the stability of Chuju flavonoids: Aliquots of flavonoid extracts from Chuju flowers were incubated under four conditions for 6 h at 25 °C: (1) natural light and aerobic condition, (2) natural light and anaerobic condition, (3) dark and aerobic condition, and (4) dark and anaerobic condition. The flavonoid contents were quantified using $Al(NO_3)_3$ spectrophotometry at 510 nm by UV-Vis spectrophotometry. Identical procedures were applied for stem and leaf extracts.

Effect of temperature on the stability of Chuju flavonoids: Aliquots of flavonoid extract from Chuju flowers, stems and leaves were transferred into sealed test tubes and incubated in constant-temperature water baths at 25 °C, 60 °C, 70 °C, 80 °C, and 90 °C. The residual flavonoid content was quantified every 2 h using $Al(NO_3)_3$ spectrophotometry at 510 nm.

Effect of pH on the stability of Chuju flavonoids: Equal aliquots of the extract were distributed into eight sealed test tubes and adjusted to pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. Each solution was diluted to 50 mL with ultrapure water. Flavonoid content was measured at 510 nm to evaluate pH-dependent stability of flavonoid from Chuju flowers, stems and leaves.

Each stability test condition was evaluated with three replicates (n = 3).

Statistical analyses

Statistical analyses were conducted with SPSS 25.0 statistical software (IBM Corp., USA). Data were expressed as the mean ± SD. Significant differences among the groups

were determined by using one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. $p < 0.05$ was considered to be statistically significant.

Results and discussion

Comparative analysis of total flavonoid content in different tissues of Chuju

Flavonoids from Chuju flowers, stems, and leaves were extracted using 60% ethanol to compare content differences among tissues. Fig. 1A shows the flavonoid extraction rate of Chuju flowers, stems, and leaves gradually decreased as the extraction times increased, with stem content nearing the detection limit after the third extraction. Fig. 1B shows significant differences in total flavonoid content among tissues ($p < 0.05$). Flowers had the highest content (73.99 ± 0.82 mg/g DW), significantly greater than leaves (51.95 ± 2.07 mg/g DW) and stems (32.72 ± 1.47 mg/g DW). The leaves, in turn, contained a significantly higher flavonoid content than the stems. These results align with Jia et al. (2019), who reported flavonoid (70.32 mg/g DW), total phenolic (53.67 mg/g DW), and polysaccharide (7.68 mg/g DW) contents in Chuju flowers, but their study did not analyze stems and leaves. Hu et al. (2017) also reported a total flavonoid content of 64.25 ± 4.80 mg/g in Chuju, and Du et al. (2015) found a wide range (1.4–87.2 mg/g DW) in Chinese chrysanthemum teas, confirming Chuju as a rich flavonoid source.

This study is the first to report flavonoid content in Chuju stems and leaves. Although individual stem and leaf contents were lower than in flowers, their combined content (84.67 mg/g) exceeded that of flowers alone, highlighting the value of non-floral tissues.

HPLC analysis of flavonoid extracts from flowers, stems, and leaves

The Chinese Pharmacopoeia (2020) identifies luteolin-7-O-glucoside, chlorogenic acid and 3,5-O-dicaffeoylquinic acid as the key bioactive constituents in *Chrysanthemum morifolium*. In this study, chlorogenic acid, rutin, luteolin-7-O-glucoside, and 3,5-O-dicaffeoylquinic acid were used as authentic standards to quantify them in Chuju extracts. As shown in Table 1, the retention time of four standards was13.39 min for chlorogenic acid, 29.66 min for rutin, 34.67 min for luteolin-7-O-glucoside, and 43.59 min for 3,5-O-dicaffeoylquinic acid. Calibration curves for each compound were established by plotting the peak area (Y) against the concentration (X, μg/mL). Calibration curves showed good linearity ($R^2 \geq 0.9992$).

Table 1. Calibration curve equations of the standards.

Compound	Retention time (min)	Calibration Curve	R^2
Chlorogenic acid	13.39	y = 9.9496x + 10.748	0.9995
Rutin	29.66	y = 11.784x + 1.2613	0.9999
Luteolin-7-O-glucoside	34.67	y = 25.006x + 15.728	0.9996
3,5-O-dicaffeoylquinic acid	43.59	y = 11.062x + 21.609	0.9992

Y = peak area, X = concentration of the standard (μg/mL).
R^2 = coefficient of determination for five calibration data points (n = 3).

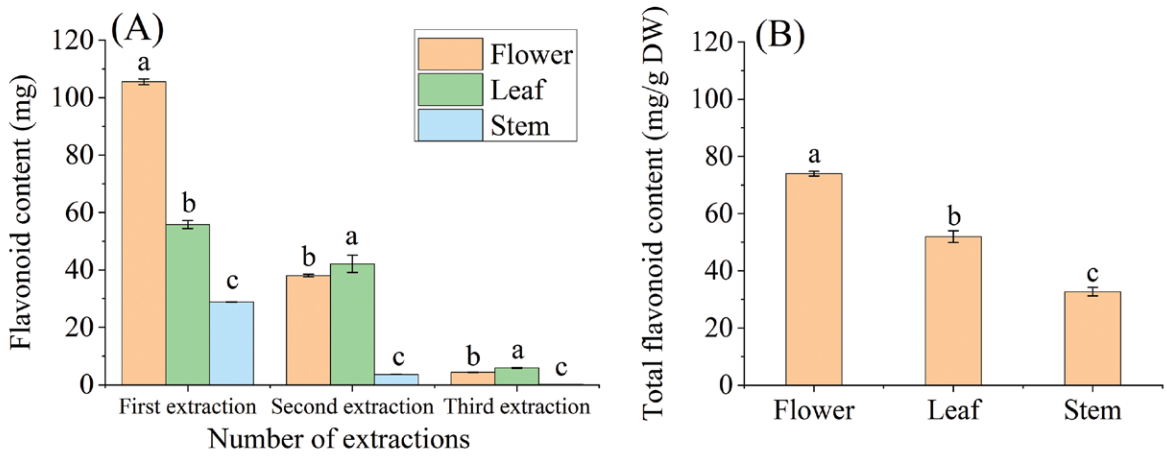


Figure 1. Total flavonoid content in different tissues of Chuju. **A.** Changes in the flavonoid content of flowers, leaves and stems during three extraction processes; **B.** Total flavonoid content of Chuju flowers, leaves and stems. Data are presented as mean ± SD (n = 3). Values labeled with different letters (a, b, c) are significantly different ($p < 0.05$) according to one-way ANOVA with Tukey's test.

As shown in Fig. 2, the flavonoid extracts from different parts of Chuju showed distinct chemical compositions. Flower extracts had the most chromatographic peaks, indicating richer composition. Leaves showed intermediate peak numbers, while stems had the fewest, suggesting a simpler flavonoid profile.

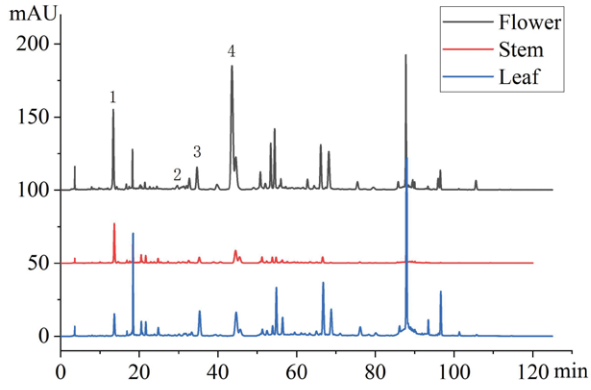


Figure 2. Chromatograms of Chuju flowers, leaves and stems. (1) Chlorogenic acid; (2) Rutin; (3) luteolin-7-O-glucoside; (4) 3,5-O-dicaffeoylquinic acid.

Table 2 shows quantitative results. Flower extracts had significantly higher ($p < 0.05$) levels of chlorogenic acid, 3,5-O-dicaffeoylquinic acid, and rutin than stems and leaves. In contrast, luteolin-7-O-glucoside was highest in leaves ($p < 0.05$), with flowers intermediate. This indicates significant accumulation of specific bioactives in non-floral tissues, especially leaves.

The flavonoid differences among organs likely reflect their distinct physiological roles. Flowers, as reproductive structures, are metabolic sinks that channel resources towards the synthesis of specialized metabolites for pollinator attraction, UV protection, and defense against pathogens during the vulnerable flowering stage (Samman and Cook 1996; Meldau et al. 2012; Cheynier et al. 2013). The high accumulation of chlorogenic acid and 3,5-O-dicaffeoylquinic acid in flowers may be linked to their roles as precursors for lignin biosynthesis in floral tissues and as potent antioxidants protecting reproductive structures from oxidative stress (Fraser and Chapple 2011). Additionally, the shikimate and phenylpropanoid pathways are highly active in flowers (Vogt 2010), leading to the abundant synthesis of these phenolic acids.

In contrast, leaves, as photosynthetic organs, are major sites for the biosynthesis of flavones like luteolin and its glycosides (Falcone Ferreyra et al. 2012). The high concentration of luteolin-7-O-glucoside in leaves suggests its crucial role in mitigating photo-oxidative damage. Leaves are exposed to high light flux, and flavonoids such as luteolin glycosides effectively scavenge reactive oxygen species generated during photosynthesis, thereby protecting photosynthetic apparatus (Williams. 2000; Brunetti et al. 2013). This ecological function aligns with the significant allocation of metabolic resources towards luteolin derivative synthesis in leaf tissues, as observed in our results.

Stems, primarily involved in structural support and transport, typically exhibit a simpler flavonoid profile and lower overall content. Their primary defense strategy often relies more on structural polymers like lignin and cellulose rather than high concentrations of phenolic antioxidants. The lower flavonoid diversity and quantity in stems may reflect a different investment strategy in chemical defense, prioritizing physical barrier formation over the biosynthesis of a broad spectrum of soluble phenolics (Meldau et al. 2012). This organ-specific partitioning of defense resources is a common adaptive strategy in plants.

Therefore, the distinct flavonoid composition in each organ likely reflects its unique developmental needs and adaptive defense strategies within the plant.

Notably, HPLC revealed several unquantified peaks in all parts, especially flowers, indicating a more complex phytochemistry than captured by the four markers. These may include other flavonoid glycosides or phenolic derivatives. They could synergistically contribute to overall bioactivity. Future LC-MS studies should identify these compounds.

DPPH and ABTS radical scavenging activity

DPPH and ABTS are two classic and widely accepted methods for evaluating antioxidant activity. The DPPH radical scavenging activities of flavonoid extracts from different parts of Chuju (flowers, stems, and leaves) were investigated (Table 3). The results demonstrated concentration-dependent antioxidant effects with a clear dose-response relationship, which was consistent with previous findings of Chuju aqueous extract by Wang et al. (2019b). At 0.1 mg/mL, DPPH scavenging rates were 48.51% for flower extracts, 36.09% for stem

Table 2. Flavonoids and phenolic acids detected in Chuju extracts.

Sample	Chlorogenic acid (mg/g DW)	Luteolin-7-O-glucoside (mg/g DW)	3,5-O-dicaffeoylquinic acid (mg/g DW)	Rutin (mg/g DW)
Flower	3.51 ± 0.01^{a}	0.61 ± 0.01^{b}	10.69 ± 0.02^{a}	0.24 ± 0.01^{a}
Stem	1.56 ± 0.01^{b}	0.14 ± 0.01^{c}	0.81 ± 0.01^{c}	0.11 ± 0.01^{c}
Leaf	0.95 ± 0.01^{c}	0.69 ± 0.01^{a}	1.70 ± 0.01^{b}	0.14 ± 0.01^{b}

Data are presented as mean ± SD (n = 3). Different superscript letters (a, b, c) within the same column indicate significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's multiple comparison test.

Table 3. DPPH and ABTS radical scavenging activities of flavonoids extracts from different organs of Chuju at various concentrations.

Plant Organ	Flavonoids concentration (μg/mL)	DPPH activity (%)	ABTS activity (%)
Flower	20	18.97 ± 2.20 [b]	12.24 ± 4.26 [b]
Stem	20	13.47 ± 1.31 [c]	9.33 ± 0.54 [c]
Leaf	20	13.91 ± 1.52 [c]	9.53 ± 0.35 [c]
Ascorbic acid (Control)	20	56.92 ± 1.18 [a]	22.85 ± 1.73 [a]
Flower	40	22.99 ± 1.79 [b]	25.83 ± 3.14 [b]
Stem	40	19.20 ± 3.54 [b]	15.82 ± 0.73 [c]
Leaf	40	21.43 ± 0.59 [b]	15.82 ± 2.26 [c]
Ascorbic acid (Control)	40	62.13 ± 1.52 [a]	39.49 ± 0.31 [a]
Flower	60	31.40 ± 3.04 [b]	32.79 ± 1.95 [b]
Stem	60	31.18 ± 2.28 [b]	28.19 ± 4.26 [bc]
Leaf	60	34.52 ± 2.07 [b]	22.92 ± 1.22 [c]
Ascorbic acid (Control)	60	63.91 ± 3.18 [a]	51.66 ± 1.64 [a]
Flower	80	38.54 ± 1.95 [b]	33.94 ± 1.83 [b]
Stem	80	33.04 ± 1.34 [c]	27.05 ± 0.62 [c]
Leaf	80	38.39 ± 1.18 [b]	26.98 ± 3.72 [c]
Ascorbic acid (Control)	80	73.51 ± 1.49 [a]	72.41 ± 1.42 [a]
Flower	100	48.51 ± 1.90 [b]	41.72 ± 0.82 [b]
Stem	100	36.09 ± 5.92 [c]	30.63 ± 0.54 [d]
Leaf	100	40.85 ± 1.56 [c]	38.07 ± 1.17 [c]
Ascorbic acid (Control)	100	81.18 ± 2.25 [a]	78.09 ± 1.13 [a]

Data are presented as mean ± SD (n = 3). Values labeled with different letters (a, b, c) are significantly different ($p < 0.05$) according to one-way ANOVA with Tukey's test.

extracts, and 40.85% for leaf extracts. Despite lower than flowers, stem and leaf extracts still showed considerable potential as natural antioxidants.

ABTS scavenging activity of all flavonoid extracts from stems, leaves and flowers showed concentration-dependent activity ($R^2 > 0.86$) in the range of 0.02–0.1 mg/mL. At 0.1 mg/mL, the scavenging rates were 41.72% (flowers), 30.63% (stems), and 38.07% (leaves), compared to 78.09% for ascorbic acid. These results indicate good antioxidant potential of chrysanthemum flavonoids. These findings align with previous reports on the leaves and flowers of six cultivars of *C. morifolium* (Doan et al. 2024), supporting the potential utilization of non-traditional plant parts as antioxidant sources.

The relationship between specific flavonoid composition and antioxidant capacity can be qualitatively discerned. The superior antioxidant activity of the flower extracts is consistent with their highest overall content of total flavonoids and key phenolic acids, such as chlorogenic acid and 3,5-O-dicaffeoylquinic acid, which are known for their strong radical-scavenging abilities (Chen et al. 2021). Notably, the leaf extracts, despite having a lower total flavonoid content than flowers, demonstrated proportionally strong antioxidant activity. This can be reasonably attributed to their uniquely high concentration of luteolin-7-O-glucoside, a flavone glycoside renowned for its potent antioxidant efficacy. This suggests that the antioxidant quality of an extract is not solely determined by the total flavonoid content, but also by the presence and abundance of specific, highly active compounds like luteolin-7-O-glucoside.

Effects of light and oxygen on flavonoid stability in stems, leaves, and flowers of Chuju

Previous studies have reported that light exposure can affect the stability of flavonoids such as quercetin and luteolin (Rameová et al. 2012). Liu et al. (2025) further demonstrated about 5% degradation in stability of total flavonoids from *Millettia speciosa* Champ under light conditions within 4 days, indicating significant light-induced flavonoid degradation. However, no studies have systematically investigated the combined effects of light and oxygen on flavonoid stability in Chuju. In this study, the effects of light and oxygen on the stability of flavonoids in the flowers, stems and leaves of Chuju were investigated.

As shown in Fig. 3, flavonoid contents decreased progressively under all storage conditions except dark without oxygen. Degradation was fastest under light with oxygen. These results demonstrated that both light and oxygen could affect flavonoid stability in Chuju. Oxygen caused more degradation than light alone. Therefore, optimal preservation of Chuju flavonoids requires storage in dark without oxygen to maintain the flavonoid quality best.

Effects of temperature on flavonoid stability in stems, leaves, and flowers of Chuju

Thermal stability analysis (Fig. 4) revealed that flavonoids from Chuju flowers, stems, and leaves remained stable at 25–60 °C but degraded significantly above 80 °C over time. These findings demonstrated the thermal lability of Chuju

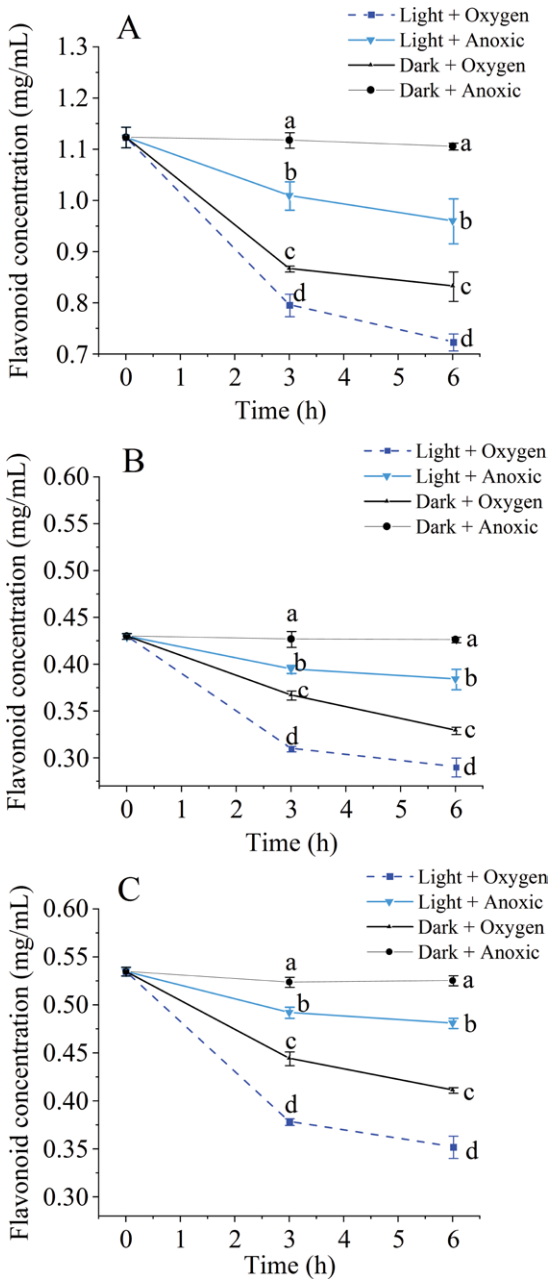


Figure 3. The influence of light and oxygen on the stability of Chuju flavonoids. **A.** Flavonoids in the flowers; **B.** Flavonoids in the stems; **C.** Flavonoids in the leaves. Data are presented as mean ± SD (n = 3).

flavonoids under elevated temperatures. Consequently, low-temperature storage conditions are recommended to prevent thermal degradation and oxidative deterioration.

Effects of pH on flavonoid stability in stems, leaves, and flowers of Chuju

The pH value had a significant impact on the stability of flavonoids. As shown in Fig. 5, stability of flavonoids in Chuju was poor under strong acid or base conditions but relatively stable in weak acid. Thus, Chuju flavonoid solutions should be stored under weakly acidic conditions.

Degradation Kinetics of Flavonoids

To gain deeper insight into the degradation kinetics of Chuju flavonoids under various stress conditions, the stability data were fitted to a first-order kinetic model. The calculated kinetic parameters are summarized in Table 4. Among all conditions, exposure to light and oxygen combined resulted in the shortest half-lives for Chuju flavonoids, confirming it as the most detrimental factor. Elevated temperature also drastically reduced flavonoid stability, with half-lives decreasing from over 85 hours at 25 °C to just 17 hours at 90 °C for the flower extracts. Interestingly, the degradation rates (k values)

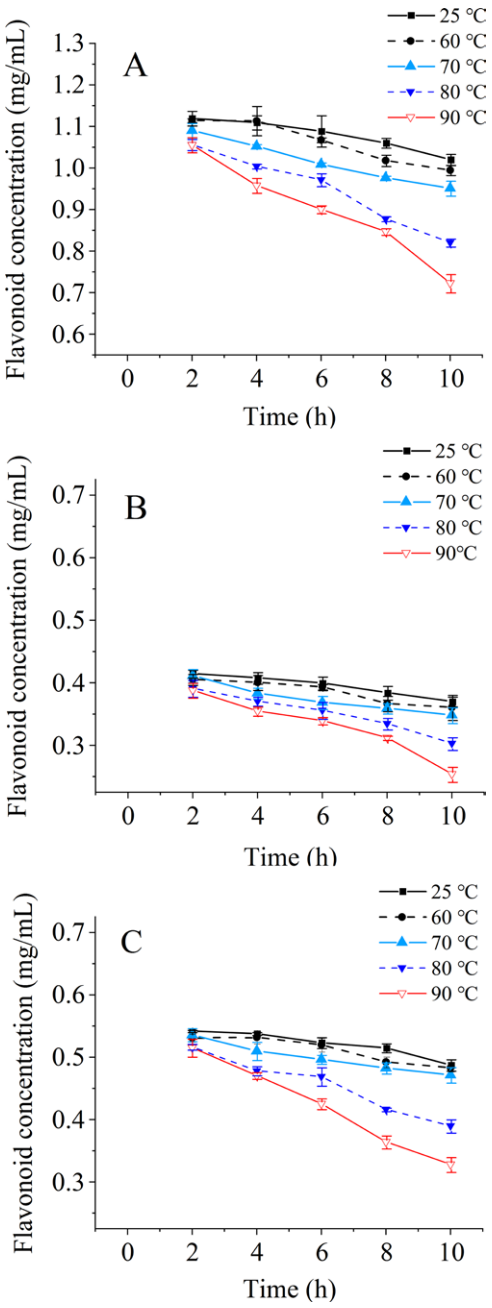


Figure 4. The influence of temperature on the stability of Chuju flavonoids. **A.** Flavonoids in the flowers; **B.** Flavonoids in the stems; **C.** Flavonoids in the leaves. Data are presented as mean ± SD (n = 3).

Table 4. Degradation kinetics parameters of total flavonoids from Chuju flowers, stems, and leaves under different stress conditions.

Stress Condition	Plant Organ	Degradation Rate Constant, k (h^{-1})	Half-Life, $t_{1/2}$ (h)	R^2
Light + Oxygen	Flower	0.0815	8.50	0.8837
	Stem	0.0741	9.35	0.8451
	Leaf	0.0815	8.50	0.8837
Light + Anoxic	Flower	0.0571	12.14	0.8185
	Stem	0.0461	15.04	0.986
	Leaf	0.0473	14.65	0.9353
Dark + Oxygen	Flower	0.0262	26.46	0.9582
	Stem	0.0207	33.49	0.9041
	Leaf	0.0197	35.19	0.8825
Dark + Anoxic	Flower	0.0025	277.26	0.9501
	Stem	0.0017	407.73	0.8457
	Leaf	0.0038	182.41	0.5520
Temperature (25 °C)	Flower	0.0081	85.57	0.8793
	Stem	0.0112	61.89	0.9345
	Leaf	0.0094	73.74	0.8756
Temperature (60 °C)	Flower	0.0113	61.34	0.8824
	Stem	0.0150	46.21	0.9435
	Leaf	0.0120	57.76	0.9243
Temperature (70 °C)	Flower	0.0158	43.87	0.9864
	Stem	0.0198	35.01	0.9739
	Leaf	0.0158	43.87	0.9864
Temperature (80 °C)	Flower	0.0303	22.88	0.9796
	Leaf	0.0307	22.58	0.9825
	Leave	0.0328	21.13	0.9753
Temperature (90 °C)	Flower	0.0403	17.20	0.9720
	Stem	0.0434	15.97	0.9390
	Leaf	0.0485	14.29	0.9726

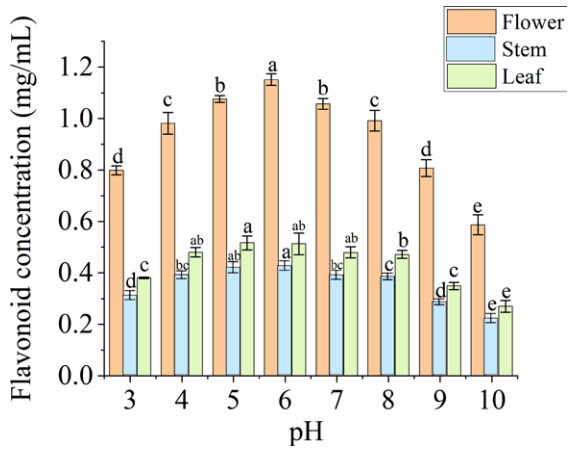


Figure 5. The influence of pH on the stability of Chuju flavonoids. Data are presented as mean ± SD (n = 3).

for flavonoids from stems and leaves across different conditions were generally comparable to those from flowers, reinforcing that all organs require similar preservation strategies. This kinetic analysis quantitatively underscores the critical need to protect Chuju flavonoids from high temperatures and oxidative environments during storage and processing.

It is important to note that the stability results presented here are based on spectrophotometric quantification of total flavonoids. While this method provides a robust overview of the overall stability profile, it does not reveal potential variations in the degradation kinetics of individual flavonoid and phenolic acid compounds. Future studies employing HPLC or LC-MS to monitor the specific degradation patterns of each major compound would provide a more granular understanding of the stability characteristics of Chuju flavonoids and identify the most vulnerable bioactive components.

Conclusion

This study analyzed the flavonoid content and quality characteristics of different parts of Chuju. The highest concentration of total flavonoids was found in the flowers (73.99 mg/g DW), followed by the leaves (51.95 mg/g DW) and stems (32.72 mg/g DW). The results demonstrate that the stems and leaves of Chuju are significant sources of flavonoids with notable antioxidant properties. While the flowers contained the highest total flavonoid content, the leaves exhibited a remarkable concentration, reaching over 70% of that in flowers, and were uniquely enriched in specific bioactive compounds such as luteolin-7-O-glucoside. All plant parts demonstrated substantial DPPH and ABTS radical scavenging activities, confirming their potent antioxidant capacity.

The flavonoids demonstrated good stability under mild processing conditions, supporting their practical utility. These findings redefine Chuju stems and leaves from agricultural waste to valuable bio-resources. They offer promising applications as natural antioxidants in functional foods and nutraceuticals. This work enhances the comprehensive utilization and economic value of Chuju, while providing a scientific basis for sustainable valorization of medicinal plant by-products.

Limitations of the study

While this study provides a comprehensive comparative assessment of flavonoids in different organs of *Chrysanthemum morifolium* 'Chuju', several limitations should be acknowledged. Firstly, the antioxidant activity was evaluated using in vitro chemical assays (DPPH and ABTS). While these are standard and valuable methods for initial screening, they cannot fully replicate the complex biological environment of an in vivo system. Future research involving cellular models or in vivo studies would be necessary to confirm the antioxidant efficacy and understand the associated mechanisms of action. Secondly, the HPLC analysis was focused on quantifying four key marker compounds. While this approach is standard for quality assessment, the chromatograms indicated the presence of other, unidentified flavonoids and phenolic substances. The contribution of these compounds to the overall bioactivity of the extracts warrants further investigation. Thirdly, our stability assessment relied on spectrophotometric measurements of total flavonoids. A more comprehensive analysis using HPLC to track the stability of individual compounds (e.g., chlorogenic acid, luteolin-7-O-glucoside) would yield deeper insights into compound-specific degradation kinetics and is a recommended direction for future research.

Conflict of interest

All authors declare that there is no conflict of interest.

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Author contributions

Conceptualization: WM. Data curation: WM, QW, KW. Formal analysis: KW. Funding acquisition: WM, HW. Investigation: HW, YL. Methodology: YL. Writing – original draft: WM, QW. Writing – review and editing: HW.

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