Postprint of a Study on Flavonoid Components in Flowers of Three Species of Camellia Section Chrysantha

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Date: 2018-12-19T00:00:00+00:00

Abstract

Using Camellia chrysantha, Camellia impressinervis, and Camellia chuongtsoensis from the Camellia sect. Chrysantha as materials, qualitative and quantitative analysis of flavonoid components and contents in their flowers was conducted using ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS). The results indicated that 15 flavonoids were detected in the three species, among which pelargonidin-3-O-glucoside, luteolin, luteolin-7-O-rutinoside, quercetin-3,7-O-diglucoside, rutin, eriodictyol, and genistein were discovered for the first time in the Camellia sect. Chrysantha; quercetin-3-O-glucoside, quercetin-7-O-glucoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside were discovered for the first time in Camellia impressinervis and Camellia chuongtsoensis. Catechin, epicatechin, quercetin-3-O-glucoside, quercetin-7-O-glucoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside constituted the main components in the three species; pelargonidin-3-O-glucoside was unique to Camellia chrysantha, quercetin-3,7-O-diglucoside was unique to Camellia chuongtsoensis; luteolin-7-O-rutinoside was primarily present in Camellia chrysantha and Camellia chuongtsoensis; luteolin was primarily present in Camellia impressinervis and Camellia chuongtsoensis. The flavonoid types were primarily catechins, quercetin derivatives, luteolin derivatives, and kaempferol derivatives; in Camellia chuongtsoensis, quercetin derivatives, luteolin derivatives, and total flavonoids were significantly higher than in Camellia chrysantha and Camellia impressinervis, catechins in Camellia impressinervis and Camellia chuongtsoensis were higher than in Camellia chrysantha, and kaempferol derivatives in Camellia chrysantha and Camellia chuongtsoensis were higher than in Camellia impressinervis.

Full Text

Preamble

Flavonoid Components in Flowers from Three Species of Section Chrysantha Chang in Camellia

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Abstract

The components and contents of flavonoids in flowers from three species of Section Chrysantha Chang in Camellia—*C. nitidissima*, *C. impressinervis*, and *C. chuangtsoensis*—were qualitatively and quantitatively analyzed using ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry. The results showed that fifteen flavonoids were detected across the three species. Among these, pelargonium-3-O-glucoside, luteolin, luteolin-7-O-rutinoside, quercetin-3,7-O-diglucoside, narirutin, eriodictyol, and genistin were identified for the first time in Section Chrysantha Chang, while quercetin-3-O-glucoside, quercetin-7-O-glucoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside were newly discovered in *C. impressinervis* and *C. chuangtsoensis*. The main flavonoid components across all three species were catechin, epicatechin, quercetin-3-O-glucoside, quercetin-7-O-glucoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside. Pelargonium-3-O-glucoside was unique to *C. nitidissima*, while quercetin-3,7-O-diglucoside was specific to *C. chuangtsoensis*. Luteolin-7-O-rutinoside was primarily found in *C. nitidissima* and *C. chuangtsoensis*, whereas luteolin was mainly present in *C. impressinervis* and *C. chuangtsoensis*. The predominant flavonoid types were catechins, quercetins, luteolins, and kaempferols. Notably, the contents of quercetins, luteolins, and total flavonoids in *C. chuangtsoensis* were substantially higher than those in *C. nitidissima* and *C. impressinervis*. The catechin content in *C. impressinervis* and *C. chuangtsoensis* exceeded that in *C. nitidissima*, while the kaempferol content in *C. nitidissima* and *C. chuangtsoensis* was higher than in *C. impressinervis*.

Keywords: *Camellia*, Section Chrysantha Chang, flowers, flavonoids, ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS)

Introduction

Plants in Section Chrysantha Chang of the genus *Camellia* produce yellow flowers that contain various bioactive substances. For instance, *C. nitidissima* flow-

ers and leaves contain flavonoids, tea polyphenols, and saponins with demonstrated hypoglycemic effects (Xia et al., 2013), antioxidant properties (Song et al., 2011), and antitumor activity (Lin et al., 2013). Research on these active flavonoid components is crucial for developing the medicinal value and economic potential of golden camellias. However, current studies have primarily focused on total flavonoid analysis (Tang et al., 2017) and component identification (Peng et al., 2011; Peng et al., 2012), while the specific content and variation characteristics of flavonoid components remain unclear, significantly limiting their exploitation and utilization.

More than 30 species in Section Chrysantha Chang exhibit yellow flowers (Guan et al., 2014). Previous chemical studies of golden camellia flowers have identified flavonoids such as quercetin and kaempferol (Peng et al., 2011). However, most research has concentrated on *C. nitidissima* (Qi et al., 2016; He et al., 2017), leaving the majority of species unexamined. *C. impressinervis* and *C. chuangtsoensis* produce abundant deep-yellow flowers, with *C. chuangtsoensis* displaying the most intense yellow coloration and a unique characteristic of flowering year-round (Guan et al., 2014). These attributes make them excellent materials for flavonoid extraction and product development. Therefore, this study employed UPLC-Q-TOF-MS to analyze the flavonoid composition and content in flowers of *C. nitidissima*, *C. impressinervis*, and *C. chuangtsoensis*, investigating their differential profiles and variation patterns to provide a scientific basis for the development and utilization of Section Chrysantha Chang plant resources.

Materials and Methods

1.1 Materials

The experimental materials consisted of three Section Chrysantha Chang species: *C. nitidissima*, *C. impressinervis*, and *C. chuangtsoensis*, sourced from the *Camellia* germplasm repository at the Research Institute of Subtropical Forestry, Chinese Academy of Forestry. Five plants with consistent growth conditions were selected, and three fresh flowers from the southern outer canopy of each plant were collected at full bloom stage.

1.2.1 Qualitative Analysis

Fresh flowers (0.6 g) were ground to powder in liquid nitrogen and extracted using the method of Hashimoto et al. (2002) with 2 mL of methanol:water:formic acid:THF (70:27:2:1, v/v/v/v) for 24 hours. The extract was filtered through a 0.22 m membrane, and the filtrate was stored at -20°C for subsequent analysis (Wang et al., 2004).

Flavonoid components were qualitatively and quantitatively analyzed using UPLC-Q-TOF-MS, comprising an ACQUITYTM UPLC I-Class system (Waters Corporation, Milford, MA, USA) coupled with an Xevo G2-XS QTof MS system (Waters Corporation, Manchester, UK). Chromatographic separation

was performed on an ACQUITY BEH C18 column (2.1 mm × 100 mm, 1.7 m) at a flow rate of 0.3 mL · min ^{1} with an injection volume of 2 L. The mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B) using the following gradient program: 0–1.5 min, 5% B; 1.5–11 min, 5–40% B; 11–14 min, 40–95% B; 14–16.5 min, 95% B; 16.5–16.8 min, 95–5% B; 16.8–20 min, 5% B. The column temperature was maintained at 40°C. Mass spectrometry was conducted using electrospray ionization (ESI) in positive ion mode with a source temperature of 120°C, desolvation gas (high-purity nitrogen) at 450°C and 600 L · h ^{1}, capillary voltage of 1 kV, cone voltage of 40 V, and scan range of 50–1200 m/z. Low-energy scans were performed at 6 eV, while high-energy scans used 20–45 eV.

1.2.2 Quantitative Analysis

Standard compounds including quercetin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-7-O-glucoside, kaempferol, kaempferol-3-O-glucoside, luteolin, eriodictyol, narirutin, and pelargonium-3-O-glucoside were purchased from Sigma-Aldrich; cyanidin from Shanghai ANPEL Laboratory Technologies; and catechin and epicatechin from Beijing Solarbio Science & Technology. All standards had purity 98%. Calibration curves were established for eight flavonoids (catechin, epicatechin, cyanidin, quercetin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, kaempferol, and kaempferol-3-O-glucoside) for quantitative calculations (Table 1). Pelargonium-3-O-glucoside was quantified using the cyanidin calibration curve, while seven other flavonoids (luteolin, luteolin-7-O-rutinoside, quercetin-7-O-glucoside, quercetin-3,7-O-diglucoside, eriodictyol, narirutin, and genistin) were analyzed using the quercetin-3-O-glucoside standard curve. All analyses were performed in five replicates to calculate component contents.

Results

2.1 Flavonoid Identification in Flowers of Three Section Chrysantha Chang Species

UPLC-Q-TOF-MS analysis identified fifteen flavonoid components in the three species (Figure 1 [Figure 1: see original paper]), with detailed mass spectrometry data presented in Table 2 . Among these, twelve compounds were verified against authentic standards, except for components 3, 4, and 13. Comparison with standards confirmed components 1 and 2 as catechin and epicatechin, respectively. Component 3 exhibited a molecular ion at m/z 595.17 and fragment ion at m/z 287.06, consistent with luteolin-7-O-rutinoside as identified by Zhang et al. (2013), and was thus inferred to be luteolin-7-O-rutinoside. Component 8 showed a molecular ion at m/z 287.06 and fragment ion at m/z 153.02, identified as luteolin based on standard comparison. Component 6 displayed a molecular ion at m/z 433.11 and fragment ion at m/z 271.06, identified as pelargonium-3-O-glucoside through standard comparison.

Component 4, with a molecular ion at m/z 627.16 and fragment ion at m/z 303.05, matched quercetin-3,7-O-diglucoside as reported by Ceska & Styles (1984) and was inferred accordingly. Standard comparisons confirmed components 5, 7, 12, and 14 as quercetin-3-O-rutinoside, quercetin-3-O-glucoside, quercetin-7-O-glucoside, and quercetin, respectively. Components 10 and 15 were identified as kaempferol-3-O-glucoside and kaempferol, while components 9 and 11 corresponded to eriodictyol and narirutin. Component 13, lacking a standard, showed a molecular ion at m/z 433.11 and fragment ion at m/z 271.06, matching genistin as described by Li et al. (2010) and was thus inferred to be genistin.

2.2 Flavonoid Content in Flowers of Three Section Chrysantha Chang Species

Flavonoid contents in the three species are summarized in Table 3 . In *C. nitidissima*, eight major components each accounted for >1% of total flavonoids, with epicatechin (33.98%), quercetin-3-O-glucoside (26.16%), and luteolin-7-O-rutinoside (11.60%) comprising 71.74% of the total. *C. impressinervis* contained seven major components, with epicatechin (42.45%), quercetin-3-O-glucoside (26.05%), and quercetin-3-O-rutinoside (12.38%) representing 80.88% of the total. *C. chuangtsoensis* had eleven major components, with quercetin-3-O-glucoside (26.06%), epicatechin (23.91%), and luteolin (11.25%) accounting for 61.23% of the total.

Catechin, epicatechin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-7-O-glucoside, and kaempferol-3-O-glucoside were the principal components shared across all three species. Pelargonium-3-O-glucoside was unique to *C. nitidissima*, while quercetin-3,7-O-diglucoside was specific to *C. chuangtsoensis*. Luteolin-7-O-rutinoside was predominantly present in *C. nitidissima* and *C. chuangtsoensis*, whereas luteolin was mainly found in *C. impressinervis* and *C. chuangtsoensis*.

Among the principal components, epicatechin content was similar across species, but catechin content in *C. chuangtsoensis* was 2.17-fold and 1.49-fold higher than in *C. nitidissima* and *C. impressinervis*, respectively. Luteolin content in *C. chuangtsoensis* was 2.61-fold higher than in *C. impressinervis*, while luteolin-7-O-rutinoside content in *C. nitidissima* was 2.31-fold higher than in *C. chuangtsoensis*. Quercetin-3-O-rutinoside content was comparable across species, and quercetin-3-O-glucoside and quercetin-7-O-glucoside contents were similar between *C. nitidissima* and *C. impressinervis*. However, quercetin-3-O-glucoside content in *C. chuangtsoensis* was 1.65-fold and 1.55-fold higher than in *C. nitidissima* and *C. impressinervis*, respectively, and quercetin-7-O-glucoside content was 7.23-fold and 8.31-fold higher. Kaempferol-3-O-glucoside content in *C. nitidissima* and *C. chuangtsoensis* was 1.69-fold and 1.43-fold higher than in *C. impressinervis*.

2.3 Flavonoid Classification in Flowers of Three Section Chrysantha Chang Species

Flavonoid classification data are presented in Table 4 . The identified flavonoids were categorized as catechins, anthocyanins, luteolins, quercetins, kaempferols, and other types. In *C. nitidissima*, catechins (39.31%), quercetins (38.20%), and luteolins (11.67%) were the most abundant, collectively representing 89.18% of total flavonoids, followed by kaempferols (5.18%) and anthocyanins (4.59%). In *C. impressinervis*, catechins (49.72%), quercetins (40.12%), and luteolins (6.70%) accounted for 96.53% of the total, with kaempferols at 2.98%. In *C. chuangtsoensis*, quercetins (47.25%), catechins (30.91%), and luteolins (14.29%) comprised 92.45% of the total, with kaempferols at 2.59%. Thus, the primary flavonoid types across all three species were catechins, quercetins, luteolins, and kaempferols.

The total flavonoid content in *C. chuangtsoensis* flowers reached 555.77 $g \cdot g^{1}$, substantially exceeding the 336.60 $g \cdot g^{1}$ and 358.75 $g \cdot g^{1}$ observed in *C. nitidissima* and *C. impressinervis* (1.65-fold and 1.55-fold higher, respectively). Catechin content in *C. impressinervis* and *C. chuangtsoensis* was 1.35-fold and 1.30-fold higher than in *C. nitidissima*. Luteolin content in *C. chuangtsoensis* was 2.02-fold and 3.31-fold higher than in *C. nitidissima* and *C. impressinervis*, respectively, while quercetin content was 2.04-fold and 1.83-fold higher. Kaempferol content in *C. nitidissima* and *C. chuangtsoensis* was 1.63-fold and 1.41-fold higher than in *C. impressinervis*. These results demonstrate that *C. chuangtsoensis* contains substantially higher levels of quercetins, luteolins, and total flavonoids compared to the other two species, while *C. impressinervis* and *C. chuangtsoensis* show higher catechin content than *C. nitidissima*, and *C. nitidissima* and *C. chuangtsoensis* exhibit higher kaempferol content than *C. impressinervis*.

Discussion and Conclusion

This study employed UPLC-Q-TOF-MS for qualitative and quantitative analysis of flavonoids in three Section Chrysantha Chang species, detecting fifteen flavonoid components including two catechins, one anthocyanin, five quercetins, two kaempferols, two luteolins, and three other compounds such as narirutin. Calibration curves for eight flavonoids were established for quantitative analysis. Pelargonium-3-O-glucoside was quantified using the cyanidin standard curve, while flavonoids lacking specific standards were analyzed using the quercetin-3-O-glucoside calibration curve. With five replications and R^2 values exceeding 0.999, the established method demonstrated effective quantification of all components within their linear ranges.

Among the fifteen flavonoids detected, seven were identified for the first time in Section Chrysantha Chang: pelargonium-3-O-glucoside, luteolin, luteolin-7-O-rutinoside, quercetin-3,7-O-diglucoside, narirutin, eriodictyol, and genistin. Pelargonium-3-O-glucoside was exclusive to *C. nitidissima*

flowers, while quercetin-3,7-O-diglucoside was specific to *C. chuangtsoensis*. Luteolin was predominantly found in *C. impressinervis* and *C. chuangtsoensis*, and luteolin-7-O-rutinoside was mainly present in *C. nitidissima* and *C. chuangtsoensis*. The principal components shared across all three species —quercetin-3-O-glucoside, quercetin-7-O-glucoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside—have been reported as major flavonoids in *C. nitidissima* flowers (Peng et al., 2011; Zhou et al., 2013), but their detection in *C. impressinervis* and *C. chuangtsoensis* represents a novel finding (He et al., 2017).

Previous research has identified cyanidin-3-O-glucoside as the primary anthocyanin in red camellia flowers (Li et al., 2007, 2008, 2009), whereas quercetin glycosides such as quercetin-3-O-glucoside, quercetin-3-O-rutinoside, and quercetin-7-O-glucoside are responsible for the yellow coloration in golden camellias (Hwang et al., 1992; Sangwan et al., 2015). In this study, quercetins accounted for 40.12% and 47.25% of total flavonoids in *C. impressinervis* and *C. chuangtsoensis*, respectively, exceeding the 38.20% observed in *C. nitidissima*, confirming that quercetins are the primary pigments responsible for yellow coloration in these species as well. Both the absolute content and relative proportion of quercetin-3-O-glucoside and quercetin-3-O-rutinoside were notably high across all three species. In *C. chuangtsoensis*, quercetin-3-O-glucoside exhibited the highest content and percentage, while in *C. nitidissima* and *C. impressinervis*, these values were second only to epicatechin. These findings indicate that quercetins, particularly quercetin-3-O-glucoside, are the key pigments determining yellow flower color in golden camellias, consistent with previous reports (Hwang et al., 1992; Miyashima, 1997). The deepest yellow coloration observed in *C. chuangtsoensis* may be attributed not only to its highest quercetin-3-O-glucoside content and proportion but also to its significantly greater total flavonoid content.

Golden camellias contain bioactive flavonoids with demonstrated pharmacological effects including tumor inhibition (Peng et al., 2012; Lin et al., 2013), hypoglycemic activity (Xia et al., 2013), antioxidant properties (Niu et al., 2015), and cardiovascular and immune system enhancement (He et al., 2015). Both *C. impressinervis* and *C. chuangtsoensis* exhibited higher total flavonoid content than *C. nitidissima*, with *C. chuangtsoensis* showing particular promise due to its high flavonoid content, extended flowering period, and abundant flower production, making it an excellent material for flavonoid product development in pharmaceutical, health care, and food applications. This study elucidated the flavonoid composition, content, and variation patterns in *C. nitidissima*, *C. impressinervis*, and *C. chuangtsoensis*, providing a scientific foundation for further development and utilization of these resources.

In summary, fifteen flavonoid components were detected across the three Section Chrysantha Chang species, with catechin, epicatechin, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, quercetin-7-O-glucoside, and kaempferol-3-O-glucoside identified as the principal shared components. *C. chuangtsoensis*

exhibited the highest total flavonoid content at 555.77 g · g ^{1}, surpassing *C. nitidissima* (336.60 g · g ^{1}) and *C. impressinervis* (358.75 g · g ^{1}). The predominant flavonoid classes were catechins, quercetins, luteolins, and kaempferols. *C. chuangtsoensis* contained substantially higher levels of quercetins and luteolins compared to the other two species, while *C. impressinervis* and *C. chuangtsoensis* showed elevated catechin content relative to *C. nitidissima*, and *C. nitidissima* and *C. chuangtsoensis* exhibited higher kaempferol content than *C. impressinervis*.

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Note: Figure translations are in progress. See original paper for figures.