

**STUDIES ON THE ANTIOXIDANT COMPONENTS AND
ACTIVITIES OF THE METHANOL EXTRACTS OF
COMMERCIALY GROWN *HEMEROCALLIS FULVA* L.
(DAYLILY) IN TAIWAN**

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ABSTRACT

*We explored the antioxidant components and activities of daylily *Hemerocallis fulva* L. flowers among two growth areas, three flower ages and two processing methods. The results showed that the growth area, flower age and processing method all significantly influenced the functional components and antioxidant activities grown in mountainous areas of Taiwan. The total phenols, flavonoids and total chlorophylls of the methanol extracts of D3DF were 59.51, 70.76 and 5.67% of the respective values of F3DF. The total phenols and anthocyanins of the methanol extracts of F1DF were 2.64 mg GAE/100 g-dried basis (db) and 0.102 μ mole/100 g-db, which were significantly higher than the others. The total flavonoid contents of F1DF, F2DF,*

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F3DF and *D3DF* were 20.83, 29.67, 31.65 and 22.30 mg *QE/100 g-db*, respectively, with the *F1DF* level as the lowest. The reducing power showed that both the fresh and dried flowers were very weak. The amounts of most flavonoids in the flowers from Hualien were greater than those from Taidong.

PRACTICAL APPLICATIONS

The aim of this research was to discuss the antioxidant activities and the main components of *Hemerocallis fulva* L. (day lily); for example, the content of total phenols, flavonoids and total chlorophylls. In particular, the study examines the content of 10 common types of flavonoids. The research used different solvents to extract *H. fulva* L. grown at different time and origins. The findings provide consumers with a better understanding of the functions of *H. fulva* L. It increases the *H. fulva* L. uptake and improves consumers' health. Consequently, it increases farmers' income.

INTRODUCTION

Flowers of *Hemerocallis fulva* L. (daylily; *jin chen* or golden needle in Chinese) contain several antioxidant compounds such as anthocyanins, flavonoids, carotenoids, chlorophylls and a potential agent for the inhibition of lipid oxidation, which may prevent atherosclerosis (Thu *et al.* 2004).

Flavonoids are polyphenol compounds of secondary metabolic compounds in plants, and their structure is a 2-phenyl-benzo- α -pyrone (Table 1). There are more than 4,000 kinds of structures in the heterocyclic ring because the substituting group of the ring produces different kinds of structures. Flavonoids in plants combine with carbohydrates to form glycosides (Hollman

TABLE 1.
OCCURRENCE OF FLAVONOIDS IN COMMON FOODS

Flavonoid subgroup	Major foods	Examples of foods
Flavones	herbs	parsley, thyme
Flavonols	vegetables, fruits, beverages	onions, kale, broccoli, apple, cherries, berries, tea, red wine
Flavanones	fruits	citrus
Catechins	fruits, beverages	apples, tea
Anthocyanidins	fruits	cherries, grapes
Isoflavones	vegetables	soy beans, legumes

Source of data: Hilhorst *et al.* (1998).

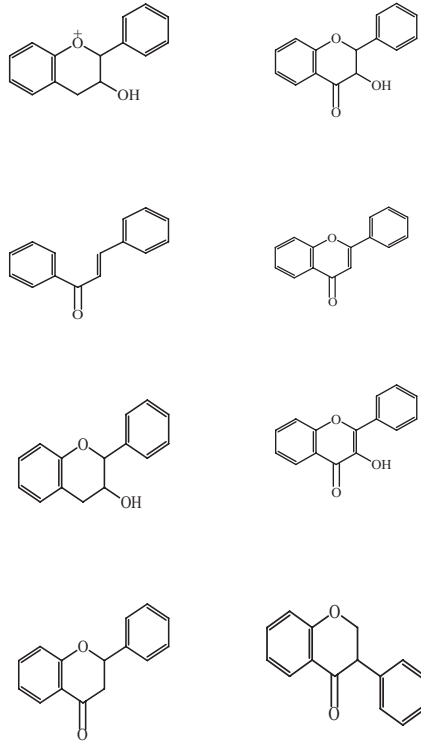


FIG. 1. EIGHT KINDS OF GLYCOSIDES BASED ON THE CHARACTER OF THE STRUCTURE

et al. 1996). Research has shown (Bailleul and Trotin 2000; Luo *et al.* 2002) that flavonoids have free radical-scavenging activity against superoxide anions, hydroxide free radicals and peroxide, and inhibit radical damage.

According to Lin (2002), farmers in Taiwan always divide the flowers according to the maturity of the flower bud that means the days before to flower, as 1-, 2-, 3-, and 4-day flowers, indicating the length of time the bud has been open. The polyphenol contents of daylily flowers are affected by the method of processing (Hsu *et al.* 2000). When *H. fulva* flowers are freeze dried, there are higher amounts of polyphenols, so they have greater reducing power, Fe^{2+} -chelating activity, DPPH-scavenging activity and lipid peroxidation inhibition power.

There are eight kinds of glycosides based on the character of the structure (Fig. 1): flavones (apigenin, luteolin, kaempferol, rutin, quercetin, galangin and chrysin) and flavanols (myricetin, morin); flavanonols (hesperidin, naringin, naringenin, pinocembrin); and flavanones, including chalcones

TABLE 2.
FLAVONOL AND FLAVONE CONTENTS OF COMMON VEGETABLES,
FRUITS AND BEVERAGES

Flavonol and flavone contents*	Foods
Low (<10 mg/kg or <10 mg/L)	cabbage, spinach, carrots, peas, mushrooms, peaches, strawberries, orange juice, white wine, brewed coffee
Medium (<50 mg/kg or <50 mg/L)	lettuce, broad beans, red pepper, tomatoes, apples, grapes, cherries, tomato juice, red wine, tea beverages
High (>50 mg/kg or >50 mg/L)	broccoli, endive, kale, French beans, celery, onions, cranberries

* Represents the sum of quercetin, kaempferol, myricetin, luteolin and apigenin.
Source of the data: Hilhorst *et al.* (1998).

(catechin, epicatechin, epigallocatechin and gallate), isoflavones (daidzein, daidzin, genistein and genistin) and anthocyanidins (cyaniding, delphinidin, malvidin, pelargonidin, peonidin and petunidin) (Table 2). The types and contents of flavonoids are not exactly similar in different plants (Li *et al.* 1998). Flavonoids are used as oxidation inhibitors, and the main functions are: (1) as a metal-chelating agent and -reducing agent; (2) to eliminate active oxygen species; (3) to end the chain-reaction of free radicals; and (4) to end the formation of singlet oxygen. In addition, flavonoids can restrict the reaction of the fat oxidation enzyme and the NADPH oxidation enzyme in the oxidizing reaction, thus preventing tumors. Their antioxidant activities have close relationships with their structures.

There are many methods for analyzing flavonoids, such as high-performance liquid chromatography (HPLC), spectrophotometry, thin-layer chromatography and capillary electrophoresis (CE). The amount needed for a sample is small, the analysis time is short and the separation effect is good for the CE method (Hilhorst *et al.* 1998). So it is suitable for analyzing the contents of flavonoids. This research used CE to examine the compositions of flavonoids in daylily flowers. The objectives of this study was to determine the effects of the growth area, the age of the flower, and the processing method on the antioxidant components and their activities.

MATERIALS AND METHODS

Sources of Samples

Fresh Daylily Flowers. One- to 3-day-before to flower were purchased from Hualien's Ye-Lie Mt. and 3-day-before to flower (F3DF) from a mountain area in Taidong.

Dried Daylily Flowers. Three-day-old daylily flowers were purchased from Hualien's Ye-Lie market.

Sample Pretreatment. The flowers were dried in a freeze dryer (40C, 80 mm Hg), ground into a powder with a mortar, and the powder was stored in a refrigerator at 4C.

Reagents

Boric Acid-Methyl Alcohol Solution. A 0.1-M boric acid solution and caustic soda solution were mixed and adjusted to pH 9.5. Methyl alcohol was added in a 9:1 proportion to the boric acid-methyl alcohol solution, and this was filtered through a 0.45- μ m filter.

Others. DPPH (1,1-biphenyl-2-picryl-hydrazyl), potassium ferricyanide ($K_3Fe(CN)_6$), 3-(2-pyridyl-5,6-bis(4-phenyl-sulfuric acid)-1,2,4-triazine (ferrozine), ethylene diaminetetraacetic acid (EDTA), folin-Ciocalteau's reagent, gallic acid and quercetin were purchased from Sigma Chemical (St. Louis, MO). $CuSO_4$ and KH_2PO_4 were purchased from Shimakyu Chemical (Osaka, Japan). $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, NaCl, NaBr, Na_2CO_3 , $AlCl_3 \cdot 6H_2O$, acetic acid, trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were purchased from Kanto Chemical (Tokyo, Japan). Acetonitrile, methanol, dichloromethane, petroleum ether, ethyl acetate, chloroform, acetone, ether, 25% ammonia water and sulfuric acid were purchased from Merck (Darmstadt, Germany).

Methods

Preparation of Methanol Extracts of *H. fulva* Flowers. The powdered flowers (0.30 g) were placed into an extractor at 45C, and spun at 150 rpm for 20 min with 10-mL methanol; and then this mixture was centrifuged at 3,500 rpm for 15 min. We collected the supernatant in 25-mL test tubes. Methanol (5 mL) was added to the residue and duplicated two times. The supernatant and filtrate were mixed. The filtrate was collected in 25-mL flasks. Finally, methanol was used to bring the volume to 25 mL.

Determination of Total Phenols and Flavonoids. According to the method of Taga *et al.* (1984), 0.2 mL of each test solution was mixed with 0.8 mL of a 7.5% Na_2CO_3 solution. Next, 1 mL of Folin-Ciocalteau's phenol reagent was added to the mixture. The mixture was placed at room temperature for 30 min in the dark, and then the absorbance at 765 nm was measured. Total phenols of the sample were expressed in gallic acid equivalents (GAE) (mg/g

extracted dry powder). According to the method of Quettier-Deleu *et al.* (2000), 1 mL of each test solution was mixed with 1 mL of a 2% methanolic $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution. The solution was placed at room temperature for 10 min, and then the absorbance at 430 nm was measured. Total flavonoids were expressed as quercetin equivalents (QE) (mg/g extracted dry powder).

Determination of Total Anthocyanins. According to the method of Padmavati *et al.* (1997), 0.5–2 g of powdered flowers was mixed with 10 mL of a methanol solution (containing 1% hydrochloric acid) and placed into an extractor at 45°C and 200 rpm for 30 min in the dark. Then this mixture was centrifuged at 3,000 rpm and 4°C for 15 min. We collected the upper transparent liquid in a 50-mL flask. A methanol solution (10 mL) was added to the residue and duplicated two times. The supernatant and methanol solution (containing 1% hydrochloric acid) were combined to bring the volume to 50 mL, then the absorbances at 627 and 530 nm were individually measured, and the absorbance coefficient was 31.6. The following equation was used to measure the content of total anthocyanins:

$$\text{Total anthocyanins } (\mu\text{mole}/100 \text{ g}) = (\text{Absorbance}_{530} - 0.33\text{Absorbance}_{657}) / 31.6 \times \text{volume (mL)} \times \text{dilution/sample weight (g)} \times 100.$$

Determination of Antioxidant Activity

Determination of DPPH-Scavenging Activity. According to the method of Shimada *et al.* (1992), 2 mL of four concentrations (1, 2.5, 5 and 10 mg/mL) of methanol extracts from powdered daylily flowers was separately mixed with 2 mL of a 1 mM DPPH solution. The test sample solution replaced by methanol was used as the control. Methanol was used to replace the DPPH solution as the blank. Each mixture was allowed to stand for 30 min in the dark, and then the absorbance at 517 nm was measured. The percentage of the DPPH-scavenging activity was expressed as $(1 - 0[\text{absorbance of the test sample} - \text{absorbance of the blank}] / \text{absorbance of the control}) \times 100\%$.

Determination of the Reducing Power. Using Oyaizu's (1986) method, 0.5 mL of 0.2 M Na_3PO_4 buffer (pH 6.6) was added to 0.1 mL of the sample extract solution. Next, 0.2 mL of 1% $\text{K}_3\text{Fe}(\text{CN})_6$ was added. Each mixture was incubated at 50°C for 20 min, and then it was rapidly cooled down with ice cubes. Next, 0.5 mL of 10% CCl_3COOH was added to the solution. The solution was centrifuged at 3,000 rpm for 10 min, and 0.5 mL of supernatant was mixed with 0.5 mL deionized water and 0.1 mL 1% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The mixture was placed in the dark for 10 min, and then the absorbance at 700 nm was measured. The reducing power was expressed as the absorbance of the test sample – the absorbance of the control.

Determination of Fe²⁺-Chelating Activity. The Fe²⁺-chelating activity of the extracts of powdered flowers was measured according to the method of Dinis *et al.* (1994). Two milliliter of four concentrations (1, 2.5, 5 and 10 mg/mL) of methanol extracts of powdered flowers was separately mixed with 0.1 mL of 1 mM FeCl₂.4H₂O and allowed to stand for 30 s. Each reaction mixture was vigorously mixed with 0.2 mL of 2.5 mM ferrozine solution and incubated for 10 min in the dark, and then the absorbance at 562 nm was measured. The percentage of ferrous ion-chelating activity was expressed as $(1 - [\text{absorbance of the test sample} - \text{absorbance of the blank}] / \text{absorbance of the control}) \times 100\%$.

Determination of the Flavonoid Contents by CE

Standard Solution. Ten grams of each sample (naringin, chrysin, pinocanbrin, hesperidin, daidzein, rutin, naringenin, isoquercetin, kaempferol, myricetin, quercetin and morin) was precisely weighed, and then dissolved in 10 mL of 80% ethyl alcohol to make the standards. The standards were diluted by 80% ethyl alcohol to 8, 12, 16 and 20 µg/mL to produce standard solutions before use.

The Boric Acid-Methyl Alcohol Solution. A 0.1-M boric acid solution was mixed with a caustic soda solution to adjust the pH to 9.5. Methyl alcohol was added in a 9:1 proportion to obtain the boric acid–methyl alcohol solution; it was filtered through a 0.45-µm microfilter.

CE Conditions. The correct amount of clear liquid was filtered through a 0.45-µm microfilter, as the examined sample, and analyzed with CE.

The total length of the analysis tube was 60 cm; the effective length was 50 cm i.d. of a 50-µm glass-capillary column, with an untreated surface, a voltage of 18 kV of voltage, a temperature of 25C and a wavelength of 214 nm. A sample was poured into the high-pressure nitrogen for 5 s, and the material was recorded and processed using P/ACE System Gold V8.0 software (Beckman Instrument, Fullerton, CA).

Determination of the Concentration. Individual volumes of samples and standard solutions were injected into the CE instrument. Peaks were identified by comparing the retention times and spectra of samples with those of the standard solutions. The following formula was used to calculate the amounts of flavonoid in the test samples: Amount of flavonoid (ppm) = $C \times V / W$, where C is the flavonoid concentration (µg/mL) calculated by the standard curve, V is the volume of the sample solution (mL) and W is the weight of the sample (g).

Statistical Analysis. Results were expressed in terms of means and standard deviations. For all measurements, results were considered statistically significant at $P < 0.05$. All statistical analyses were conducted by using the method of Statsoft (Kim and Kohact 1975); the comparisons were made by analysis of variance.

RESULTS AND DISCUSSION

Antioxidant Components and the Yield of *H. fulva* Methanol Extracts

As shown in Table 3, the yields of the methanol extracts of *H. fulva* flowers were about 29.47–30.78%. There were no significant differences between the sources and among the ages of the flowers. Total phenols and total chlorophylls of the methanol extract of D3DF (dried 3-day-before to flower) were 1.22 mg GAE/100 g-dried basis (db) and 0.013 $\mu\text{g}/100$ g-db, which were significantly lower than those of the fresh flower methanol extracts. Values of the yield, total phenols and total chlorophylls of D3DF were 40.49, 92.73 and 94.32% lower compared with the respective values for F3DF. The total phenols and anthocyanins of the methanol extracts of F1DF (fresh 1-day-old flowers) were 2.64 mg GAE/100 g-db and 0.102 $\mu\text{mole}/100$ g-db, which were significantly higher than the others. The total flavonoid contents of F1DF, F2DF, F3DF and D3DF were 20.83, 29.67, 31.65 and 22.30 mg QE/100 g-db, respectively, with F1DF being the lowest. The results showed that the antioxidant components differed because of the age of the flower.

Distribution of Antioxidant Activities

The DPPH-scavenging activities of none of the tested methanol extracts of *H. fulva* flowers significantly differed. The IC_{50} values were about 0.500–0.625 mg/mL (Table 4), while the concentration was 2,375 ppm for both methanol extracts of the tested flowers which produced 95% DPPH-scavenging activity (Fig. 2).

The Fe^{2+} -chelating activity of the methanol extracts showed that D3DF was significantly lower than the others, while F1DF was the highest (Fig. 3). The IC_{50} of D3DF was 2.560 mg/mL, while that of F1DF was 1.125 mg/mL (Table 4). This suggests that the total phenol, flavonoid and chlorophyll contents of daylily flowers decreased during the drying process. Those components had Fe^{2+} -chelating activity. That was the reason the Fe^{2+} -chelating activity of D3DF was significantly lower.

The reducing power of the methanol extracts of *H. fulva* flowers was examined. All of the test samples exhibited very low reducing powers when the

TABLE 3.
YIELDS AND ANTIOXIDANT CONTENTS OF THE METHANOL EXTRACTS OF *HEMEROCALLIS FULVA* L FLOWERS

	Yield (% db)	Total phenols (mg GAE/100 g-db)	Flavonoids (mg QE/100 g-db)	Anthocyanins (μ mole/100 g-db)	Total chlorophylls (μ g/100 g-db)
F1F1DF	30.38 \pm 0.23	2.64 \pm 0.11 ^{a*}	20.83 \pm 1.21 ^d	0.102 \pm 0.007 ^a	0.201 \pm 0.034 [#]
F2DF	30.13 \pm 0.67	2.16 \pm 0.07 ^b	29.67 \pm 1.15 ^b	0.087 \pm 0.004 ^b	0.221 \pm 0.012 ^a
F3DF	31.78 \pm 0.51	2.05 \pm 0.03 ^c	31.65 \pm 0.55 ^a	0.071 \pm 0.003 ^c	0.229 \pm 0.001 ^a
D#D3DF	29.47 \pm 0.14	1.22 \pm 0.01 ^d	22.30 \pm 0.25 ^c	0.084 \pm 0.002 ^b	0.013 \pm 0.002 ^b

* Each value is the mean \pm standard deviation of triplicate analysis; values in the same column with different superscripts significantly differ at $P < 0.05$. F1DF, fresh 1 day before to flower; F2DF, fresh 2 days before to flower; F3DF, fresh 3 days before to flower; D3DF, dried 3 days before to flower; GAE, gallic acid equivalents; QE, quercetin equivalents; db, dried basis.

TABLE 4.
ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS OF *HEMEROCALLIS FULVA* FLOWERS

	DPPH-scavenging activity IC ₅₀ (mg/mL)	Fe ²⁺ -chelating activity IC ₅₀ (mg/mL)
F1DF	0.595 ± 0.04*	1.125 ± 0.04 ^c
F2DF	0.580 ± 0.24	1.920 ± 0.72 ^b
F3DF	0.625 ± 0.16	1.670 ± 0.51 ^b
D3DF	0.500 ± 0.21	2.560 ± 0.83 ^a

* Each value is the mean ± standard deviation of triplicate analysis; values in the same column with different superscripts significantly differ at $P < 0.05$.

F1DF, fresh 1 day before to flower; F2DF, fresh 2 days before to flower; F3DF, fresh 3 days before to flower; D3DF, dried 3 days before to flower.

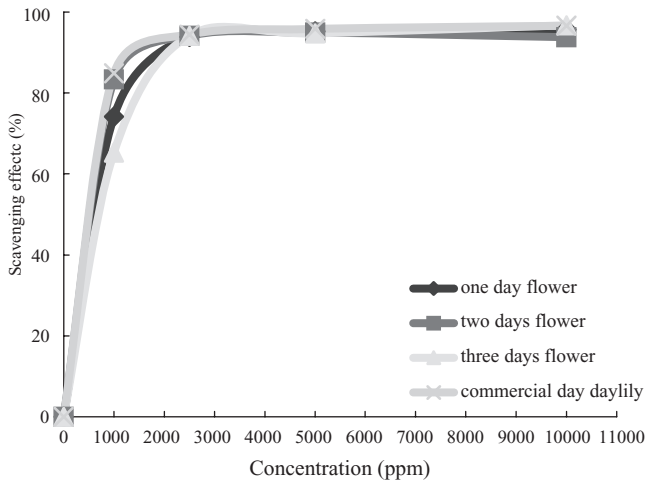


FIG. 2. THE DPPH-SCAVENGING EFFECTS OF DAYLILY METHANOL EXTRACTS

concentration was 10,000 ppm. Both F3DF and D3DF were nearly zero, while F1DF and F2DF were only 0.17 and 0.14 (Fig. 4).

Flavonoids Content Analysis

CE Spectrum of *H. fulva* Flavonoids. There were 12 different flavonoids separated by CE (Fig. 5), including naringin, chrysin, pinocembrin, hesperidin, daidzein, rutin, naringenin, isoquercetin, kaempferol, myricetin, quercetin and morin.

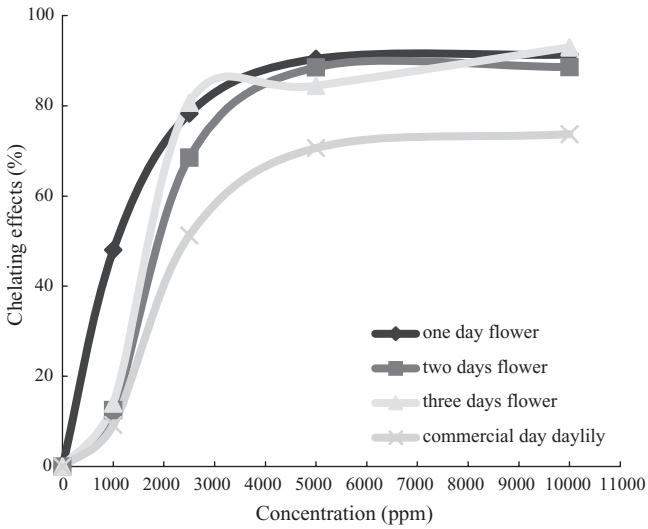


FIG. 3. EFFECTS ON THE FERROUS ION-CHELATING ABILITY OF THE METHANOL EXTRACTS OF DAYLILY FLOWERS

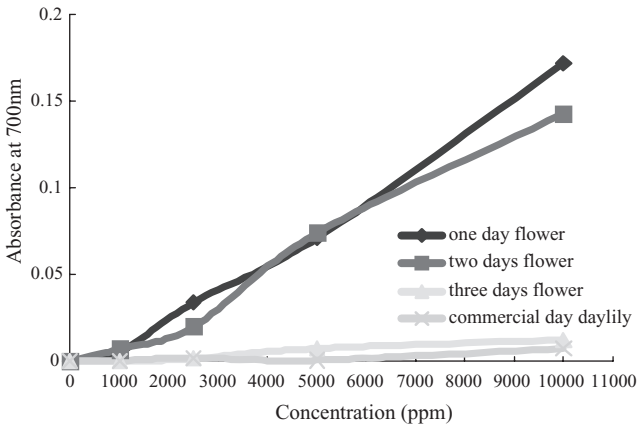


FIG. 4. REDUCING POWER OF THE METHANOL EXTRACTS OF DAYLILY FLOWERS

Standard Curve of Flavorids. The concentration of the flavorid standards were 4–20 $\mu\text{g}/\text{mL}$, after individual CE. We used the concentration of flavorid standards against the absorbance and calculated the correlation coefficient (R^2), which were 0.9845–0.9988, indicating quite-good linear relationships as shown in Table 5.

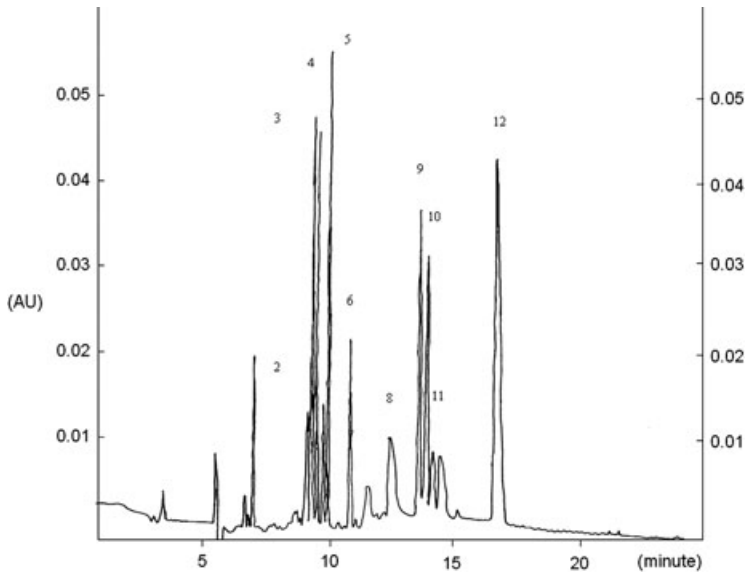


FIG. 5. CAPILLARY ELECTROPHORESIS SPECTRUM OF *HEMEROCALLIS FULVA* FLAVONOIDS

1. naringin, 2. chrysin, 3. pinocembrin, 4. hesperidin, 5. daidzein, 6. rutin, 7. naringenin,
8. isoquercetin, 9. kaempherol, 10. myricetin, 11. quercetin, 12. morin.

TABLE 5.
CORRELATION COEFFICIENTS (R^2) OF FLAVORIDS BY
CAPILLARY ELECTROPHORESIS ANALYSIS

Component	R^2	Component	R^2
Luteolin	0.9979	Vitexin	0.9980
Chrysin	0.9985	Kaempherol	0.9976
Galangin	0.9964	Naringin	0.9962
Apigenin	0.9988	(±)Naringenin	0.9943
Quercetin	0.9978	Morin	0.9983
Rutin	0.9980	Myricetin	0.9845
Isoquercetin	0.9980	Hesperidin	0.9845
Pinocembrin	0.9982	Daidzein	0.9981

Detection Limits. Based on a signal/noise (S/N) ratio of 3 as the detection limit, the following detection limits were obtained (Table 6).

Flavonoid Contents of the Methanol Extracts of *H. fulva* Flowers. Table 7 shows that the flavonoid contents of daylily flowers significantly differed because of the growth area and the age of the flowers. Although

TABLE 6.
THE DETECTION LIMITS OF FLAVORIDS BY CAPILLARY ELECTROPHORESIS
ANALYSIS

Component	Detection limits $\mu\text{g/mL}$	Component	Detection limits $\mu\text{g/mL}$
Luteolin	0.10	Vitexin	0.12
Chrysin	0.17	Kaempferol	0.1
Galangin	0.15	Naringin	0.3
Apigenin	0.08	(\pm)Naringenin	0.08
Quercetin	0.14	Morin	0.3
Rutin	0.25	Myricetin	0.2
Isoquercetin	0.15	Hesperidin	0.18
Pinocebrin	0.13	Daidzein	0.2

TABLE 7.
FLAVONOID CONTENTS OF THE METHANOL EXTRACTS OF *HEMEROCALLIS FULVA*
FLOWERS (PPM)

Flavonoid	TD-F3DF	HL-F1DF	HL-F2DF	HL-F3DF
Naringin	—	—	—	1.86 \pm 0.45
Chrysin	167.80 \pm 10.21 ^{a*}	—	26.51 \pm 2.78 ^c	38.01 \pm 2.76 ^b
Pinocebrin	—	—	—	5.59 \pm 1.69
Hesperidin	107.59 \pm 9.34 ^a	5.12 \pm 1.35 ^d	31.64 \pm 1.76 ^c	57.51 \pm 2.54 ^b
Rutin	81.55 \pm 1.24	—	—	—
Naringenin	—	42.92 \pm 3.98	—	—
Kaempferol	360.21 \pm 8.69	—	—	—
Myricetin	110.59 \pm 8.57 ^c	103.92 \pm 9.47 ^c	142.14 \pm 10.34 ^b	651.48 \pm 8.67 ^a
Quercetin	475.05 \pm 13.56 ^b	154.82 \pm 10.45 ^c	468.15 \pm 8.98 ^b	763.32 \pm 9.76 ^a
Total	1301.79 \pm 12.53 ^b	306.78 \pm 10.54 ^d	668.44 \pm 10.66 ^c	1517.76 \pm 10.02 ^a

* Each value is the mean \pm standard deviation of triplicate analysis; values in the same row with different superscripts significantly differ at $P < 0.05$.

TD-F3DF, Taidong fresh 3 days before to flower; HL-F1DF, Hualien fresh 1 day before to flower; HL-F2DF, Hualien fresh 2 days before to flower; HL-F3DF, Hualien fresh 3 days before to flower.

the total flavonoid contents of 3-day-old flowers were higher than 1- or 2-day-old flowers, that of the 3-day-old flowers grown in Hualien were significantly higher than that grown in the Taidong area; the total flavonoid content from Hualien was 1,517.76 ppm, while that from Taidong was 1,301.79 ppm; that from Hualien area was significantly 16.5% higher. The total flavonoid contents of flowers grown in Hualien area were also in the order of 3-day-old > 2-day-old > 1-day-old flowers; the total flavonoid contents of 3-day-old flowers were significantly 394% higher than 1-day-old flowers and 127% higher than 2-day-old flowers.

As to the individual flavonoid contents, we found that the content of 1-day-old flowers from Hualien was highest in quercetin (154.82 mg/kg), followed by myricetin (103.92 mg/kg) and naringenin (42.92 mg/kg); the 2- and 3-day-old flowers were highest in quercetin (468.15 and 763.32 mg/kg), followed by myricetin (142.14 and 651.48 mg/kg) and hesperidin (31.64 and 57.51 mg/kg); but the 3-day-old flowers from Taidong were highest in quercetin (475.05 mg/kg), followed by kaempferol (360.21 mg/kg) and chrysin (167.80 mg/kg). Chu (1998) once pointed out that the Dutch scholar used hydrolysis to extract carbohydrates and used HPLC to examine the composition of flavonoids in vegetables. The results showed that the main flavonoids in the vegetable part were quercetin, followed by kaempferol and quercetin which was quite high in the onion (347 mg/kg).

CONCLUSIONS

The results showed that the growth area, the age of the flower and the processing method significantly influenced the functional components and antioxidant activities of the flowers of *H. fulva* grown in mountainous areas of Taiwan. The amounts of most flavonoids from Hualien-grown flowers were greater than those of Taidong-grown ones. Total phenols, anthocyanins of the methanol extract of fresh 1 day before to flower were significantly higher than those of 2- and 3 day before to flower. The Fe²⁺-chelating activity of the methanol extracts of fresh *H. fulva* L. flowers was 2,250 µg, while that of dried flowers was 5,120 µg. The reducing powers of both fresh and dried flowers were very weak.

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