# *In vitro* antioxidant capacity of daylily (*Hemerocallis disticha*) flowers cultivated in Taiwan

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**Abstract:** Daylilies (*Hemerocallis disticha*) are very popular for vegetarian cuisine in Taiwan and have been long used as a nutritious food and/or traditional medicine in Chinese society. Therefore, the antioxidant capacity of daylily flowers was investigated with a number of established in vitro assays. The results showed that DPPH radical scavenging activity, hydrogen peroxide scavenging activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity all increased with increasing concentrations of daylily flowers extract. Moreover, the $IC_{50}$ values of daylily flowers extract from the hydrogen peroxide, superoxide radical, hydroxyl radical scavenging assays were 1.45 ± 0.11, 1.23 ± 0.12 and 18.55 ± 1.57 μg/ml, respectively. Taken together, these results clearly indicate that daylily flower has significant potential as a natural antioxidant agent.
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## 1. Introduction

Daylily (*Hemerocallis disticha*), a kind of perennial monocotyledon, are widely distributed in Asia. Daylilies are very popular for vegetarian cuisine in Taiwan and have been long used as a nutritious food and/or traditional medicine in Chinese society. All parts of daylilies have also been used as an oriental medicine for treating a variety of diseases including depression, inflammation, and insomnia (1). It has also been reported that *Hemerocallis* extracts possesses antioxidant (2), inhibited lipid peroxidation (3), inhibited fibroblast proliferation (4) and induced human leukemia cells to undergo differentiation (5). Further, daylilies can favor neurological changes in sleeping mice (6) and impact motor activity in rats as a result of alteration to the normal levels of several central nervous system neurotransmitters (7).

Previous phytochemical works on daylily was isolated and identified an assortment of chemical constituents including phenolic (8), carotenoids (9), anthocyanins (10) and fulvanine lactams (11).

Although daylily flowers contain abundant phytochemicals, only limited information is available about its antioxidant properties. Therefore, the present study was designed to investigate the antioxidant properties of daylily flowers. The antioxidant activities of the daylily flowers extract for 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging assay and using chemiluminescence to measure scavenging activities of hydrogen peroxide, superoxide anion and hydroxyl radical were investigated in this study.

## 2. Material and Methods

### Chemicals

Gallic acid and ferrous sulfate heptahydrate were purchased from Sigma Chemical Company (St. Louis, MO, USA). Deionized water (dd $H_2O$) was prepared by Mill-RQ and Milli Q-UV water purification system (Millipore Co., Ltd. Taipei, Taiwan). All other chemicals and reagents used were obtained from local sources and were of analytical grade.

### Plant material and extraction

Fresh daylily flowers were gathered in September from a farm located in the Hualien city of Taiwan. The daylily flowers were picked in the early morning, then transported to our lab, and stored at 4 °C prior to drying. Before drying, fresh daylily flowers were putted in the -80℃ refrigerator for one day and than were dried directly in the freeze-dryer (-42 °C, below $133\times10^{-3}$ mBAR) for 48 h. After freeze-dried, samples were took into the grinder to smash in powder for extraction. Samples (20 g) were extracted with boiling water (500 ml) for 5 min. The extracts were filtered through Whatman No. 1 filter paper and the filtrates were freeze-dried.

### DPPH radical scavenging activity

The method was referred to that reported by Epsin et al. (12). An aliquot of each sample (30 μL, 0.5-40 mg/mL) in acetone/MeOH (1/1, v/v) was mixed with 200 μL of 100 μM DPPH (prepared with methanol). The mixture was shaken vigorously and then left to stand at room temperature for 60 min in the dark. The absorbance was measured by UV/Vis spectrophotometer at 520 nm against an acetone/

MeOH (1/1, v/v) blank. The lower absorbance indicated the stronger scavenging activity. $IC_{50}$ value (mg sample/mL), the effective concentration at which 50% of the DPPH radicals were scavenged, was obtained from the plot of scavenging activity against the concentration of sample. The scavenging activity was calculated based on the percentage of DPPH radical scavenged.

**Hydrogen peroxide scavenging activity**

Reduction of hydrogen peroxide was determined according to Yoshiki et al. (13) with some modifications. For scavenging of hydrogen peroxide ($H_2O_2$), reaction mixture consisting of $H_2O_2$ and MeCHO were added to distilled water in stainless steel cell in chemiluminescence analyzing system (CLA 2100, Tokoyo Electronic Indust. Co., Ltd., Japan) and measurement of chemiluminescence intensity was started. At the 60-second mark, various concentrations of daylilies flowers extracted were injected into the cell and the chemiluminescence intensity was measured continuously for a further 180 seconds. The total amount of chemiluminescence intensity was calculated by integrating the area under the curve and subtracting the background level. Gallic acid was used as reference compound.

**Superoxide anion scavenging activity**

Superoxide radicals were generated by the xanthine-xanthine oxidase system as previously described (14) and modified further. In brief, xanthine oxidase grade I (0.25U; one unit converts 1μmol of xanthine to uric acid per min at pH 7.5 at 25ºC), lucigenin and various concentration of daylilies flowers extracted were added to phosphate-buffer saline (PBS), pH 7.4 in a special chamber unit that included a stainless steel cell with a magnetic stirrer and stirrer bar in a dark chamber of the chemiluminescence analyzing system. At the 60-second mark, xanthine was injected into the cell and the superoxide radical-induced lucigenin chemiluminescence was measured continuously for a total of 180 seconds. Vitamin C was used as reference compounds.

**Hydroxyl radical scavenging activity**

Hydroxyl radical was generated by the addition of ferrous iron to the buffer solution (15). Freshly prepared $FeSO_4$ (in 0.9%NaCl) and various concentrations of daylilies flowers extracted were added to phosphate-buffered saline, pH 7.4 in the stainless steel cell and measurement of chemiluminescence intensity began. At the 60-second mark, luminol was injected to the cell and the chemiluminescence system was measured continuously for a further 180 seconds. The total amount of the chemiluminescence intensity was calculated by integrating the area under the curve and subtracting the background level. Quercetin was used as reference compounds.

**Statistical analysis**

All antioxidant capacity assays were carried out in triplicate and the mean values were calculated. Statistically significant differences between groups were defined as $p < 0.05$.

**3. Results and discussion**

The aim of the present study was to investigate the antioxidant activity of daylily flowers extracts and reference compounds evaluated using four antioxidant models. DPPH radical scavenging assay is widely used for relatively rapid evaluation for antioxidant activities compared to other methods. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and the discoloration is visualized from purple to yellow (16). For DPPH radical scavenging assay, daylily flowers extract had significantly higher effect than reference compound, trolox (Fig. 1). The daylily flowers extract proved to be an effective scavenger for the DPPH radicals and at a concentration range from 0.30 to 2.70 mg/mL, the scavenging abilities on DPPH radicals could be increased from 8.12 to 94.39 %. Moreover, the $IC_{50}$ values of the scavenging abilities on DPPH radicals of the daylily flowers extract and trolox were 1.46 and 3.65 mg/mL, respectively.

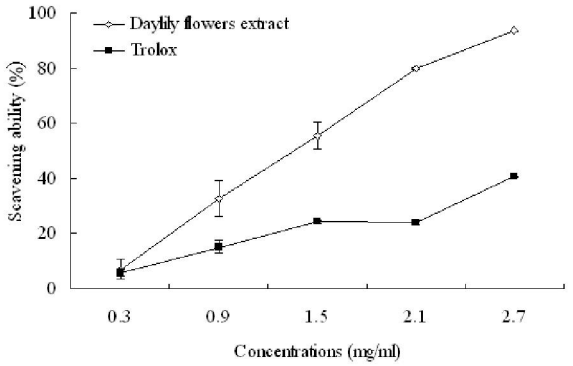


Fig. 1. Effects of daylily flowers extract and trolox on DPPH radical scavenging activities. Values are means ± SDs for triplicate in each concentration.

The chemiluminiescence assay is another very sensitive and convenient method to determine the potency of the antioxidant activity of the nature product, as well as other antioxidants. For $H_2O_2$ scavenging activities, daylily flowers extract had significantly higher effect than reference compound, gallic acid (Fig. 2). The daylily flowers extract proved to be an effective scavenger for the $H_2O_2$ and at a

concentration range from 0.17 to 16.52 mg/mL, the scavenging abilities on $H_2O_2$ could be increased from 13.90 to 81.14 %. Table 1 shows the $IC_{50}$ value of the scavenging activities of DPPH radical, hydrogen peroxide, superoxide anion and hydroxyl radical of the daylily flowers extract and reference compound. The antioxidant capacity of the daylily flowers extract was significantly higher ($p < 0.05$) than of reference compound and $IC_{50}$ value of the scavenging activity of hydrogen peroxide, superoxide anion and hydroxyl radical of the daylily flowers extract were 1.45 ± 0.11, 1.23 ± 0.12 and 18.55 ± 1.57, respectively. The gallic acid, vitamin C and quercetin were used as positive control of scavenger for ROS and they exhibited the $IC_{50}$ value of scavenging activity were 12.31 ±0.82, 18.39 ± 2.67, and 54.23 ± 3.77 μg/mL of the $H_2O_2$, superoxide anions radical and hydroxyl radical, respectively.

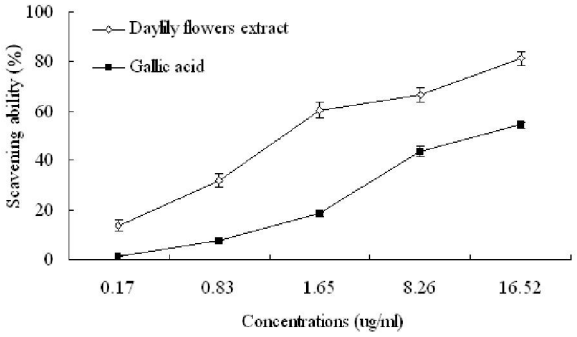


Fig. 2. Effects of daylily flowers extract and gallic acid on $H_2O_2$ scavenging activities. Values are means ± SDs for triplicate in each concentration.

Table 1. $EC_{50}$ of DPPH radical quenching activity, hydrogen peroxide scavenging activity, superoxide anion radical scavenging activity and hydroxyl radical scavenging activity of the daylily flowers extract and reference standards.

| | $EC_{50}$ | | | |
|---|---|---|---|---|
| | DPPH (mg/ml) | $H_2O_2$ (μg/ml) | (μg/ml) | •OH (μg/ml) |
| Daylily flowers extract | 1.46 ± 0.12 | 1.45 ± 0.11 | 1.23 ± 0.12 | 18.55 ± 1.57 |
| Trolox | 3.65 ± 0.14 | | | |
| Gallic acid | | 12.31 ± 0.82 | | |
| Vitamin C | | | 18.39 ± 2.67 | |
| Quercetin | | | | 54.23 ± 3.77 |

Values are the mean ± SD for triplicate in each concentration.

Hsu et al. (9) reported that daylily flowers contained abundant lutein and zeaxanthin and had high antioxidant activities, successfully scavenging $H_2O_2$, superoxide anion and hydroxyl radicals. Jimenéz and Pick (17) suggested that carotenoids are proved as a protective agent against oxidative stress damage and carotenoids scavenge several active oxygen species such as superoxide anion, $H_2O_2$, peroxy radicals, and hydroxy radicals (18). The carotenoids are stronger scavenger of ROS and the lutein and zeaxanthin with hydroxyl group substitutions on both sides of the compound also exhibited high $H_2O_2$ scavenging potential (19). Therefore, it can be concluded that daylily flower is an available source of natural antioxidants that provides the expected health benefits.

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**References**

1. Uezu, E. (1997). A philological and experimental investigation of the effects of Hemerocallis as food in man and ddy mice. Bulletin of College of Education, University of the Ryukyus, 51, 231-238.
2. Cichewicz, R.H., Nair, M.G. (2002). Isolation and characterization of stelladerol, a new antioxidant naphthalene glycoside, and other antioxidant glycosides from edible daylily (Hemerocallis) flowers. Journal of Agricultural and Food Chemistry, 50 (1), 87-91.
3. Zhang Y., Cichewicz R.H., Nair M.G. (2004). Lipid peroxidation inhibitory compounds from daylily (Hemerocallis fulva) leaves. Life Sciences, 75, 753-763.
4. He, C.X. (1994). Effects of extracts from Hemerocallis citrina Barroni (EHCB) and epidermal growth factor (EGF) on human dermal fibroblast proliferation. Zhonghua Pifuke Zazhi, 27, 218-220.
5. Hata, K., Ishikawa, K., Hori, K. (1998). Differentiation-inducing activities of human leukemia cell line (HL60) by extracts of edible wild plants in Akita. Nature Medicine, 52, 269-272.

6. Uezu, E. (1998). Effects of *Hemerocallis* on sleep in mice. Psychiatry and Clinical Neurosciences, 52, 136-137.
7. Hsieh, M.T.; Ho, Y.F.; Peng, W.H.; Wu, C.R.; Chen, C.F. (1996). Effects of *Hemerocallis fla*V*a* on motor activity and the concentration of central monoamines and its metabolites in rats. Journal of Ethnopharmacology, 52, 71-76.
8. Que, F., Mao, L., Zheng, X. (2007). *In vitro* and *vivo* antioxidant activities of daylily flowers and the involvement of phenolic compounds. Asia Pacific journal of clinical nutrition, 16, 196-203.
9. Hsu, Y.W., Tsai, C.F., Chen, W.K., Ho, Y.C., Lu, F.J. (2011). Determination of lutein and zeaxanthin and antioxidant capacity of supercritical carbon dioxide extract from daylily (*Hemerocallis disticha*). Food Chemistry, 129, 1813-1818.
10. Griesbach, R.J.; Batdorf, L. (1995). Flower pigments within *Hemerocallis ful*V*a* L. fm. *ful*V*a*, fm. *rosea*, and fm. *disticha*. HortScience, 30, 353-354.
11. Inoue, T.; Konishi, T.; Kiyosawa, S.; Fujiwara, Y. 2,5-dihydrofuryl-*ç*-lactam derivatives from *Hemerocallis ful*V*a* L. var. *kwanzo* Regel. II. Chemical and Pharmaceutical Bulletin, 42, 154-155.
12. Epsin, J.C., Soler-Rivas, C., Wichers, H.J. (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-2-picrylhydrazyl radical. Journal of Agricultural and Food Chemistry, 48, 648-656.
13. Yoshiki, Y., Yamanaka, T., Satake, K., Okubo, K. (1999). Chemiluminescence properties of soybean protein fraction in the hydroperoxide and hydrogen donor system. Luminescence, 14, 315-319.
14. Yeung, S.Y., Lan, W.H., Huang, C. S., Lin, C. P., Chan, C. P., Chang, M.C., Jeng, J.H. (2002). Scavenging property of three cresol isomers against $H_2O_2$, hypochlorite, superoxide and hydroxyl radicals. Food and Chemistry Toxicology, 40, 1403-1413.
15. Yıldız, G., Demiryürek, A.T. (1998). Ferrous iron-induced luminol chemiluminescence: a method for hydroxyl radical study. Journal of Pharmacological and Toxicological Methods, 39, 179-184.
16. Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G.I., Nishioka, I. (1998). Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochemical Pharmacology, 56, 213–222.
17. Jimenez, C., Pick, U. (1993). Differential reactivity of β-carotene isomers from Dunaliella bardawil toward oxygen radicals. Plant Physiology, 101, 385-390.
18. Krinsky, N.I. (1989). Antioxidant functions of carotenoids. Free Radical and Biology Medicine, 7, 617-635.
19. Tian, B., Xu, Z., Sun, Z., Lin, J., Hua, Y. (2007). Evaluation of the antioxidant effects of carotenoids from Deinococcus radiodurans through targeted mutagenesis, chemiluminescence, and DNA damage analyses. Biochimica et Biophysica Acta, 1770, 902-911.

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