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ANTIOXIDANT ACTIVITY AND MECHANISM IN FLOWER OF *HYLOCEREUS UNDATUS* (HAW.) BRITT. ET ROSE

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Hylocereus undatus flower is commonly used as food or for medicinal purposes in south China. To study its antioxidant activity and mechanism we used antioxidant and chemical assays to compare two commercial samples from different locations (Shenjing, Qixing). The difference in antioxidant levels corresponded with differences in chemical content (including total phenolics, total flavonoids, kaempferol and quercetin) between Shenjing and Qixing. The antioxidant ability of *H. undatus* flower seems attributable to total phenolics (mainly total flavonoids). Kaempferol is one of the main bioactive components. *H. undatus* flower exerts its antioxidant effects through metal chelation and radical scavenging via hydrogen atom (H•) and electron (e) donation.

Key words: *Hylocereus undatus* flower, antioxidant activity, total phenolics, total flavonoids, kaempferol, quercetin.

INTRODUCTION

Hylocereus undatus (Haw.) Britt. et Rose is a climbing vine cactus species widely distributed in many countries including Australia, Israel, Malaysia, Nicaragua, Taiwan, Vietnam, and south China. Its flower (Fig. 1) is commonly used to prepare various healthful, tasty soups. In traditional Chinese medicine (TCM) these soups are thought to exert effects including clearing "heat-fire," moisturizing the lung, eliminating phlegm and relieving cough (Ye, 1999; Zhou, 2001) Phytochemical analyses revealed that *H. undatus* flower contains three glycosides (undatusides A, B, C) (Wu et al., 2011) and several flavonoids (Yi et al., 2012). There are no reports on its antioxidant activity. In this study we investigated its antioxidant activity, and the mechanism of it, in two typical commercial samples of *H. undatus* flower, Shenjing Bawanghua flower and Qixing Jianhua flower.

MATERIALS AND METHODS

PLANT MATERIAL

Shenjing flower is grown on Shenjing Changzhou Island, Guangzhou city, and the dried Shenjing flower was purchased from the Changzhou vegetable market, Guangzhou city, Guangdong Province, China. Qixing flower is widely cultivated in Zhaoqing city in Guangdong Province, and dried Qixing flower was obtained from Dinghutang Food Processing Factory, Guangdong Province, China. Voucher specimens are deposited in our laboratory.

CHEMICALS

DPPH• (1,1-diphenyl-2-picrylhydrazyl radical), ABTS [2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt], BHA (butylated hydroxyanisole), Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], linoleic acid, ferrozine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate], murexide (5,5'-nitrilodibarbituric acid monoammonium salt), pyrogallol and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Shanghai Trading Co., China. Kaempferol and quercetin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and water were HPLC grade. Other chemicals used in this study were analytic grade.

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Fig. 1. *Hylocereus undatus* (Haw.) Britt. et Rose and its flower

PREPARATION OF EXTRACTS

Shenjing flower and Qixing flower were soaked in 70% ethanol at room temperature for a month, then concentrated under reduced pressure to yield SJFH (ethanol extract of Shenjing flower) and QXFH (ethanol extract of Qixing flower). They were refrigerated until used for analysis.

ANTI-LIPID PEROXIDATION

The anti-lipid peroxidation effect was investigated in linoleic acid emulsion (Li et al., 2009). Briefly, 1.5 mL linoleic acid emulsion was mixed with 0.15 mL sample methanolic solution (0.4–2.0 mg/mL) and 0.35 mL 30% ethanol (v/v). The reaction mixture (total 2 mL) was incubated at room temperature for 72 h. Then 0.15 mL of the mixture was added to 3.65 mL 75% ethanol (v/v), 0.1 mL NH_4SCN (30%, w/w), and 0.1 mL $FeCl_2$ (0.02 M in 3.6% HCl). Absorption at 500 nm was measured with a Unico 2100 spectrophotometer. The inhibition percentage was calculated by the equation:

$$\text{Inhibition \%} = (A_0 - A)/A_0 \times 100\%$$

where A_0 is the absorbance of the control without sample, and A is the absorbance of the reaction mixture with sample.

$\bullet O_2^-$ RADICAL SCAVENGING ASSAY

Measurement of superoxide anion ($\bullet O_2^-$) scavenging activity was based on our method (Li, 2012). In brief, 1 mg/mL sample ethanolic solution (× mL) was mixed with Tris-HCl buffer (2.92-× mL, 0.05 M, pH 7.4) containing EDTA (1 mM). When 80 μL pyrogallol (60 mM in 1 mM HCl) was added, the mixture was shaken rapidly at 37°C. The absorbance of the mixture was measured every 30 s for 5 min at 325 nm. The $\bullet O_2^-$ scavenging ability was calculated using the formula (Li, 2012):

$$\text{Inhibition \%} = [(\Delta A_{325nm,control}/T) - (\Delta_{A325nm,sample}/T)]/(\Delta A_{325nm,control}/T) \times 100\%$$

Here, $\Delta A_{325nm,\ control}$ is the increment in A_{325nm} of the mixture without the sample and $\Delta A_{325nm,\ sample}$ is that with the sample; T = 5 min.

Fe^{2+} CHELATION ASSAY

Fe^{2+} chelation activity was estimated by the method of Li et al. (2012a). Briefly, 0.2 mL sample methanolic solution (260–1040 μg/mL) was added to 0.1 mL $FeCl_2$ aqueous solution (250 μM). The reaction was initiated by adding 150 μL ferrozine aqueous solution (1 mM) and the total volume was adjusted to 1.0 mL with methanol. Then the mixture was shaken vigorously and stood at room temperature for 10 min. Absorption at 562 nm was measured spectrophotometrically. The chelation percentage was calculated by the formula (Li et al., 2012a):

$$\text{Chelating effect \%} = (A_0 - A)/A_0 \times 100\%$$

where A_0 is the absorbance of the control without sample, and A is the absorbance of the reaction mixture with sample.

Cu^{2+} CHELATION ASSAY

The Cu^{2+} chelation effect was analyzed by the method of Li et al. (2012a). Briefly, 0.06 mL $CuSO_4$ aqueous solution (20 mM) was added to hexamine-HCl buffer (pH 5.0, 30 mM) containing 30 mM KCl and 0.20 mM murexide. After incubation at room temperature for 1 min, 0.030–0.18 mL sample methanolic solution (13 mg/mL) was added. The final volume was adjusted to 1.5 mL with methanol. Then the mixture was shaken vigorously and left at room temperature for 10 min. Absorption at 485 nm and 520 nm was measured spectrophotometrically. The absorbance ratio (A_{485}/A_{520}) reflected the free Cu^{2+} content. The cupric chelation percentage was therefore calculated as the equation (Li et al., 2012a):

$$\text{Chelating effect \%} = [(A_{485}/A_{520})_{max} - (A_{485}/A_{520})]/[(A_{485}/A_{520})_{max} - (A_{485}/A_{520})_{\ min}] \times 100\%$$

where (A_{485}/A_{520}) is the absorbance ratio of the sample, while $(A_{485}/A_{520})_{max}$ is the maximum absorbance ratio and $(A_{485}/A_{520})_{min}$ is the minimum absorbance ratio in the test.

DPPH• RADICAL SCAVENGING ASSAY

DPPH• radical scavenging activity was determined as described (Li et al., 2012a). Briefly, 1 mL DPPH• ethanolic solution (0.1 mM) was mixed with 0.5 mL sample ethanolic solution (0.6–3.0 mg/mL) and kept at room temperature for 30 min. Then absorption at 519 nm was measured with a spectrophotometer. The DPPH• inhibition percentage was calculated by the formula described in "ANTI-LIPID PEROXIDATION."

$ABTS^+$• RADICAL SCAVENGING ASSAY

$ABTS^+$• scavenging activity was measured as described by Li et al. (2012b). Briefly, 1.2 mL diluted $ABTS^+$• reagent was added to 0.3 mL sample ethanolic solution (0.08–0.4 mg/mL). After incubation for 6 min, absorbance at 734 nm was read with a spectrophotometer and the inhibition percentage was calculated using the formula described in "ANTI-LIPID PEROXIDATION."

CHEMICAL ANALYSIS

Total phenolics content was determined using the Folin-Ciocalteu method (Li et al., 2012b). Briefly, 0.5 mL sample methanolic solution (1 mg/mL) was added to 0.5 mL Folin-Ciocalteu reagent (0.25 M). The mixture stood for 3 min, then 1.0 mL Na_2CO_3 aqueous solution (15%, w/w) was added. After incubation at ambient temperature for 30 min the mixture was centrifuged at 3500 rpm for 3 min. The absorption of the supernatant at 760 nm was measured with a spectrophotometer. The standard curve was prepared using different concentrations of quercetin and the result was expressed as quercetin equivalent in milligrams per gram extract.

Total flavonoid content was measured using the $NaNO_2$-Al $(NO_3)_3$ method (Li et al., 2012a). Briefly, 1 mL sample methanolic solution (10 mg/mL) was mixed with 0.15 mL $NaNO_2$ aqueous solution (5%, w/w). The mixture stood for 6 min, followed by the addition of 0.15 mL Al $(NO_3)_3$ aqueous solution (10%, w/w). After incubation for another 6 min, 2 mL NaOH aqueous solution (4%, w/w) was added, then the mixture was adjusted to 5 mL with distilled water. Absorption at 508 nm was read with a spectrophotometer. The standard curve was obtained using standard quercetin and the result was also expressed as quercetin equivalent in milligrams per gram extract.

Kaempferol and quercetin were identified by comparing their retention times using HPLC, performed with a Syltech P510 system (Los Angeles, CA, USA) equipped with a Dikma Diamonsil C18 column (250 mm × 4.6 mm, 5 μm) (Beijing, China). The mobile phase consisted of methanol and water (50:50, v/v), the flow rate was 0.5 mL/min, and the wavelength was 360 nm. Kaempferol and quercetin content in the extracts was calculated on the basis of the calibration curves of kaempferol and quercetin standards.

STATISTICAL ANALYSIS

Data are given as means ±SD of three measurements. The IC_{50} values were calculated by linear regression analysis. All linear regressions in this paper used Origin 6.0 software. The significance of differences was checked with the t-test ($p < 0.05$) using SPSS software (v.12, SPSS, USA).

RESULTS AND DISCUSSION

Under oxidative stress, unsaturated fatty acids may undergo lipid peroxidation (LPO) and subsequently form reactive aldehyde species that are both cytotoxic and genotoxic through their ability to covalently modify proteins and DNA (Sowell et al., 2004). Many diseases are associated with lipid peroxidation, such as cardiovascular disease (Halliwell, 2000), bronchitis (Ignatova et al.,1998), protitis (Filipenko and Salii, 2007), pulmonary tuberculosis (Novitskii et al., 2005) and atherosclerosis (Sdvigova et al., 1993).

We used a linoleic acid emulsion system to assess the anti-lipid peroxidation ability of *H. undatus* flower; both SJFH and QXFH exhibited anti-lipid peroxidation activity in a concentration-dependent manner (Fig. 2a). The IC_{50} values were 3.62 ± 0.32 μg/mL for SJFH and 3.42 ± 0.10 μg/mL for QXFH (Tab. 1). It suggested that *H. undatus* flower can effectively prevent lipid peroxidation.

Lipid peroxidation may occur nonenzymatically through the reaction of linoleic acid with reactive oxygen species (ROS) (Sowell et al., 2004). For example, $\bullet O_2^-$, one form of ROS, can directly damage lipids oxidatively. In addition, $\bullet O_2^-$ can further generate •OH radicals via the Haber-Weiss reaction (Fang and Zheng, 2002).

The hydroxyl radical (•OH) with high reactivity can extract •H from lipid (LH) to produce L•, LO•, LOO• and LOOH. Therefore $\bullet O_2^-$ scavenging is considered to be a mechanism of preventing lipid peroxide from forming. Both SJFH and QXFH exhibited stronger $\bullet O_2^-$ radical scavenging ability than Trolox in the study (Tab. 1), suggesting that *H. undatus* flower exerted anti-lipid peroxidation action, perhaps via $\bullet O_2^-$ scavenging.

Transition metals, especially Fe and Cu, play an important role in ROS generation. We examined the metal chelation abilities of SJFH and QXFH. The dose-response curves confirmed that both SJFH

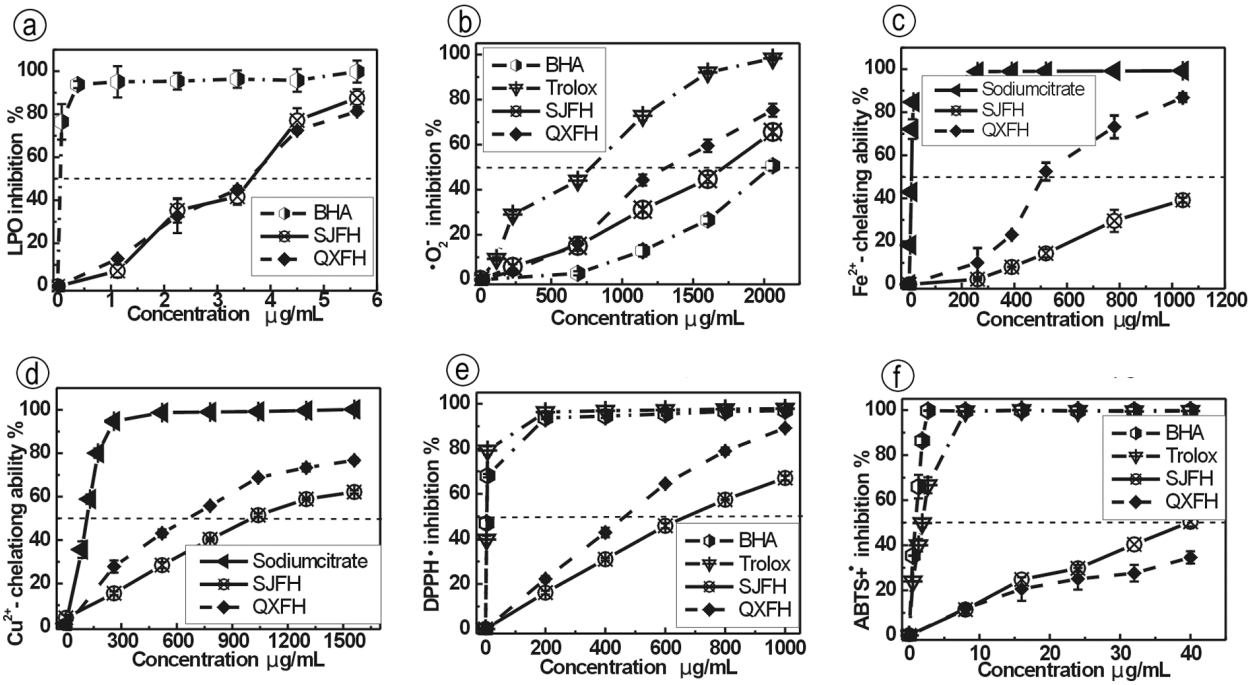

Fig. 2. Dose-response curves of antioxidant assays for SJFH, QXFH and positive controls. SJFH – ethanol extract of Shenjing Bawanghua flower; QXFH – ethanol extract of Qixing Jianhua flower. (**a**) Anti-lipid peroxidation, (**b**) $\cdot O_2^-$ radical scavenging, (**c**) Fe^{2+} chelation assay, (**d**) Cu^{2+} chelation assay, (**e**) DPPH• radical scavenging, (**f**) ABTS•$^+$ radical scavenging. Values are means ± SD (n = 3).

TABLE 1. IC_{50} values for SJFH, QXFH and positive controls (μg/mL)

Assay	SJFH	QXFH	Positive control	
			BHA	Trolox
LPO	3.62±0.32^b	3.42±0.10^b	0.012±0.01^a	nd
$\cdot O_2^-$	1678.39±37.75^c	1356.53±53.18^b	2087.40±53.16^d	715.91±36.08^a
Fe^{2+}-chelating	1428.53±255.25^c	489.11±34.04^b	9.31±0.35^{*,a}	nd
Cu^{2+}-chelating	1033.49±15.13^c	658.89±24.56^b	101.05±8.83^{*,a}	nd
DPPH•	672.09±15.38^c	465.64±16.43^b	3.79±0.38^a	4.20±0.14^a
ABTS$^+$•	39.81±1.31^c	51.96±9.30^c	0.91±0.05^a	1.89±0.03^b

IC_{50} value is defined as the concentration for 50% effect, calculated by linear regression analysis and expressed as means ±SD (n = 3). Means with different superscripts in the same row differ significantly at $p < 0.05$. *The positive control is sodium citrate. BHA – butylated hydroxyanisole; SJFH – ethanol extract of Shenjing flower, QXFH – ethanol extract of Qixing flower, nd – not detected.

and QXFH possessed effective metal chelation ability (Fig. 2c,d). The chelation ability can be attributed mainly to polyphenols and especially flavonoids (Li et al., 2012a). For example, kaempferol isolated from *H. undatus* flower (Yi et al., 2011) can bind Cu^{2+} via -OH and C=O groups in *ortho* positions (Torreggiani et al., 2005). Metal chelation may be another mechanism to prevent LPO.

To further study the radical scavenging mechanism, SJFH and QXFH were subjected to DPPH and ABTS assays. Our data showed that both extracts effectively scavenge DPPH• and ABTS$^+$• radicals

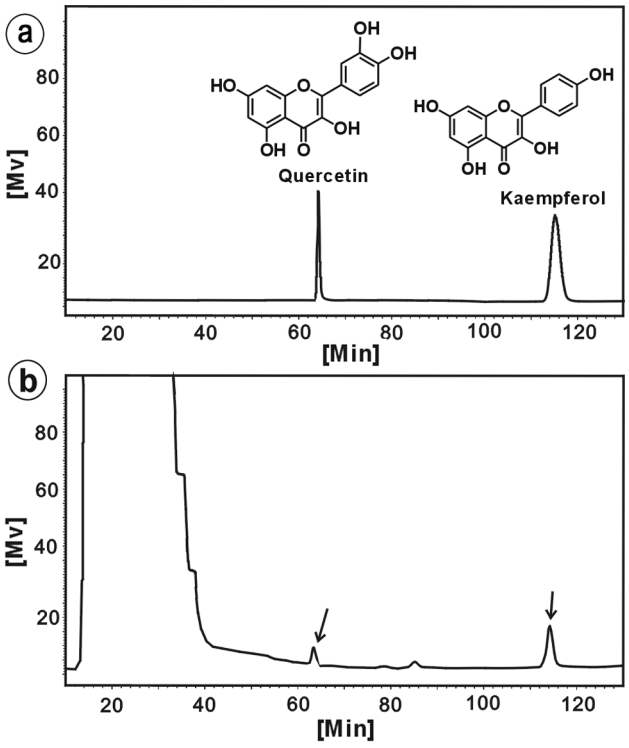

Fig. 3. HPLC chromatogram of standards (**a**) and typical HPLC profile of extract from flower of *Hylocereus undatus* (Haw.) Britt. et Rose (**b**). Syltech P510 system (Los Angeles, California, USA), Dikma Diamonsil C18 column (250 mm × 4.6 mm, 5 μm) (Beijing, China), methanol-water (50:50, v/v) mobile phase, 0.5 mL/min flow rate, 360 nm wavelength.

(Fig. 2e,f). Previous research suggested that DPPH• may be scavenged by an antioxidant through hydrogen atom (H•) donation to form a stable DPPH-H molecule that does not absorb at 519 nm (Bondet et al., 1997). For example, kaempferol could transfer H· to DPPH• and then transform into a semiquinone, even stable quinine (Dimitrios and Vassiliki, 2006; Khanduja and Anjana, 2003). However, $ABTS^{\bullet+}$ scavenging is considered an electron (e) transfer reaction (Aliaga and Lissi, 1998). The fact that both SJFH and QXFH can effectively scavenge DPPH• and $ABTS^{\bullet+}$ suggests that *H. undatus* flower exerts radical scavenging action by donating hydrogen atoms (H•) and electrons (e).

Earlier work suggested that *H. undatus* flower can be used as an adjuvant therapy for atherosclerosis (Liang et al., 1995), cardiovascular diseases, pulmonary tuberculosis, bronchitis, parotitis (Zhonghua Bencao, 2004) and other conditions. These pharmacological effects may be related to antioxidant action (Zheng and Huang, 2007).

We also used spectrophotometry and HPLC to analyze the chemical content of SJFH and QXFH,

TABLE 2. Chemical content of SJFH and QXFH

	SJFH	QXFH
Total phenolics*	25.27±1.28 [a]	53.34±7.41 [b]
Total flavonoids*	10.34±0.21 [a]	21.28±0.18 [b]
Kaempferol (mg/g)	0.29±0.040 [a]	0.94±0.14 [b]
Quercetin (mg/g)	0.065±0.0078 [a]	0.087±0.0044 [b]

Values are means ±SD (n = 3). Values with different superscripts in the same row differ significantly at $p < 0.05$. SJFH – ethanol extract of Shenjing flower, QXFH – ethanol extract of Qixing flower. *Expressed as mg quercetin/g extract.

including total phenolics, total flavonoids, kaempferol and quercetin. As seen in Table 2, the chemical content of SJFH was generally lower than that of QXFH. The difference in chemical content paralleled the difference in their antioxidant levels. It can be inferred that the antioxidant ability of *H. undatus* flower is attributable to its chemical components. This conclusion is expected, as similar observations have been reported (Li et al., 2009). Total phenolics include flavonoids, phenolic acids, tannins, anthocyanins and others. In our study the high ratios of total flavonoids versus total phenolics (10.34:25.27 for SJFH and 21.28:53.34 for QXFH) suggest that flavonoids are the main form of total phenolics (Tab. 2), so the antioxidant ability of *H. undatus* flower seems mainly the effect of flavonoids. Thirteen flavonoids have been isolated from *H. undatus* flower, including kaempferol, quercetin, isorhamnetin and others (Yi et al., 2011). Our results indicate that kaempferol content was much higher than quercetin content (Tab. 2, Fig. 3); kaempferol is one of the main bioactive antioxidant components of *H. undatus* flower.

CONCLUSIONS

As an edible or medicinal plant material, *H. undatus* flower has antioxidant effects. Its antioxidant action is due mainly to its content of total flavonoids, among which kaempferol is a principal bioactive component. It exerts its antioxidant effect through metal chelation, and radical scavenging via hydrogen atom (H•) and electron (e) donation.

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