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-picyrylhydrazyl 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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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 NH₄SCN (30%, w/w), and 0.1 mL FeCl₂ (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:

\[
\text{Inhibition} \% = \frac{(A_0-A)}{A_0} \times 100\%
\]

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

\(\bullet\)O₂ RADICAL SCAVENGING ASSAY

Measurement of superoxide anion (\(\bullet\)O₂) scavenging activity was based on our method (Li, 2012). In brief, 1 mg/mL sample ethanolic solution (\(\times\) mL) was mixed with Tris-HCl buffer (2.92-\(\times\) mL, 0.05 M, pH 7.4) containing EDTA (1 mM). When 80 \(\mu\)L 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 \(\bullet\)O₂ scavenging ability was calculated using the formula (Li, 2012):

\[
\text{Inhibition} \% = \left(\frac{|(\Delta A_{325\text{nm},\text{control}}/T) - (\Delta A_{325\text{nm},\text{sample}}/T)|}{(\Delta A_{325\text{nm},\text{control}}/T)}\right) \times 100\%
\]

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

Fe²⁺ CHELATION ASSAY

Fe²⁺ chelation activity was estimated by the method of Li et al. (2012a). Briefly, 0.2 mL sample methanolic solution (260–1040 \(\mu\)g/mL) was added to 0.1 mL FeCl₂ aqueous solution (250 \(\mu\)M). The reaction was initiated by adding 150 \(\mu\)L 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):

Chelating effect \% = \(\frac{(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.

Cu²⁺ CHELATION ASSAY

The Cu²⁺ chelation effect was analyzed by the method of Li et al. (2012a). Briefly, 0.06 mL CuSO₄ 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 Cu²⁺ content. The cupric chelation percentage was therefore calculated as the equation (Li et al., 2012a):

Chelating effect \% = \(\frac{(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."

**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), proptisis (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 IC₅₀ 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, •O₂⁻, one form of ROS, can directly damage lipids oxidatively. In addition, •O₂ 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 •O₂ scavenging is considered to be a mechanism of preventing lipid peroxide from forming. Both SJFH and QXFH exhibited stronger •O₂ radical scavenging ability than Trolox in the study (Tab. 1), suggesting that *H. undatus* flower exerted anti-lipid peroxidation action, perhaps via •O₂ 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...
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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.

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>SJFH</th>
<th>QXFH</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>BHA</td>
</tr>
<tr>
<td>LPO</td>
<td>3.62±0.32b</td>
<td>3.42±0.10b</td>
<td>0.012±0.01a</td>
</tr>
<tr>
<td>•O_{2}^{-}</td>
<td>1678.39±37.75c</td>
<td>1356.53±53.18b</td>
<td>2087.40±53.16d</td>
</tr>
<tr>
<td>Fe^{2+}-chelating</td>
<td>1428.53±255.25c</td>
<td>489.11±34.04b</td>
<td>9.31±0.35a</td>
</tr>
<tr>
<td>Cu^{2+}-chelating</td>
<td>1033.49±15.13c</td>
<td>658.89±24.56b</td>
<td>101.05±8.33a</td>
</tr>
<tr>
<td>DPPH•</td>
<td>672.09±15.38c</td>
<td>465.64±16.43b</td>
<td>3.79±0.38a</td>
</tr>
<tr>
<td>ABTS••</td>
<td>39.81±1.31c</td>
<td>51.96±9.30c</td>
<td>0.91±0.05a</td>
</tr>
</tbody>
</table>

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.

**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) •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).
Previous research suggested that DPPH• may be scavenged by an antioxidant through hydrogen atom (H•) donation to form a stable DPPH-Hmolecule 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•+ 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•+ suggests that H. undatusflower exerts radi cal scavenging action by donating hydrogen atoms (H•) and electrons (e).

Earlier work suggested that H. undatusflower 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, 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. undatusflower 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. undatusflower seems mainly the effect of flavonoids. Thirteen flavonoids have been isolated from H. undatusflower, 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. undatusflower.

**CONCLUSIONS**

As an edible or medicinal plant material, H. undatusflower 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.

**ACKNOWLEDGMENTS**

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