ANTIOXIDANT ACTIVITY OF GOLDEN BARREL CACTUS (ECHINOCACTUS GRUSONII HILDM.) CRUDE EXTRACTS

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Highlights
- Golden barrel cactus (Echinocactus grusonii) known as the Tang Thong in Thailand is the most popular cultivar in Thailand.
- Crude extracts from Golden barrel cactus aged 3 years has significantly higher antioxidant activity (p < 0.05) than extracts from Golden barrel cactus aged 6 years.

Abstract- Presently, international scientists have studied cactus extract from various species for different phytochemicals that benefit human health. In this study, we focus on the Golden barrel cactus, a Thai cultivar cactus known as the Tang Thong in Thailand, at differing maturities. This work studies on proximate composition and antioxidant activity of Golden barrel cactus stem extract. Total phenolic contents was 3,545.35 and 2,557.96 mg gallic acid equivalent per 100g of dry weight for Golden barrel cactus aged 3 and 6 years crude extracts, respectively. The crude extracts of Golden barrel cactus aged 3 and 6 years were evaluated with DPPH, ABTS and FRAP assay. Extracts from Golden barrel cactus aged 3 years showed IC50 values of 112.60 mg/ml, 44.62 mg/ml and 0.014 mmol Fe²⁺/g of dry weight, while the crude extracts of Golden barrel cactus aged 6 years showed IC50 values of 191.90 mg/ml, 81.84 mg/ml, and 0.011mmol Fe²⁺/g of dry weight for each assay. In conclusion, the total phenolic and antioxidant activity of 3 years old Golden barrel cactus extract is significant higher (p < 0.05) than 6 years old crude extract of Golden barrel cactus.

Keywords- Golden barrel cactus (Echinocactus grusonii), Antioxidant activity

I. INTRODUCTION

Nowadays, international scientists have studied the cactus extract for different phytochemicals that are beneficial to human health. Van Heerden (2008) studied Hoodia species which is found in the arid west of Southern Africa Namibia, and Botswana. Hoodia traditionally has been used by the San people of South Africa and Namibia to suppress appetite and thirst during long hunting trips in the Kalahari desert. This ability to suppress appetite and thirst comes from the steroid glycoside derived from Hoodia. Therefore, Hoodia gordonii was an interesting candidate for developing a weight control drug. Van Heerden, Horak, Maharaj, Vleggaar, Senabe and Gunning (2007) studied the appetite-suppressant activity of Hoodia species extracts in rats by oral gavage at 6.25–50 mg/kg for an eight day period. The results showed that all doses resulted in a decrease of food consumption over an eight-day period and body mass decrease when compared to the control sample receiving only the vehicle.

Guevara-Figueroa, Jiménez-Islas, Reyes-Escogido, Mortensen, Laursen, Lin et al. (2010) studied the proximate composition, phenolic acid, and flavonoid characterization in two commercial and eight wild Opuntia spp., as well two processed products (nopal powder & tablets). They showed that differences in proximate composition ‘wild nopal blanco’ collected from the High Plateau had the highest protein content, while ‘tapón-II’ had the highest fiber content. Nopal tablets had low protein and carbohydrate content but had the highest ash content. The wild morado, tempranillo, blanco, and cristalino varieties had the highest total phenolic acid content, while the commercial varieties had the highest total flavonoid contents.

Hernández-Urbiola, Contreras-Padilla, Pérez-Torrero, Hernández-Quevedo, Rojas-Molina, Cortes et al. (2010) studied the development of the of nopal (Opuntia ficus-indica, cv. Redonda) composition at advanced maturity stages in order to evaluate the age related changes in the nutritional composition to suggest its potential use for human consumption. Insoluble dietary fiber, calcium increased from 17.95% to 34.40% from 40-135 days respectively. In addition the ash and phosphorus content also increased. The soluble dietary fiber in nopal decreased as age progressed from 40 to 135 age days. The results show that older nopal is an important source of calcium and dietary fiber. Nopal can be an economic alternative for food supplement mainly at advanced maturation stage i.e. at 135 days and can be ameliorated to prevent chronic and degenerative disease. As mentioned previously, a lot of interest has been garnered to study the Golden barrel cactus or Thailand call “Tang Thong”, a plant in the family of Cactaceae originating in Mexico same as O. ficus – indica. Currently, it is the most popular cacti cultivated in Thailand. Nevertheless, Golden barrel cactus has been less intensively studied. Recently, Huang, Qiu and Guo (2014) have been reported that...
the Golden barrel cactus spine has highly aligned fiber content. However, they did not study antioxidant activity in stems of Golden barrel cactus. Therefore, this study studies the proximate composition, antioxidant activity of Golden barrel cactus stems extracts.

II. MATERIALS AND METHODS

2.1 Materials
Golden barrel cactus was collected at the age of 3 and 6 years in Uncle Chorn’s Cabin Garden, Pathumthani province, Thailand to be used as raw material. The plants were trimmed and cleaned, chopped and tray drying (Kluay Nam Tai Wong Op, Bangkok, Thailand) at 60°C for 12 hrs, after which they were grounded with an Ultra Centrifugal Mill Model ZM-1000 (Retsch, Haan, Germany), sieved by mesh size 0.2 mm and stored in vacuum package at -20°C until use.

2.2 Preparation of acetonitrile extract of Golden barrel cactus
Golden barrel cactus powder was extracted by the method described by Vermaak, Hamman and Viljoen (2010) with slight modifications. First 500 mg of Golden barrel cactus powder was dissolved in 15 mL of acetonitrile, the solution was stirred for 1 min and then sonicated in an ultrasonic bath (Elma Ultrasound, Germany) at 100% power at temperature of 25°C for 20 min, after that the solution was centrifuged (Thermo electron LED GmbH D-37520, Germany) at 3000×g for 10 min and then filtered using Whatman No.1 paper. Extraction was repeated two times. Supernatant was filtered, combined, and volume adjusted to be 50 mL. Solvent was evaporated by rotary evaporator (Buchi Rotavapor R-114, USA) and dried by nitrogen gas at room temperature. Samples were kept at -20°C until used.

2.3. Total phenolic compounds quantification
Total phenolic concentration was determined by the Folin Ciocalteu procedure described by Oonsivilai et al. (2007) and gallic acid (Sigma Aldrich Co.) was used as a standard. Aliquot of 0.02 mL gallic acid standard/sample/blank was mixed with 1.58 mL of deionized water. Folin-Ciocalteu reagent of 0.1 mL was added, and the tube was stirred and allowed to stand at room temperature for 5 mins. Then, 0.3 mL of Na2CO3 (20%/w/v) was added to mixture and stored in the absence of light for 2h at room temperature. Absorbance was measured at 765 nm using spectrophotometer (Biochrom Libra S22 S/N 97765, UK). Gallic acid (0, 50, 100, 250, 500 and 750 mg/L) was used for calibration of a standard curve. Results were expressed as mg gallic acid equivalents (GAE)/100g of dry weight of material. Triplicate measurements were taken and mean values calculated.

2.4 Antioxidant activity of crude extract of Golden barrel cactus
2.4.1 DPPH free radical scavenging activity
Scavenging activity of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method described by Oonsivilai, Ferruzzi and Ningsanond (2008). Briefly, 100 μM violet solution of 0.1 mM DPPH in methanol was prepared. Aliquots 0.1 mL of standard/sample/blank transferred into test tube. After addition of 1.90 mL DPPH solution, solution was mixed and then left to stand for 15 mins. Absorbance of solution at 515 nm was read. BHT and ascorbic acid in methanol solution were used as positive controls. IC50 value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from a calibration curve by linear regression.

2.4.2 Scavenging activity of ABTS radical cation
ABTS radical scavenging activity of extracts was determined according to Ksouri, Falleh, Megdiche, Trabelsi, Mhamdi, Chaieb et al. (2009). ABTS•+ cation radical was produced by a reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate (K2S2O8) solution, stored in the dark at room temperature for 12-16 hrs. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 2 mL, the reaction mixture comprised of 1,900 µL of ABTS•+ solution and 100 µL of the sample extracts at various concentrations and distilled water for control. Reaction mixture was homogenized and its absorbance was recorded at 734 nm at 6 min after mixing. Similarly, mixture of standard group reaction was obtained by mixing 1,900 µL of ABTS•+ solution with 100 µL of BHT or ascorbic acid for antiradical activity. ABTS scavenging ability was expressed as IC50 (mg/mL).

2.4.3 Ferric reducing antioxidant power assay
FRAP assay was carried out according to the procedure (Oonsivilai et al., 2007) with slight modifications. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v) respectively. FRAP reagent was prepared fresh daily and warmed at 37°C in a water bath prior to use. Fifty microliters of sample was added, along with 150 µL of deionized water to 1.5 mL of FRAP reagent. Reaction mixture absorbance was then recorded at 593 nm at 4 min. Standard curve was constructed using ferrous sulphate solution (100–1000 μM), and results were expressed as mM ferrous (II)/g dry weight of plant material. All measurements were taken in triplicate and mean values were calculated.

2.5 Statistical analysis
All of the samples were performed at least in triplicate. All statistical analysis was performed using SPSS (version 16.0, SPSS Inc., USA). Data is presented as mean ± standard deviation (SD). Means
were compared by independent-samples t-test. Differences were considered significantly at p < 0.05.

III. RESULTS

Antioxidant activity of crude extract of Golden barrel cactus

3.1. DPPH free radical scavenging activity
Antioxidant activity of crude extracts of Golden barrel cactus at age 3 and 6 years tested by DPPH radical scavenging activity reveal antioxidant potency based on IC50 values when compared with BHT and ascorbic acid as positive control. Antioxidant activity in each sample extract is presented in Table 3. We found that antioxidant activity of Golden barrel cactus extract extracted at age 3 years is higher than age 6 years at IC50 values 112.60 mg /ml and 191.90 mg /ml, respectively. IC50 values were 0.08 mg/ml for ascorbic acid. BHT showed 50% inhibition at 0.35 mg/ml.

3.2 Scavenging activity of ABTS radical cation
ABTS+ radical scavenging activity of Golden barrel cactus crude extract at age 3 and 6 years reveal antioxidant potency based on IC50 values when compared with BHT and ascorbic acid as positive control showed in table 4. The antioxidant potential is inversely proportional to IC50 value, low value of IC50 indicates higher scavenging activity. ABTS+ radical scavenging activity of Golden barrel cactus crude extract extracted at age 3 years was higher than age 6 years at IC50 values 44.62mg /ml and 81.84 mg /ml, respectively.

3.3 Ferric reducing antioxidant power assay (FRAP) Antioxidant activity by FRAP assay in each sample extract is presented in Table 5. We found that antioxidant activity of Golden barrel cactus crude extract extracted at age 3 years was higher than age 6 years at FRAP value 0.014mmol Fe2+/g dry weight and 0.01 mmol Fe2+/g dry weight, respectively.

IV. DISCUSSION

Antioxidant activity of crude extract of Golden barrel cactus

4.1 DPPH free radical scavenging activity
Antioxidant activity by DPPH assay of Golden barrel cactus of both ages were lower than two species dragon fruits, Hylocereus undatus (white dragon fruit) and Hylocereus polyrhizus (red dragon fruit) (0.30 and 0.40 mg/mL) reported by Ruzlan, Kamarudin, Idid, Idid, Mohamed Rehan and Koya (2008). Antioxidant activity of both ages was less than BHT and ascorbic acid as positive control.

Table 1 Antioxidant activities of Golden barrel cactus at age 3 and 6 years extracts on DPPH assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 of DPPH radical scavenging activity (mg /ml)</th>
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</thead>
<tbody>
<tr>
<td>Cactus at age 3 years</td>
<td>112.60±0.142</td>
</tr>
<tr>
<td>Cactus at age 6 years</td>
<td>191.90±0.52</td>
</tr>
<tr>
<td>BHT</td>
<td>0.35±0.04</td>
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<tr>
<td>Ascorbic acid</td>
<td>0.08±0.01</td>
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</tbody>
</table>

4.2 Scavenging activity of ABTS radical cation
The antioxidant activity by ABTS assay of both crude extract of Golden barrel cactus were lower antioxidant activity than medicinal plants (IC50 values were 5.79- 19.78 mg/ml) from previously reported by Al-Laith, Alkhuzai and Freije (2015). While the antioxidant activity of both ages were less than BHT and ascorbic acid as positive control.

Table 2 Antioxidant activities of Golden barrel cactus at age 3 and 6 years extracts on ABTS assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 of ABTS radical scavenging activity (mg /ml)</th>
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</thead>
<tbody>
<tr>
<td>Cactus at age 3 years</td>
<td>44.62±2.43</td>
</tr>
<tr>
<td>Cactus at age 6 years</td>
<td>81.84±0.42</td>
</tr>
<tr>
<td>BHT</td>
<td>0.09±0.003</td>
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<tr>
<td>Ascorbic acid</td>
<td>0.04±0.002</td>
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4.3 Ferric reducing antioxidant power assay (FRAP) Crude extract of Golden barrel cactus at age 3 and 6 years were tested for antioxidant activity by using the FRAP assay, which is a simple assay that gives fast, reproducible results (Benzie et al., 1996). In this assay, antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron. Results were expressed as mmol ferrous iron (Fe2+) equivalents per gram dry weight. Antioxidant activity of both crude extract of Golden barrel cactus were less than BHT and ascorbic acid (positive control), when compared with Chinese medicinal plants from a previous study of Wong et al. (2006). It was found that the antioxidant activity of both crude extract of Golden barrel cactus was less than Chinese medicinal plants (0.092 and 0.059 mmol Fe2+/g for boiling water and methanol extracts, respectively).

Table 3. Antioxidant activities of Golden barrel cactus at age 3 and 6 years extracts on FRAP assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>FRAP values of Ferric reducing antioxidant power assay (mmol Fe2+/ g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cactus at age 3 years</td>
<td>0.01±0.002a</td>
</tr>
<tr>
<td>Cactus at age 6 years</td>
<td>0.01±0.002a</td>
</tr>
<tr>
<td>BHT</td>
<td>2.5±0.32b</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>8.6±0.14d</td>
</tr>
</tbody>
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Note: Data are means ± SD; Data in the same column with different superscripts are significantly different (p<0.05).

CONCLUSION

In conclusion, it is found that the phytochemical profile of the crude extracts from the Golden barrel cactus at age 3 and 6 years presented lutein, total phenolics and antioxidant activity of the crude extract from 3-years-old Golden barrel cactus is...
significantly higher ($p < 0.05$) than the 6-years-old. Total phenolic, total chlorophylls, and lutein contents of the crude extract of 3-years-old golden barrel cactus is significantly higher ($p < 0.05$) than the 6 years old. For future research in development Golden barrel cactus as a dietary supplement, it is suggested that younger Golden barrel cactus should be used as better source of total phenolic.

ACKNOWLEDGEMENTS

This research was supported by the Research and Development Institute Funds, Suranaree University of Technology, Nakhon Ratchasima, and National Research Council Thailand.

REFERENCES


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