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Antioxidant and antibacterial activities of red (*Hylocereus polyrhizus*) and white (*Hylocereus undatus*) dragon fruits

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Abstract

Dragon fruit belongs to the genus *Hylocereus* of the Cactaceae family. There are two species that are commonly cultivated; *Hylocerues polyrhizus* and *Hylocereus undatus* that have the same red skin but different flesh colours, red and white respectively. Although from the same genus, the phytochemical contents and bioactivities of both fruits may not be the same. This study aims to compare the phytochemical contents, antioxidant and antibacterial activities of *H. polyrhizus* and *H. undatus* to help consumers better choose nutritional fruits and to explore potential natural preservatives. The fruit samples were extracted using 50% ethanol and later were subjected to phytochemical, antioxidant and antibacterial assays. The phytochemical contents were determined using Folin Ciolcalteu and aluminium chloride methods for total phenolic and total flavonoid respectively. The antioxidant activity was determined using diphenyl-picryl hydrazine (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Disk diffusion method was performed to evaluate antibacterial activities against two food-borne pathogens, *Escherichia coli* and *Staphylococcus aureus*. *H. polyrhizus* showed to contain significantly higher phenolic content (p<0.05), while *H. undatus* had significantly higher flavonoid content (p<0.05). Comparison of antioxidant activities in both fruit samples indicated higher activities were observed in *H. polyrhizus* and both fruit extracts showed inhibition zones against the tested bacteria with *H. polyrhizus* extract was able to inhibit at lower concentration. The results suggest that *H. polyrhizus* may have higher bioactivities compared to *H. undatus* due to the significantly higher phenolic content.

1. INTRODUCTION

Dragon fruits have been promoted as one of the natural resources for food additives due to their antioxidant properties (Nurliyana *et al.*, 2010). The main principle of antioxidant activity is the ability of a compound to neutralize cell damaging free radicals produced from the oxidation process. The dragon fruits contain high amount of polyphenols such as flavonoids and tannins (Nurliyana *et al.*, 2010), that are beneficial as preservatives to control the growth of bacteria and fungus (Mostafa *et al.*, 2018). The spoilage of food and cosmetic products usually can be prevented using chemical preservatives as antibacterial agents. However, chemical preservatives may cause adverse effect to the human health and has potential to cause bacterial resistance (Nurmahani *et al.*, 2012).

Preservatives can be classified into two classes. Class I is the natural preservatives such as salt, sugar, vinegar, spices and honey, while Class II is the chemical or synthetic preservatives such as sorbates, benzoates, nitrites, glycerides and glutamates. Class II preservatives need to be used according to the standard and regulations for food, cosmetic and pharmaceuticals respectively, in which one Class II preservatives can only be applied in one product. Most preservatives used today are artificial preservatives rather than natural preservatives with some of the synthetic preservatives have concerning health issues. Previous research has reported that benzoates, sorbates, nitrates, butylated hydroxytoluene (BHT), (beta hydroxy acid (BHA) and others can cause serious health

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problems such as allergy, hyperactivity, hypersensitivity, asthma and cancer (Anand and Sati, 2013).

Natural preservatives from plants, animals and minerals possess antioxidant and antimicrobial properties that can prolong the shelf life of food, cosmetic and pharmaceutical products (Anand and Sati, 2013). This research focused on the comparison of antioxidant and antibacterial activities of *H. polyrhizhus* and *H. undatus* extracts. The findings can benefit manufacturers to produce a cost-effective natural preservative and allow consumers to have more choices when purchasing natural antioxidant.

2. MATERIALS AND METHODS

2.1. Sample preparation

H. polyrhizus and *H. undatus* fruits were purchased from local supermarket in Kota Bharu, Kelantan, Malaysia. The fruits were washed and wiped dry. Later, the fruits were cut into smaller pieces. 100 g of fresh weight (FW) fruits were weighed and crushed into paste using a PANASONIC blender (MX-GM1011H). The homogenized samples were transferred into a 100 mL volumetric flask and 50% ethanol was added to obtain 100 mL of sample solution. The solution was mixed manually for 10 min before filtration. In situation where the filtrate appeared to be very cloudy, the filtrate was centrifuged at 1500 × g at 4 ℃ for five minutes to obtain a clear supernatant liquid before storage at -20 ℃. All tests were performed within a week (Lim *et al.*, 2007).

2.2. Determination of total phenolic content (TPC)

Total phenolic content (TPC) was determined using Folin Ciocalteu reagent (McDonald *et al.*, 2001). A volume of 0.5 mL of fruit extract or gallic acid (standard phenolic compound) was mixed with 5 mL Folin Ciocalteu reagent diluted with distilled water and aqueous Na_2CO_3 (4 mL, 1 M). The mixtures were allowed to stand for 15 minutes before absorbance reading at 765 nm using UV-Vis spectrophotometer (Genesys 20). Gallic acid standard (50-160 µg/mL) was prepared in 50 % ethanol and was used to form a calibration curve. TPC were expressed as gallic acid equivalent per 100 g fresh weight (GAE /100 g FW).

2.3. Determination of total flavonoids content (TFC)

Aluminum chloride colorimetric method was used to determine flavonoid contents (Chang *et al.*, 2002). Extracts (0.5 mL) were mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was left at room temperature for 30 min and the absorbance was measured at 415 nm with a UV/Visible spectrophotometer (Genesys 20). Experiment was conducted in triplicates. Quercetin (50-125 µg/mL) was used to obtain a calibration curve. TFC was expressed as quercetin equivalent per 100 g fresh weight (QE/100 g FW).

2.4. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted according to previous method with some modifications (Blois, 1958). 240 µL of various concentrations of extract were added into 2160 µL DPPH solution (50 µg/mL). After 30 min incubation, absorbance was determined at wavelength 515 nm using UV-Vis spectrophotometer (Genesys 20). Distilled water was used as blank, DPPH solution (50 µg/mL) as control and ascorbic acid (AA) as standard. Experiment was conducted in triplicates. The DPPH scavenging activity was expressed as ascorbic acid equivalent (AAE) in /100 g FW.

2.5. 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt (7.6 mM) and potassium persulfate (2.5 mM) were prepared in distilled water and left in dark room for 12 hours. The two solutions were mixed and incubated for 30 minutes. The mixture was kept in the refrigerator (4 ℃) for 24 hours. 150 µL extract were added into 2850 µL ABTS solution and incubated for 15 min. The absorbance was read at wavelength 734 nm using UV-Vis spectrophotometer (Genesys 20). Distilled water was used as blank, ascorbic acid as standard and ABTS solution as a control (Li *et al.*, 2011. Experiment was performed in triplicates. The ABTS scavenging activity was expressed as ascorbic acid equivalent (AAE) in /100 g FW.

2.6. Antibacterial activity

2.6.1. Bacterial strains and growth conditions

Two bacterial strains used in this study were *Staphylococcus aureus* and *Escherichia coli*. Bacterial strains were cultivated at 37 °C and maintained on a nutrient agar slant at 4 °C. The working cultures were grown on nutrient agar at 37 °C for 24 hours before each experiment.

2.6.2. Disc diffusion assay

The disc diffusion assay was performed to observe the inhibitory spectrum of the extracts against two pathogenic bacteria. Single isolated colonies were picked from an overnight plate culture and inoculated into saline suspension at room temperature. The turbidity was adjusted to 0.5 McFarland standards (108 CFU/mL) and was streaked onto Mueller-Hinton agar plates using sterile cotton swabs. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with 10 µL of the extracts. A 30 mg/mL chloramphenicol disc was used as positive control. An extraction solvent (distilled water) was used as negative control. The plates were incubated at 37 °C for 24

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hours. The tests were conducted in triplicates and repeated for three times (Al-Zoreky, 2009). The results were reported as inhibition zone by using the formula below;

$$\text{Inhibition zone (mm)} = [A - B] \quad (1)$$

where; A is the diameter of clear zone surrounding the paper disc including the paper disc and B is the diameter of paper disc.

2.7. Statistical analysis

The GraphPad Prism version 7.0 was used to analyse all the data. All the samples were tests triplicates, and the results were expressed as means ± standard deviation from the triplicates values and comparison was performed by t-test. Data was considered statistically significant when $p \leq 0.05$ level.

3. RESULTS AND DISCUSSION

3.1. Total phenolic contents (TPC)

Figure 1 shows a bar graph from t-test analysis of gallic acid equivalent to determine if there is a significant difference between the means of total phenolic content between *H. polyrhizus and H. undatus* extracts. The total phenolic content between the *H. polyrhizus* extract and *H. undatus* extract showed significant difference at the p <0.0001. The graph shows that the total phenolic content of *H. polyrhizus* was significantly higher than *H. undatus* with 20.50 ± 0.016 mg GAE/100 g FW and 11.47 ± 0.007 mg GAE/100 g FW respectively.

This result was supported by previous research that *H. polyrhizus* extract had higher total phenolic content than *H. undatus*, where the *H. polyrhizus* contain 16.70 mg GAE/g of sample and *H. undatus* contain 11.02 mg gallic equivalent/g of sample (Mohd Adzim Khalili *et al.*, 2012). The slightly difference of the total phenolic content between this present study and the previous study was due to the different solvent used for the sample's extraction, where 50 % ethanol was used as compared to pure methanol, and these solvents have different polarity index. Methanol is more polar than ethanol with a polarity index of 6.6, while ethanol has a polarity index of 5.2. The total phenolic content has been shown to be higher in extraction using polar solvent and lower in non-polar solvent (Abarca-Vargas *et al.*, 2016). Even though methanol is more polar than ethanol, the presence of water (dH_2O) with a polarity index of 9.0 in 50 % ethanol extract may contribute to the higher total phenolic observed in this present study.

The phenolic compounds are abundantly found in these plants. The phenolic acids such as gallic acid, and polyphenols such as flavonoids are widely being exploited because of their biological activities potential such as anticarcinogenicity, antimutagenicity and antiaging besides of antioxidant activity (Kosem *et al.*, 2007). Previous study reported that antioxidant activity is highly correlated with the total phenolic content (Bertoncelj *et al.*, 2007). Folin-Ciocalteu (FC) assay works based on the oxidation and reduction reactions observable from colour change from yellow to dark blue colour in the presence of phenolic compounds (Verzelloni *et al.*, 2007; Wojdyło *et al.*, 2007). *H. polyrhizus* extracts developed much darker colour than *H. undatus* in the FC assay reaction mixture suggesting greater amount of phenolics presence in the extract. The result may be explained by the presence of the betalain in *H. polyrhizus* that gives the red purple colour to the species as compared to *H. undatus* extract (Wu *et al.*, 2006; Ramli *et al.*, 2014).

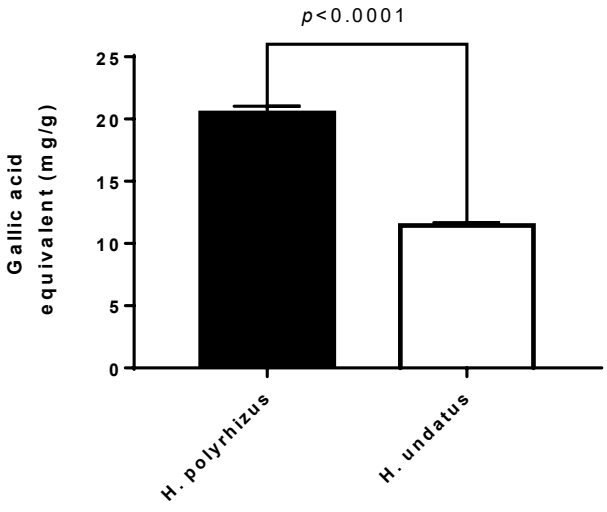

Figure 1: Comparison of total phenolic content between *H. polyrhizus* and *H. undatus* fruits. $p \leq 0.05$ is considered as significant.

Table 1: Total phenolic contents of *H. polyrhizus* and *H. undatus*. Values are mean ± standard deviations.

Dragon Fruit	Total Phenolic Content (mg Gallic Acid Equivalent /100g FW)
H. polyrhizus	20.50 ± 0.02
H. undatus	11.47 ± 0.01

3.2 Total Flavonoid Contents (TFC)

Figure 2 shows the comparison of the mean of TFC between *H. polyrhizus* and *H. undatus* extracts, where significantly higher TFC was observed in *H. undatus* (4.18 ± 0.009 mg of QE/100 g FW) than *H. polyrhizus* (2.96 ± 0.006 mg of QE/100 g FW). Flavonoid has ortho di-OH at benzene ring that will form a complex in the aluminium (III) chloride. The di-ortho structure in the B-ring of a flavonoid contributes in electron transfer and metal chelating properties that play a role in antioxidant activities (Amic *et al.*, 2007; Heim *et al.*, 2002). Thus, the result

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suggests that *H. undatus* may contain flavonoids with the ortho di-OH as compared to *H. polyrhizus* resulting in higher TFC in aluminium (III) chloride assay. The total flavonoid content of *H. undatus* was highly influenced from the flesh of the dragon fruit because the flavonoid compound mostly found in the flesh of the dragon fruit (Paixão *et al.*, 2007). Flavonoid is a subset of phenolic compounds that contribute to the antioxidant activity (Ramli *et al.*, 2014).

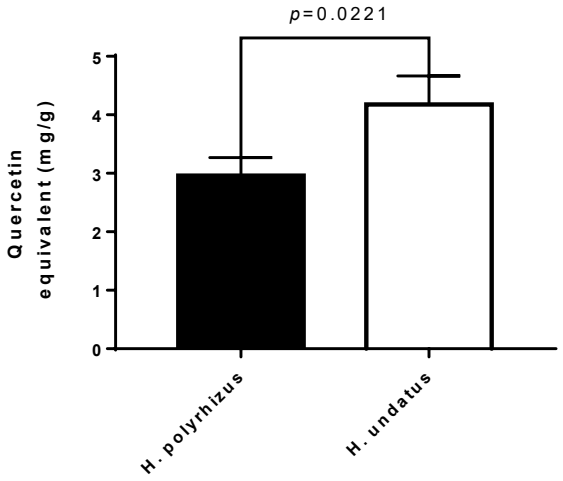

Figure 2: Comparison of total flavonoid content between *H. polyrhizus* and *H. undatus*. $p \leq 0.05$ is considered as significant.

Table 2: Total flavonoid contents of *H. polyrhizus* and *H. undatus.* Values are mean ± standard deviations.

Dragon Fruit	Total Flavonoid Content (mg Quercetin Acid Equivalent/100g FW)
H. polyrhizus	2.96 ± 0.01
H. undatus	4.18 ± 0.01

3.3 Antioxidant activities

In the present study, the antioxidant activities of *H polyrhizus* and *H. undatus* were measured using DPPH and ABTS assays, where the results were expressed as ascorbic acid equivalent (AAE) in mg /100 g of FW. *H. polyrhizus* was found to have similar antioxidant activity with *H. undatus* with both results from DPPH and ABTS assays showed no significant differences between *H. polyrhizus* and *H. undatus* activities, p>0.05 (Table 3). DPPH assay is a stable free radical solution that reacts with antioxidant compounds by transferring hydrogen to the DPPH radical. The assay measures colour changes from yellow to purple, which is measurable at 515-517 nm (Li *et al.*, 2011). Increasing concentration of antioxidants in a sample causes the absorbance readings to decrease because all the DPPH radicals will accept hydrogen from antioxidants. However, the result showed *H. polyrhizus* had slightly higher ascorbic acid content with 5.41 ± 1.40 mg AAE/100 g FW, and *H. undatus* had 5.08 ± 1.50 mg AAE/100 g FW. This result was supported by the previous study where the *H. polyrhizus* was found to have higher ascorbic acid content with 18.94 ± 2.51 mg AAE/100 g of sample, while the ascorbic acid content for *H. undatus* was 11.56 ± 1.25 mg AAE/100 g of sample (Sim Choo and Khing Yong, 2011). It is suggested that the difference in ascorbic content is mostly influence by the environmental growth variation and the maturity stage of the dragon fruits. Similar result was observed in ABTS assay, where the ascorbic acid content of *H. polyrhizus* was 1.98 ± 0.60 mg AAE/100 g FW and the ascorbic acid content for *H. undatus* was 1.53 ± 0.33 mg AAE/100 g FW. Again, *H. polyrhizus* showed a slightly higher ascorbic acid content than *H. undatus*. The total antioxidant capacities by both DPPH and ABTS assays have previously been shown to be correlated with the total phenolic content (Chun *et al.*, 2003).

Table 3: Ascorbic acid contents of *H. polyrhizus* and *H. undatus.* Values are mean ± standard deviations.

Dragon Fruit	Ascorbic Acid Content (mg Ascorbic Acid Equivalent/100g FW)	
	DPPH Assay	ABTS Assay
H. polyrhizus	5.41 ± 1.40	1.98 ± 0.60
H. undatus	5.08 ± 1.50	1.53 ± 0.33

3.4 Antimicrobial activity

In the present study, two food borne pathogens were tested for their sensitivity to *H. polyrhizus* and *H. undatus* extracts using disc diffusion method. Table 4 shows the presence of inhibition zone exhibited by each extract at effective concentration towards the selected bacteria. Both Gram-positive and Gram-negative bacteria were used in this experiment. Gram-positive bacteria was represented by *Staphylococcus aureus* and the Gram-negative bacteria was represented by *Escherichia coli*. The positive control used in this experiment was chloramphenicol and the negative control was distilled water (DW). For this experiment, four different concentrations of *H. polyrhizus* and *H. undatus* (0.5 g FW/ml, 1 g FW/ml, 2 g FW/ml and 10 g FW/ml) were tested on the *E. coli* and *S. aureus*.

Table 4: Effect of fruit extracts on bacteria

Fruit Extracts (g FW/ml)	Gram-negative (*E. coli*)				Gram-positive (*S. aureus*)			
	0.5	1	2	10	0.5	1	2	10
H. polyrhizus	+	-	-	-	-	-	-	+
H. undatus	+	-	-	-	-	-	-	+

\+ = Presence of inhibition zone
\- = Absence of inhibition zone

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For *E. coli*, there were inhibition zones at 0.5 g FW/ml which were 2.00 ± 3.46 mm and 1.33 ± 2.31 mm for both *H. polyrhizus and H. undatus* extract respectively. The antibacterial activity observed maybe due to a mixture of compound presence in the extract. However, some may act as antagonist of antibacterial activities at certain concentrations (Fidrianny *et al.*, 2017). Therefore, this explains the observed results.

However, both *H. polyrhizus* and *H. undatus* extracts inhibit *S. aureus* at 10 g FW/ml and no inhibition zones observed at lower concentrations extract. At 10 g FW/ml, the diameter of inhibition zones were 3.33 ± 2.31 mm for *H. polyrhizus* and 3.00 ± 1.00 mm for *H. undatus* extracts. This result is coherent with previous study in which antibacterial activity on *E. coli* and *S. aureus* was observed at 10 g FW/ml. It has been suggested that, the solvent used in the extraction method also play a role in the antibacterial activities exhibited because it will affect the chemical profile of the extract (Fidrianny *et al.*, 2017). Gram-positive bacteria are supposed to be more sensitive as compared to Gram-negative because of the differences in their cell wall structures (Ahmad and Beg, 2001). The effect of the different concentrations of extracts used during on the pathogens was clearly observed in the present study. The differences in the antibacterial activity of dragon fruits extracts could partially due to variations in phenolic content of extracts, strain sensitivity and antibacterial procedures adopted for the tests (Al-Zoreky, 2009). In the present study, *H. polyrhizus* and *H. undatus* was shown to successfully inhibit the growth of Gram-positive bacteria at concentration 10 g FW/ml and Gram-negative bacteria at concentration 0.5g FW/ml.

4. CONCLUSION

The total antioxidant activity of *H. polyrhizus* was found to be similar with *H. undatus* in both DPPH and ABTS assays. This present study also found that the total phenolic content in *H. polyrhizus* was higher than *H. undatus*. Meanwhile, *H. undatus* was found to have higher total flavonoid content than *H. polyrhizus* extract. So, these compounds might be the major contributor to the total antioxidant activity of the *H. polyrhizus* and *H. undatus* extracts. The result also suggests that *H. polyrhizus* and *H. undatus* have antibacterial activities depending on the concentration used as observed from the zone of inhibition for the two tested bacteria. For future study, solvent with different polarity can be used for extraction to study their effect on the antioxidant and antibacterial activities.

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