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Determination and Evaluation of Antioxidative Activity in Red Dragon Fruit (*Hylocereus undatus*) and Green Kiwi Fruit (*Actinidia deliciosa*)

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Abstract: Problem statement: Dragon fruit or pitahaya (*Hylocereus undatus*), is believed to be a healthy source of vitamins, fiber and antioxidants, especially the red-fleshed varieties which contain lycopene. **Approach:**Compared to green kiwi fruit (*Actinidia deliciosa*), which already known contain high antioxidant activity. **Results:**The antioxidant capacity of *Hylocereus undatus* (*H. undatus*) and *Actinidia deliciosa* (*A. deliciosa*) in three different solvent extraction; ethanol, methanol and aqueous, was estimated by DPPH free radical scavenging assay. The inhibition of free radical by *A. deliciosa* is almost 90% compared with *H. undatus* which only 60-10% in different solvents. Additionally, their total phenolic contents were analyzed by folin-ciocalteau method. The result showed that *A. deliciosa* (533.70 mg L^{-1} in ethanol, 460.87 mg L^{-1} in methanol and 420.652 mg L^{-1} in distilled water) seemed to be better sources of antioxidant compounds then *H. undatus* (179.35 mg L^{-1} in ethanol, 160.87 mg L^{-1} in methanol and 157.61 in distilled water). **Conclusion:** When compared between the three different solvent, extract in ethanol shown the most highly antioxidant content followed by methanol and water. The experiment showed the potential of dragon fruit and kiwi extracts high rich in antioxidant which can scavenge free radical in human body. Further study on isolation of individual antioxidant in both extracts can be providing for commercialize the extracts in jus form.

Key words: Phenolic contents, *Hylocereus undatus*, *Actinidia deliciosa*, distilled water, radical scavenging, gallic acid, Acid Equivalent Antioxidant Capacity (AEAC), dragon fruit, different solvent, gallic acid equivalent, Total Antioxidant Capacity (TAC), antioxidative activity, Gallic Acid Equivalent (GAE), DPPH, Lipid-Soluble Antioxidants (LSAs)

INTRODUCTION

Dragon fruit (*Hylocereus undatus*), also known locally as pitahaya fruit, is grown commercially in many farm in Malaysia. The plant climb on everything from trees to brick walls using aerial roots and can grow to about 20 ft high with ribbed stems, green and growing spinier with age. The fruits vary in size, color and flavor depending on the variety. There are two identified type of fruit, the red dragon fruit and white dragon fruit. It is rich in ascorbic acid (vitamin C), at levels far higher than most imported and local fruits. The fruit, especially the red-fleshed varieties contain fair amount of lycopene. Some vitamin B such as thiamin (B1), riboflavin (B2), niacin and B3 are also found in the fruit. In addition, it also contains a fair amount of carotene, calcium and zinc (Lim and Khoo, 1990). Researchers have concluded that the red dragon fruit varieties are high in antioxidant activity, as compared to the white dragon fruit varieties (Charles, 2006).

The fruit is believed to be a healthy source of vitamins, fiber and antioxidants, especially the red-fleshed varieties which contain lycopene. Researchers have concluded that the red dragon fruit varieties are high in antioxidant activity, as compared to the white dragon fruit varieties (Charles, 2006). The dragon fruit flowers bloom once every 15 days, around the 1st and 15th days of the lunar calendar. These huge, beautiful and fragrant flowers with diameter up to 30 cm only bloom for one precious night, thus earning for them the name of the "night-blooming cactus". The vitamins found in dragon fruits are vitamins B1, B2, B3 and C. Other nutrients include carotene, calcium and zinc (Charles, 2006).

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Dragon fruit, as in many other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Vaiserman, 2008; Grodstein *et al*., 2003) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. These include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non radicals such as hydrogen peroxide and hypochlorous acid. They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and singlet oxygen. Among the most abundant antioxidants in fruits are polyphenols and ascorbic acid. The polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms.

Actinidia deliciosa also known as green kiwi fruit is a member of *Actinidiaceae* family. It is a member of the *Magnoliophyta* order of climbing woody vines. The genus *Actinidia* contains species with edible fruits. One of these, *Actinidia deliciosa*, widely known as kiwi fruit, has become a major crop worldwide. *Actinidia* species were brought from China to Europe during the second half of the 19th century and the early 20th century. Which are taken to New Zealand at the beginning of the 20th century, where selection revealed commercially interesting (Margarida and Lena, 2005). The first commercial plantings of kiwi fruit were made in New Zealand in the early 1930s. This fruit is not yet planted in Malaysia commercially compared with dragon fruit and it was imported fruit from Australia. The objective of the study is to determine the total antioxidant content and their oxidative activities in locally grown dragon fruit. For comparison, similar analyses were carried out on kiwi fruit which are imported fruit.

MATERIALS AND METHODS

Sample preparation: Distilled water, acetone (Merck), ethanol (Merck), methanol(Merck), gallic acid as the standard, follin-ciocalteu reagent, Na_2CO_3, DPPH (α,α-diphenyl-β-picrylhydrazyl) solution were used for free radical scavenging assay. The samples were extracted using high speed blender and centrifuge machine. Then the assays were carried out using Genesys20 ThermoSpectronic, spectrophotometer.

Red pitahaya fruit (*Hylocereus undatus*) was obtain from local farmer at Mantin, Negeri Sembilan and the green kiwi fruit (*Actinidia deliciosa*) which is imported fruit from Australia was obtain from local hypermarket (TESCO, Shah Alam).

Table 1: Dilution for gallic acid standards

Final Concentration (ppm)	Volume stock (μL)	Volume of dH_2O (μL)
0	-	1000
50	20	980
100	40	960
250	100	900
300	120	880
375	150	850
500	200	800

Table 2: Sample solution dilution

Dilution factor	Volume of sample (μL)	Volume of solvent (μL)
5	100	800
10	200	900

Extraction of soluble free phenolics in the samples: After washing and cutting, equal amounts of each fruit were pooled, mixed and homogenized under nitrogen in a high speed blender. A precisely weighed amount of the homogenized sample (~1 g) was extracted with 4 mL of water under agitation for 15 min at room temperature, centrifuged at 1000 rpm for 10 min and the supernatant collected. The extraction was repeated with 2 mL of water and the two supernatants were combined. The pulp residue was re-extracted by the addition of 4 mL of acetone under agitation for 15 min at room temperature, centrifuged at 1000 rpm for 10 min and the supernatant collected. The extraction was repeated with 2 mL of acetone and the two supernatants were combined. All fruit extracts were adequately diluted in the distilled water, ethanol and methanol at 5X and 10X dilution and stored in -20°C before analyzed in duplicate for their antioxidant capacity.

Standard preparation: A stock solution of gallic acid was prepared by dissolving 2.5 mg of gallic acid in 1.0 mL of distilled water. The test tubes were labeled according to the concentration of the solution contained as shown in Table 1. Standards of varying concentrations were prepared by dilution of the stock solution based on the Table 2.

The sample extracts of *Hylocereus undatus* and *Actinidia deliciosa* were also diluted according to the Table 2, making the final volume to be 100 μL using different solvent such as distilled water, ethanol and methanol.

Determination of Total Phenolic Content (TPC): About100 μL of sample extract were pipetted into a clean test tube and 0.2 mL of follin-ciocalteau reagent, 2.0 mL of distilled water and 1.0 mL of Na_2CO_3 added. The mixture was vortexed to ensure thorough mixing and then left to incubate for 2 h at room temperature. At the end of the incubation period,

the sample solutions were measured for absorbance at wavelength of 765 nm using Genesys20 ThermoSpectronic, spectrophotometer (Ng and Choo, 2010). A calibration curve with gallic acid standard solutions was plotted. The phenolic acid content of sample extracts was expressed as Gallic Acid Equivalent (GAE).

Free radical scavenging assay: The antioxidant activity of all extracts was evaluated with DPPH scavenging assay. This method is rapid, sensitive, reproducible and require simple conventional laboratory equipment. They were selected for their different characteristics. In fact, the DPPH test is particularly suitable for the evaluation of antioxidant activity of crude extracts.

A stock DPPH solution was prepared by weighing out 0.000125 mg of DPPH and dissolving it in 500 mL of 100% Methanol. This was also stored in an aluminum foil-wrapped glass bottle. The DPPH solutions were stored in the refrigerator. Gallic acid standard was prepared at the concentration of 100 mg L^{-1}. The *Hylocereus undatus* in distilled water sample solution was prepared at concentration of 100 ppm by pipeting 634.48 µL of sample into an eppendorf tube and diluting it with 365.52 µL of distilled water, ethanol sample solution was prepared at concentration of 100 ppm by pipeting 557.58 µL of sample into an eppendorf tube and diluting it with 442.42 µL of ethanol and methanol sample solution was prepared at concentration of 100 ppm by pipeting 621.62 µL of sample into an eppendorf tube and diluting it with 378.38 µL of methanol. The *Actinidia deliciosa* in distilled water sample solution was prepared at concentration of 100 ppm by pipeting 237.73 µL of sample into an eppendorf tube and diluting it with 762.27 µL of distilled water, ethanol was prepared at 100 ppm by pipetting 187.37 µL of sample into an eppendorf tube and diluting it with 812.63 µL of ethanol and methanol sample solution was prepared at concentration of 100 ppm by pipeting 216.98 µL of sample into an eppendorf tube and diluting it with 783.02 µL of methanol. Then the sample was centrifuged at 1000 rpm for 10 min and the supernatant collected to be use for free radical scavenging assay.

After setting up the spectrophotometer program to autorate assay, the sample cell cuvette was filled with 900 µL of DPPH, the autorate was initiated and after the first reading was recorded and 100 µL test solution (sample at 100 ppm) was injected into the cuvette. The mixture was quickly pipetted to facilitate the reaction. Absorbance at 515 nm (Ng and Choo, 2010) was recorded at a 15 sec interval for 2 min. The procedure was repeated using other tests sample.

Calculation and analysis: The total phenolic contents were expressed in gallic acid equivalents (mg per 100 gram fresh fruit). The gallic acid standard line has the equation y = 0.0023x (R^2 = 0.955), where y is absorbance at 765 nm and x is concentration of gallic acid in mg L^{-1}.

According to Adesegun *et al*. (2007), the total content of phenolic compounds in the extract in Gallic Acid Equivalents (GAE) was calculated by the following formula:

$$T = \frac{C : V}{M}$$

Where:

T = Total content of phenolic compounds, milligram per gram fruit extract, in GAE
C = The concentration of gallic acid established from the calibration curve, milligram per milliliter
V = The volume of extract, milliliter
M = The weight of fruit extract, gram

However according to Lim *et al*. (2006) the free radical scavenging activity of the fruit extracts was measured by the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of the extract. The antioxidant activity was expressed as:

$$\% \text{ disappearance} = [(\text{A control-A sample})/\text{A control}]\times 100\%$$

where, A is absorbance. The 'A control' value is referred to as that of the "control", that is, in the absence of any sample, such as may be used to confirm the stability of the measuring system. It is also presumed that the total concentration of DPPH is kept constant in the measurement sequence. 'A sample' is the value for added sample concentration. This value of 'A sample' should be that in the cuvette (or other mixing vessel) in the absence of any DPPH and should take into account the dilution of the original sample solution by the added DPPH solution.

RESULTS

Total phenolic contents: Compared between the two samples extract, *Actinidia deliciosa* have high amount of antioxidant than *Hylocereus undatus*. The extract in ethanol solvent showed the highest volume of antioxidant compared to the extract in methanol and distilled water, respectively as shown in Table 3.

Small amounts of phenolic were detected in *Hylocereus undatus* and extract in ethanol, 179.348 ± 0.02 mg L^{-1} shown the highest phenolic content extract compared to extract in methanol, 160.870 ± 0.03 mg L^{-1} and distilled water, 157.609 ± 0.25 mg L^{-1} as shown in Fig. 1.

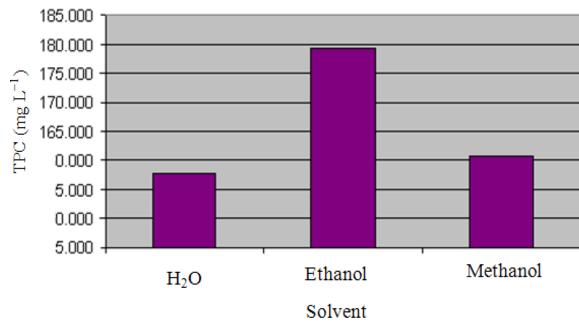

Fig. 1: Total phenolic content of *Hylocereus undatus* in different solvents of extraction

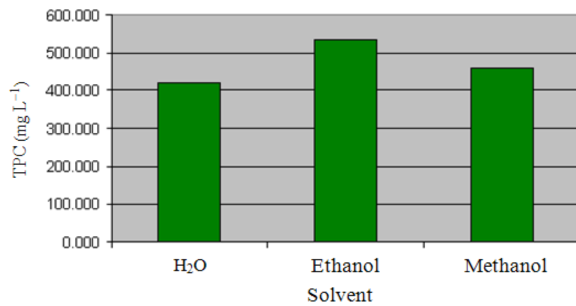

Fig. 2: Total phenolic content of *Actinida deliciosa* in different solvents

Table 3: Total antioxidant in *hylocereus undatus* and *actinidia deliciosa* in different solvent

Species	Type of solvent	Total antioxidant (mg L^{-1})
Hylocereus undatus	Distilled water	157.609±0.25
	Ethanol	179.348±0.02
	Methanol	160.870±0.03
Actinidia deliciosa	Distilled water	420.652±0.02
	Ethanol	533.696±0.03
	Methanol	460.870±0.02

In *Actinidia deliciosa* extracts shown that the phenolic compound are higher than in the *Hylocereus undatus* and extraction in ethanol, 533.696 ± 0.03 mg L^{-1} shown the highest phenolic content extract compared to extraction in methanol, 460.870 ± 0.02 mg L^{-1} and distilled water, 420.652 ± 0.02 mg L^{-1} (Fig. 2).

Free radical scavenging activities: The assay is base on the reduction of 2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH), a stable free radical. As the electron of a radical pairs off with hydrogen donation from the free radical scavenging antioxidant, the absorption strength will be decreased. This resulted in decolorization that is stoichiometric within the number of electron captured:

% DPPH = [(control abs.-extract abs.)/control abs.]×100

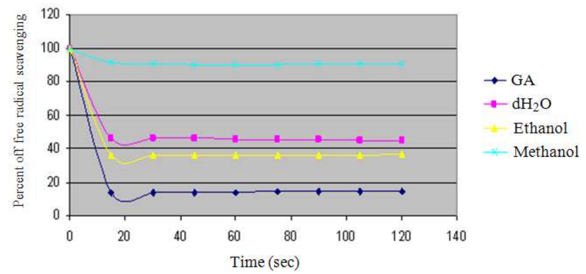

Fig. 3: Free radical scavenging of the *Hylocereus undatus*

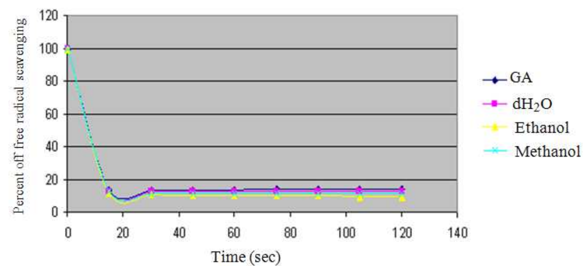

Fig. 4: Free radical scavenging of the *Actinida deliciosa*

Based on the Fig. 3 the free radical scavenging activity in *Hylocereus undatus* shown that the extract in ethanol have the highest scavenging activity compared to the extract in distilled water and methanol. GA is gallic acid (as a control), dH_2O is extract in distilled water, ethanol is extract in ethanol and methanol is extract in methanol. After two minutes of reaction between DPPH free radical and *Hylocereus undatus* in three different solvent, there are only 63.44% of DPPH remaining in the sample with ethanol solvent and 55.04% in distilled water. However, the *Hylocereus undatus* in methanol solvent shown very low reaction compared to others sample use, which there are still 8.82% of DPPH remain in the cuvette. Based on the graph (Fig. 4) the free radical scavenging activity in *Actinida deliciosa* shown that the extract in ethanol have the highest scavenging activity compared to the extract in methanol and distilled water. G.A is gallic acid (as a control), dH_2O is extract in distilled water, ethanol is extract in ethanol and methanol is extract in methanol. After two minutes of reaction between DPPH free radical and *Actinidia deliciosa* in three different solvent, there are only 90.34% of DPPH remain in the sample with ethanol solvent, followed by 87.39% in distilled water and 88.65% in methanol solvent.

DISCUSSION

The approach of using three type of solvent for both fruits extraction, generally used in Total Antioxidant Capacity (TAC), may estimate TAC values because antioxidant compounds at the extremities of the lipophilic or hydrophilic scale are completely extracted. In this study, fruit extracts obtained from two species with three different solvents were analyzed separately and their total phenolic content reported in the Table 3.

The approach of summing values of lipophilic and hydrophilic extracts permits the inclusion of the different contributors to the TAC of the fruit. However, it cannot be excluded that there may be a synergistic interaction between water and lipid-soluble antioxidants that is not evaluated by simply summing the components.

As a primer alcohol, methanol and ethanol imposes polar region, -OH group and nonpolar hydrocarbon chain (Arani and Valery, 2009). The present of polar and nonpolar region in ethanol explain the high total phenolic compared to the others solvent. Ethanol is a good extraction solvent for polar and nonpolar which it can bind with the hydrophilic and hydrophobic extract in the sample. However, as the carbon chain increases across the alcohol homologous series, the hydrophobic property of the chain becomes dominant and rendering higher alcohols insoluble in water (Arani and Valery, 2009; Mohd *et al*., 2010) such as lipophilic antioxidants Diphenylamine (DPA), tocopherol and Lipid-Soluble Antioxidants (LSAs) which include at least tocopherol and carotenoids. Compared with extract in distilled water only, the total antioxidant compounds were not extracted completely. This is due to the solvent only extract the hydrophilic compound such as quercetin, gallic acids, free and bound cuticular phenolics (Claudina *et al*., 2004). Phenolics, including simple phenols (mostly phenolic acids), flavonoids and anthocyanins, are hydrophilic compounds with antioxidant activity *in vitro* (Ganiyu and Joao, 2007). While in the methanol solvent, the short chain structure of methanol when compared to the ethanol explains the lower antioxidant extraction in methanol solvent.

In case of total phenolic content analysis, it was found that *Actinidia deliciosa* contained the highest amount of phenolic compounds, especially in ethanol solvent followed by distilled water and methanol. *Hylocereus undatus* contain small amount of phenolic compounds, from ethanol solvent followed by methanol and distilled water respectively when compared to *Actinidia deliciosa*. The most common antioxidants present in fruit are vitamins C and E, carotenoids, flavonoids and thiol (SH) compounds. There were several reports that the contribution of phenolic compounds to antioxidant activity was much greater than those of vitamin C and carotenoids (Kanjana *et al*., 2005).

Phenolics in fruits are present in both free and bound forms (Ganiyu and Joao, 2007). Bound phenolics, mainly in the form of B-glycoside, may survive human stomach and small intestine digestion and reach the colon intact, where they are released and exert bioactivity (Diane and Jeffrey, 2007). However, most of the previous investigations determined primarily free phenolics on the basis of the solvent-soluble extraction. The phenolic content in both fruit are well extracted in ethanol solvent rather than in the methanol and distilled water. Phytochemicals, especially plant phenolics constitute a major group of compounds that act as primary antioxidants (Grodstein *et al*., 2003). They can react with active oxygen radicals, such as hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals and inhibit lipid oxidation at an early stage (John *et al*., 2002). They also can inhibit yclooxygenase and lipoxygenase of platelets and macrophages, thus reducing thrombotic tendencies in vivo (Adesegun *et al*., 2007).

Even though the total phenolic content in methanol is higher than in the distilled water, in the free radical scavenging assay, it shown that the antioxidant activity of *Hylocereus undatus* in distilled water is higher than in methanol. The possible reasons may be able to account for this: First, it has been reported that reaction of DPPH with certain phenols such as eugenol and its derivatives is reversible, resulting in low readings for antioxidant activity (% disappearance). The second possible reason could be due to the slow rate of the reaction between DPPH and the substrate molecules. The third possible explanation (for the relatively low reducing power) could be that certain phenols in the langsat extract have a higher redox potential than that of other fruit extracts. To clarify this anomaly further work is necessary (Lim *et al*., 2007).

Regarding the solvent used, the method seems to work equally well with methanol or ethanol, neither of which seems to interfere with the reaction. The use of other solvent systems, such as almost neat extracts in water or acetone, seems to give low values for the extent of reduction.

From the study, the *Actinidia deliciosa* shown the highest free radical activity in all type of solvent tested. The inhibition of free radical is almost 90% compared with *Hylocereus undatus* which only 60±10% in different solvents. It shown that *Actinidia deliciosa* have more antoxidant capacity than *Hylocereus undatus*.

CONCLUSION

This study shows that although the analyzed fruits contained phenolic compounds, their contents are markedly different between *Hylocereus undatus* and *Actinidia deliciosa*, the two types of fruits and three different solvent extractions. All solvent extractions represent a potential source of natural antioxidants, but the ethanol solvent extraction showed a better performance compared to methanol and distilled water. *Actinidia deliciosa* can be considered a rich source of dietary antioxidants and its several antioxidant compounds give its products (juice and pulp) characteristics that favor preservation without the need for synthetic antioxidants. The phenolic content of *Hylocereus undatus* was 179.35 mg L^{-1} in ethanol solvent, 160.87 mg L^{-1} in methanol solvent and 157.61 mg L^{-1} in distilled water in term of GAE. The phenolic content of *Actinidia deliciosa* extract was 533.70 mg L^{-1} in ethanol solvent, 460.87 mg L^{-1} in methanol solvent and 420.65 mg L^{-1} in distilled water terms of GAE.

Hylocereus undatus extract demonstrated low antioxidant activity, free radical scavenging when compared with *Actinidia deliciosa*. Antioxidant activity of *Hylocereus undatus* in three different solvent, there are only 63.44% of DPPH remaining in the sample with ethanol solvent, 55.04% in distilled water and 8.82% of DPPH remain in methanol solvent. In *Actinidia deliciosa,* there are only 90.34% of DPPH remain in the sample with ethanol solvent, followed by 87.39% in distilled water and 88.65% in methanol solvent respectively. Purification of the extract may lead to increased activity in its bioactive compounds. The antioxidant activities of *Hylocereus undatus* and *Actinidia deliciosa* extract may be due to its proton donating capability as shown in DPPH radical scavenging results. Acting as an electron donor that can react with free radicals, it converts them to more stable products and terminates radical chain reactions. This mechanism may explain the role of antioxidant and its use for the treatment of cancers and other diseases.

A further study of antioxidant activity in *Hylocereus undatus* and *Actinidia deliciosa* are recommended due many other specific compound of antioxidant present in both fruit and others analysis method can be used to measure the antioxidant activity in fruits. By studying the specific antioxidant compound in the sample, such as beta-carotene, lutein, alpha lipoic acid, lycopene and astaxanthin, we can determine the potential antioxidant in the sample and help us to improve the nutrition value in the fruit. A wide range of assays can be used for assessment of the antioxidant activity of *Hylocereus undatus* and *Actinidia deliciosa*.

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