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Antioxidant, anti-tyrosinase, and anti-angiogenic activities of dragon fruit (*Hylocereus* spp.)

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Abstract

The potential health benefits of native and exotic fruits have been the subject of many recent studies in the Philippines as fruits are chief sources of biologically active substances such as polyphenols, carotenoids, anthocyanins and flavonoids. Dragon fruit (*Hylocereus* spp.), an exotic fruit originating from South America, has become increasingly popular in the Philippines these past few years. Relatively new to the country, its full health benefits are yet to be discovered. In this study, the anti-tyrosinase and antioxidant activities of the methanolic and aqueous extracts of the flesh and peel of red and white dragon fruits were evaluated while the anti-angiogenic potential of the ethanolic and aqueous extracts of the flesh and peel of white dragon fruit were screened using the duck embryo chorioallantoic membrane (CAM) assay. The methanolic extract of the red dragon fruit peel showed potent inhibition against the diphenolase activity with the percentage of inhibition being significantly higher than that of Rutin at 20 mg/mL. Furthermore, it exhibited better antioxidant activity than ascorbic acid in the radical scavenging assay as well as the ability to chelate with ferrous ions and reduce the ferric ion. The observed activities may be attributed to its high total phenolic content and the observed presence of tannins. In CAM assay, treated duck embryo showed evident suppression of veins at increasing concentrations of ethanolic and aqueous extracts of the white dragon fruit flesh and the aqueous extract of the white dragon fruit peel (250 ppm to 2000 ppm), with almost no vascularization at 2000 ppm; the trends of vascularization were almost comparable to that of quercetin, a known anti-angiogenic compound. These results contribute to the increasing repertoire of potential health benefits of dragon fruit.

1. Introduction

Dragon fruit is an exotic fruit native to southern Mexico and Central America but has lately gained interest and is now being cultivated in tropical countries such as Thailand, Vietnam, and the Philippines due to it being a low-calorie fruit with high fibre content and a good amount of vitamins, minerals, and natural antioxidants such as polyphenolics, hydroxycinnamates, and flavonoids that protect cells from damage. The peels of *Hylocereus polyrhizus* (red-flesh dragon fruit) and *Hylocereus undatus* (white-flesh dragon fruit) contain the free radical scavenger β-amyrin, hypolidipemic agent γ-sitosterol, and antiangiogenic campesterol (Luo *et al.*, 2014). A one-cup serving (227 g) of dragon fruit has been reported to provide 7 g of fibre, 29 g of carbohydrates, iron 8% of recommended dietary intake (RDI), magnesium 18% of RDI, vitamin C 9% of RDI, and Vitamin E 4% of RDI (Meixner, 2018).

Betalains indicaxanthin and betanin were found in the plasma of volunteers fed with the fruit which protected low-density lipoprotein from ex vivo-induced oxidation (Tesoriere *et al.*, 2004). Potential health benefits are also evident as it was found to contain oligosaccharides that stimulate the growth of lactobacilli and bifidobacteria in the gut, potentially improving metabolic health (Wichienchot *et al.*, 2010). In a study by Song *et al.* (2016), administration of *H. polyrhizus* fruit betacyanins to mice reduced the high-fat diet-induced visceral obesity and insulin resistance. Furthermore, the supplement increased the relative abundance of Akkermansia, a beneficial microbe that potentially affects glucose metabolism and intestinal

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immunity. Dragon fruit extract also effectively decreased the aortic stiffness, measured by pulse wave velocity, in rats with streptozotocin-induced diabetes (Anand Swarup *et al.*, 2010), showing its potential against cardiovascular complications. Aside from the fruit being popular as a part of a healthy diet, some use its extract as a vital ingredient in beauty regimes to prevent premature ageing and brighten skin (Ghodke *et al.*, 2017). Also, as dragon fruit is rich in Vitamins C and B3, it is added to face creams to treat acne and moisturize dry sunburned skin (Ghodke *et al.*, 2017).

The peel of dragon fruit which contains betalains and phenolic compounds and was found to possess some antioxidant activities are usually discarded and thus its potential benefits are wasted. In the Philippines, only a few are involved in dragon fruit production due to challenges in producing quality fruit and high investment cost (Eusebio and Alaban, 2018). Furthermore, consumers are limited due to it being sold at a higher price (Php 150-200) compared to other locally grown fruits. Detailing the pharmacological uses of dragon fruit may further promote its consumption and encourage local farmers to develop a technology to reduce production costs and enhance quality. As the full health benefits of dragon fruit are yet to be discovered, the main objective of this study was to investigate the anti-tyrosinase and antioxidant activities as well as anti-angiogenic potential of two species of dragon fruit, i.e. white-flesh dragon fruit and red-flesh dragon fruit.

2. Materials and methods

2.1 Plant material

Red and white dragon fruits were harvested from a private farm in Los Baños, Laguna. The fruits were sent to the Bureau of Plant Industry for authentication. For chorioallantoic membrane (CAM) assay, white dragon fruit was purchased from a local market and sent to the National Museum for authentication.

2.2 Preparation of flesh and peel extracts

Red and white dragon fruits were washed and the peel was removed. The flesh and peels were blended and serial extraction was done by soaking the samples in hexane, 80% methanol, and distilled water at a 1:4 (w/v) ratio for 72 hrs with shaking. For CAM assay, white dragon fruit peel and flesh were soaked in 95% ethanol at a 1:4 (w/v) ratio for 4 hrs at room temperature. The hexane, methanolic, and ethanolic extracts were filtered and dried using the rotary evaporator at 40˚C. Methanolic and ethanolic extracts were further lyophilized to dry. The aqueous extracts were lyophilized to dry.

2.3 Phytochemical screening

Crude extracts were spotted in 6 separate thin layer chromatography (TLC) plates. The plates were developed in the chamber containing a solvent system composed of chloroform and methanol at a 1:5 ratio. The spray reagents and observable results for a positive test are seen in Table 1.

Table 1. Spray reagents used for phytochemical screening and the observable result for positive tests (Aguinaldo *et al.*, 2005).

Phytochemical Tested	Spray Reagent	Observable Result for a positive test
Flavonoids	Antimony(III) chloride	intense yellow to
Tannins	Potassium ferricyanide	blue spots
Alkaloids	Dragendorff's reagent	brown-orange spots immediately on
Anthroquinones	Magnesium acetate	orange-violet color
Indoles	Van Urk-Salkowski test	blue-violet spots
Higher alcohols Phenols	Vanillin-Sulfuric acid	blue-violet spots

2.4 Tyrosinase inhibition assay

Methanolic samples were dissolved in dimethyl sulfoxide, while aqueous samples were dissolved in 50 mM Potassium phosphate buffer (pH 6.5) to a final concentration of 20 mg/mL. At room temperature, 140 µL of each sample was mixed with 60 µL of tyrosinase (333 U/mL in phosphate buffer, pH 6.5) and 580 µL of distilled water. After 5 mins of incubation, 220 µL of the substrate, L-3,4-dihydroxyphenylalanine (L-DOPA, 12 mM in 50 mM potassium phosphate buffer (pH 6.5)), was added and incubated for 30 mins. Rutin was a positive control and distilled water was the negative control. The reaction mixture without the substrate served as the blank. Absorbances were read at 492 nm. The per cent tyrosinase inhibition was calculated using the formula:

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

where $A_{control}$ is the absorbance of distilled water and A_{sample} is the absorbance of the reaction mixture containing the extract or rutin (Momtaz *et al.*, 2008; Fawole *et al.*, 2012).

2.5 Radical-scavenging activity

Each sample (20 µL) in 20 mg/mL was added to 1000 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 0.1 mM in absolute ethanol), vigorously stirred, and allowed to sit at room temperature for 30 mins. Ascorbic acid (20 mg/mL) was a positive control. The reaction mixture using distilled water as a sample served as the blank. Absorbances were read at 517 nm. The percentage of

DPPH radical scavenging activity (RSA) was calculated using the formula:

$$RSA(\%) = [(A_{blank} - A_{test})/A_{blank}] \times 100$$

Where, A_{blank} is the absorbance of the blank containing distilled water only and A_{test} is the absorbance of the reaction mixture containing the extract (Paulpriya *et al.*, 2015).

2.6 Ferrous ion chelating assay

A total 30 µL of 2 mM ferrous chloride ($FeCl_2$) was added to 1.5 mL of sample (5 mg/mL) followed by 40 µL of 5 mM Ferrozine. The reaction mixture was shaken well and then incubated at room temperature for 10 mins. The reaction mixture with distilled water served as the blank. Absorbances were read at 562 nm. To compute the ferrous ion chelating activity of the samples, a standard calibration curve was prepared using varying concentrations of ethylenediaminetetraacetic acid (EDTA) (Singh and Rahini, 2004; Fawole *et al.*, 2012).

2.7 Ferric ion reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) solution contains 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 5 mL of 20 mM ferric chloride ($FeCl_3$). Each extract (100 µL) at 20 mg/mL was added to 900 µL of FRAP solution and incubated in the dark for 30 mins. The reaction mixture with distilled water served as the blank. Absorbances were read at 593 nm. The FRAP activity of samples was calculated through a standard calibration curve using varying concentrations of $FeCl_2$ (Benzie and Strain, 1996; Fawole *et al.*, 2012).

2.8 Total phenolic content

Each extract (100 µL) at 20 mg/mL was added to 450 µL of 10% Folin reagent. After shaking, 450 µL of 7.5% sodium carbonate was added. After a 40-minute incubation, absorbances were read at 725 nm. The total phenolic content of the samples was computed through a standard calibration curve using varying concentrations of Tannic acid (Makkar *et al.*, 1993; Fawole *et al.*, 2012).

2.9 Duck embryo chorioallantoic membrane assay

Fertile 7-day duck eggs were cleaned and incubated at 37°C for 2 days prior to use. A total of two eggs were used for each: positive control (quercetin), negative control, and the extracts (125, 250, 500, 1000, and 2000 ppm).

The eggs were placed in front of a light bulb to view the air space part of the egg. A small hole in the shell opposite the air space was made by puncturing to expose the CAM. The test solutions were introduced through the hole then the holes were sealed with parafilm. After 4 days of incubation, the membrane was observed for formation or suppression of the development of veins. The thickness and areas covered by the veins were noted.

2.10 Statistical analysis

All data were presented as mean values (±SEM) with one-way ANOVA using Microsoft Excel and GraphPad Prism. Tukey's and Dunnett's multiple comparisons tests were performed using GraphPad Prism.

3. Results

3.1 Phytochemical screening

The results of the phytochemical screening are seen in Table 2. Tannins were found in the methanolic extract of red dragon fruit peel (RP MeOH) and the aqueous extracts of red dragon fruit peel (RP H_2O), red dragon fruit flesh (RF H_2O), and white dragon fruit flesh (WF H_2O). Alkaloids were found to be present in the aqueous extracts of white dragon fruit peel (WP H_2O), WF H_2O, and RF H_2O; while phenolics were present in the methanolic extracts of the flesh and the aqueous extracts of the peel and flesh of both variants. Indoles were found to be present in the methanolic extracts of the flesh and the aqueous extracts of the peel and flesh of both variants.

3.2 Tyrosinase inhibition

In this study, an inhibition activity percentage above 50% was described as good tyrosinase inhibition. As seen in Figure 1, RP MeOH (91.759±3.645%) and the

Table 2. Phytochemical analysis of *Hylocereus* spp. methanolic and aqueous flesh and peel extracts

Phytochemicals	Methanolic Extracts				Aqueous Extracts			
	Red Flesh	Red Peel	White Flesh	White Peel	Red Flesh	Red Peel	White Flesh	White Peel
Flavonoids	-	-	-	-	-	-	-	-
Tannins	-	+	-	-	+	+	+	-
Alkaloids	-	-	-	-	+	-	+	+
Anthraquinones	-	+	-	-	-	+	-	+
Indoles	+	-	+	-	+	+	+	+
Phenols	+	-	+	-	+	+	+	+

methanolic extract of white dragon fruit peel (WP MeOH, 76.170±10.145%) exhibited good inhibition against the diphenolase activity at 20 mg/mL. Based on the one-way ANOVA test and Dunnett's multiple comparisons test, a significant difference was observed for WP MeOH and RP MeOH against rutin (44.357±5.263%).

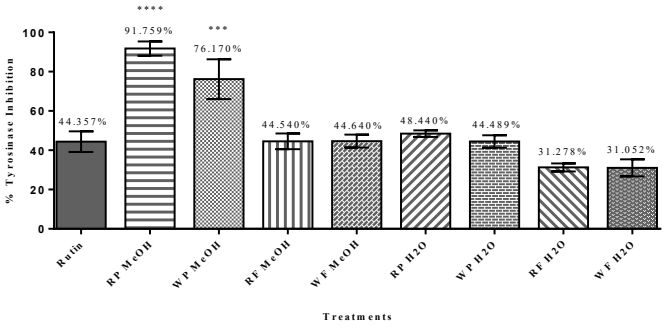

Figure 1. Tyrosinase-inhibition activities of the crude extracts relative to the positive standard, rutin. Abbreviations: RF H_2O = Aqueous extract of red dragon fruit flesh; RF MeOH = Methanolic extract of red dragon fruit flesh; RP H_2O = Aqueous extract of red dragon fruit peel; RP MeOH = Methanolic extract of red dragon fruit peel; WF H_2O = Aqueous extract of white dragon fruit flesh; WF MeOH = Methanolic extract of white dragon fruit flesh; WP H_2O = Aqueous extract of white dragon fruit peel; WP MeOH = Methanolic extract of white dragon fruit peel. All data are reported as mean±SEM (n = 5) . ****P values < 0.0001, ***P values = 0.0002.

Six different solvents were used for partial purification of RP MeOH and among these, ethyl acetate and methanol showed the most bands. Thus, gradient elution using these solvents was performed. Fractions A-F were collected using 100% ethyl acetate, 100%-60% ethyl acetate, 40%-100% methanol, 100%-50% methanol and water, 50% methanol and 50% water, and 100% water, respectively. As seen in Figure 2, the tyrosinase inhibition activity of the fractions was reduced compared to the crude RP MeOH extract.

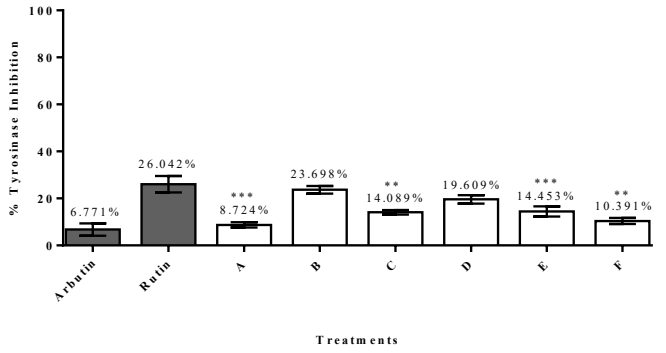

Figure 2. Tyrosinase-inhibition activities of partially purified extracts relative to the positive standard, rutin. Elution solvents: Fraction A — 100% ethyl acetate; Fraction B — 100%-60% ethyl acetate; Fraction C — 40%-100% methanol; Fraction D — 100%-50% methanol & water; Fraction E — 50% methanol, 50% water; Fraction F — 100% water. All data are reported as mean±SEM (n = 5) . ***P values = 0.0002, **P values = 0.0021.

Fraction B (23.698±1.623%) and Fraction D (19.609±1.780%) exhibited tyrosinase-inhibition activities statistically comparable to that of rutin (26.042±3.504%) at 20 mg/mL. Fractions A (8.724±1.123%), C (14.089±0.973%), E (14.453±2.175%), and F (10.391±1.281%), on the other hand, showed significantly lower tyrosinase inhibition activity compared to rutin.

3.3 Antioxidant capacity

Figure 3 shows the RSA of 8 crude extracts. Considering RSA of 50% as good activity, poor RSAs were exhibited by all extracts at 20 mg/mL, except that of RP MeOH (54.607±1.139%). The RSA of the ascorbic acid (93.789±2.372%), however, was still significantly higher. WP H_2O had a negative activity, making results for this sample highly inconclusive.

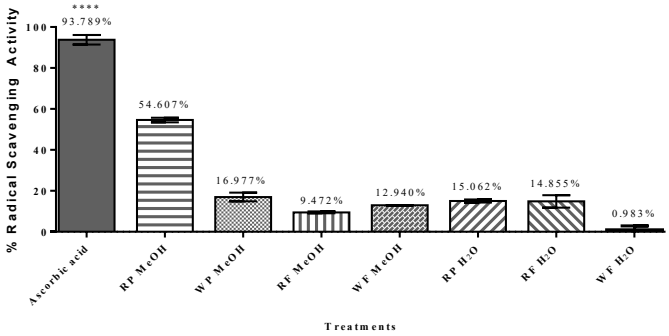

Figure 3. Radical scavenging activity of the crude extracts and the positive standard ascorbic acid at 20 mg/mL. Abbreviations: RF H_2O = Aqueous extract of red dragon fruit flesh; RF MeOH = Methanolic extract of red dragon fruit flesh; RP H_2O = Aqueous extract of red dragon fruit peel; RP MeOH = Methanolic extract of red dragon fruit peel; WF H_2O = Aqueous extract of white dragon fruit flesh; WF MeOH = Methanolic extract of white dragon fruit flesh; WP MeOH = Methanolic extract of white dragon fruit peel. All data are reported as mean±SEM (n=3) . ****P values < 0.0001.

Figure 4 shows the ferrous ion chelating (FIC) and ferric ion reducing abilities of the crude extracts. WP H_2O (0.172±0.007 mM EDTA equivalents) and WP MeOH (0.169±0.003 mM EDTA equivalents) showed comparable FIC activity with RP MeOH (0.152±0.001 mM EDTA equivalents), but significantly higher activity than the rest.

As seen in Figure 5, RF H_2O (855.000±108.448 mM $FeCl_2$ equivalents), RP MeOH (832.067±178.537 mM $FeCl_2$ equivalents), RP H_2O (781.800±0.000 mM $FeCl_2$ equivalents), WP MeOH (643.800±1.405 mM $FeCl_2$ equivalents), and the methanolic extract of white dragon fruit flesh (WF MeOH, 542.200±5.718 mM $FeCl_2$ equivalents) showed effective and comparable ferric ion

reducing capacity at 20 mg/mL, with RF H_2O showing activity significantly higher than that of the methanolic extract of red dragon fruit flesh (RF MeOH) and WP H_2O.

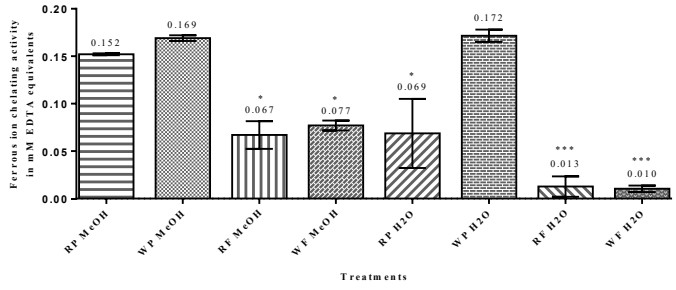

Figure 4. Antioxidant activity of the dragon fruit extracts (at 20 mg/mL) determined by FIC Assay. Abbreviations: RF H_2O = Aqueous extract of red dragon fruit flesh; RF MeOH = Methanolic extract of red dragon fruit flesh; RP H_2O = Aqueous extract of red dragon fruit peel; RP MeOH = Methanolic extract of red dragon fruit peel; WF H_2O = Aqueous extract of white dragon fruit flesh; WF MeOH = Methanolic extract of white dragon fruit flesh; WP H_2O = Aqueous extract of white dragon fruit peel; WP MeOH = Methanolic extract of white dragon fruit peel. All data are reported as mean±SEM (n=3). ***P values = 0.0002, *P values = 0.0332.

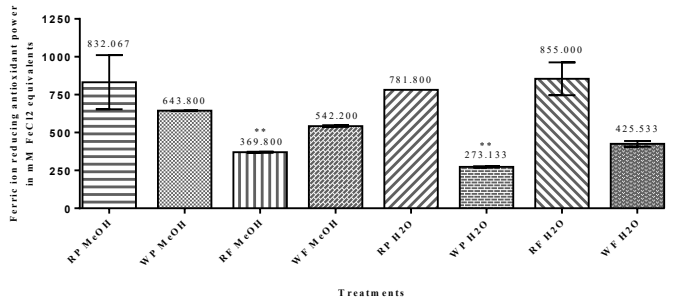

Figure 5. Antioxidant activity of the dragon fruit extracts (at 20 mg/mL) determined by FRAP Assay. Abbreviations: RF H_2O = Aqueous extract of red dragon fruit flesh; RF MeOH = Methanolic extract of red dragon fruit flesh; RP H_2O = Aqueous extract of red dragon fruit peel; RP MeOH = Methanolic extract of red dragon fruit peel; WF H_2O = Aqueous extract of white dragon fruit flesh; WF MeOH = Methanolic extract of white dragon fruit flesh; WP H_2O = Aqueous extract of white dragon fruit peel; WP MeOH = Methanolic extract of white dragon fruit peel. The values are expressed in mM $FeCl_2$ equivalents. All data are reported as mean±SEM (n=3). **P values = 0.0021.

3.4 Total phenolic content

The antioxidant activity of a plant extract is largely attributed to the presence of phenolic compounds. Figure 6 shows the total phenolic content (TPC) of the 8 crude extracts. All extracts tested had a concentration of 20 mg/mL except for the WP MeOH and RP MeOH, which were at a concentration of 1 mg/mL due to the minimum amount available, thus values were adjusted and expressed per gram of sample. After the corrected absorbances, RP MeOH had the significantly highest amount of total phenolic content at 82.460±1.014 µg/mL Tannic acid equivalents per gram sample.

The good antioxidant and tyrosinase-inhibition activities of RP MeOH may be attributed to its high TPC, thus showing its potential as a skin-lightening agent.

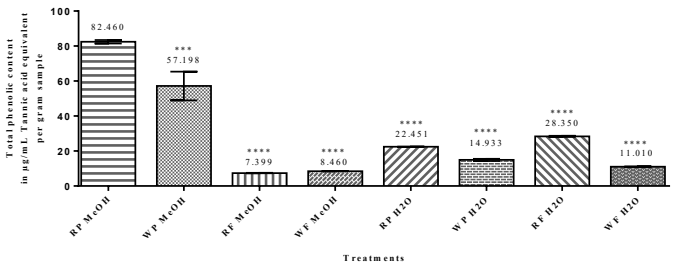

Figure 6. The total phenolic content of 8 crude extracts. The corrected values are expressed as µg/mL Tannic acid equivalents per gram of sample. Abbreviations: RF H_2O = Aqueous extract of red dragon fruit flesh; RF MeOH = Methanolic extract of red dragon fruit flesh; RP H_2O = Aqueous extract of red dragon fruit peel; RP MeOH = Methanolic extract of red dragon fruit peel; WF H_2O = Aqueous extract of white dragon fruit flesh; WF MeOH = Methanolic extract of white dragon fruit flesh; WP H_2O = Aqueous extract of white dragon fruit peel; WP MeOH = Methanolic extract of white dragon fruit peel. All data are reported as mean±SEM (n=3). ****P values < 0.0001, ***P values = 0.0002.

3.5 Anti-angiogenic effects of dragon fruit extracts

As seen in Figure 7B, the vascularization in the untreated duck embryos was evident in the CAM, with dense blood vessels radially converging towards the centre. In Figure 7A, however, treatment with WF H_2O and the ethanolic extract of white dragon fruit flesh (WF EtOH) inhibited the angiogenic process. In duck embryos treated with quercetin, WF EtOH, and WF H_2O,

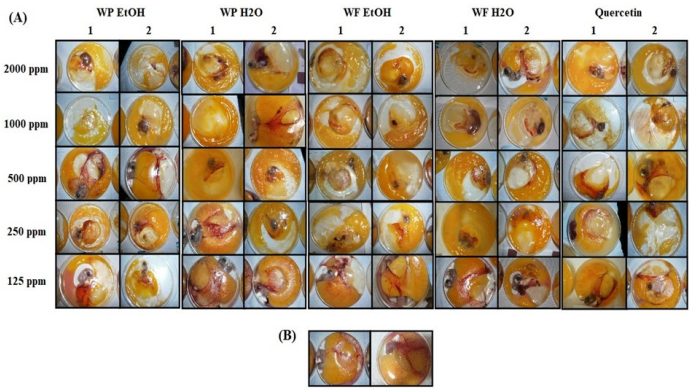

Figure 7. Duck embryos (A) treated with the test solutions at 125, 250, 500, 1000, and 2000 ppm and (B) untreated. Abbreviations: WF EtOH = Ethanolic extract of white dragon fruit flesh; WF H_2O = Aqueous extract of white dragon fruit flesh; WP EtOH = Ethanolic extract of white dragon fruit peel; WP H_2O = Aqueous extract of white dragon fruit peel. Assay was done in duplicate.

dense vascularization was observed only at 125 ppm which decreased with the increase in concentration. Notably, the blood vessels in the CAM were almost not noticeable after treatment with quercetin, WF EtOH, WF H_2O, and the ethanolic extract of white dragon fruit peel (WP EtOH) at 2000 ppm.

4. Discussion

Phenolics and indoles were found to be present in the methanolic extracts of the flesh and the aqueous extracts of the peel and flesh of both variants, while alkaloids were found mostly in the aqueous extracts of white dragon fruit and tannins were found mostly in the red dragon fruit variant, most notably in RP MeOH (Table 2). Condensed tannins have been reported to have the potency to treat tumours and can reduce the risk of cancer (Ghosh, 2015), which may be related to their ability to inhibit free radicals produced by many carcinogens and/or mutagens (Amarowicz, 2007). Condensed tannins also are potent mushroom tyrosinase inhibitors mainly via chelation (Chen *et al.*, 2014). Linear relationships were observed between the total alkaloid/phenolic contents and ferric reducing antioxidant potential and lipid peroxidation inhibition ability (Gan *et al.*, 2017), showing their importance for antioxidation. Furthermore, phenolic compounds and their derivatives were characterized as potent tyrosinase inhibitors (Zolghadri *et al.*, 2019). Indole derivatives were shown to be effective scavengers of reactive oxygen (ROS) and nitrogen species, with the radical dissipation inside the indolic system being mandatory for the observed antioxidant activity (Estevão *et al.*, 2010). Synthesized indole derivatives have also shown tyrosinase inhibitory properties, with the substituent at the C-3 position playing a key role in activity (Ferro *et al.*, 2016).

Tyrosinase, a copper-containing glycoprotein produced by melanocyte cells, is a primary enzyme responsible for the production of melanin which is the main source of skin and hair colour. Aside from this, studies proposed that tyrosinase over-expression may contribute to the neuromelanin formation in substantia nigra (Carballo-Carbajal *et al.*, 2019) and could be central to dopamine neurotoxicity as well as contribute to the neurodegeneration associated with Parkinson's disease (Xu *et al.*, 1997). Thus, tyrosinase has become an important target for the development of hypopigmented agents as well as medicinal products. In this study, RP MeOH exhibited good inhibition against the diphenolase activity at 20 mg/mL (Figure 1). It is known that the ability to form complexes with proteins is a unique characteristic of tannins. Thus, the tannin content of RP MeOH could have an effect on the reduced tyrosinase activity observed herein. When RP MeOH was subjected to partial purification, however, the tyrosinase inhibition activity was reduced which is possibly due to the separation of the active components. Fractions B and D exhibited tyrosinase-inhibition activities comparable to that of rutin at 20 mg/mL (Figure 2). Fraction B, eluted with 100%-60% ethyl acetate, might contain non-polar components such as lipids and steroids, which according to studies, can also act as tyrosinase-inhibitors (Chang, 2012). Fraction D, eluted with 100%-50% methanol and water, most probably contains the polyphenols. Tannins contain non-polar (aromatic rings) and polar (hydroxyls) moieties and studies showed that they can be obtained using ethyl acetate (Benzidia *et al.*, 2019) and methanol: water (Yuliana *et al.*, 2014) as solvent.

An antioxidant compound may be classified according to its mechanism of action: electron transfer such as FRAP assay, hydrogen atom transfer such as DPPH assay, and chelation of transition metals such as FIC assay. The DPPH test is an indirect method for determining the antioxidant activity which is based on the reduction of the stable free radical DPPH by the action of hydrogen atom donating antioxidants in the extract. In the FIC assay, the ability of plant components to chelate transition metals such as iron and copper that are involved in the formation of ROS is determined. FRAP is based on the reduction of ferric(III)-TPTZ complex to ferrous(II)-TPTZ complex, formed by the action of electron-donating antioxidants in the extract. In this study, RP MeOH notably exhibited antioxidant activity in all three assays as well as high TPC (Figure 3 - 6). Therefore, the good antioxidant and tyrosinase-inhibition activities of RP MeOH may be attributed to the presence of tannins and its high TPC.

Angiogenesis is one of the indicators of cancer, playing an essential role in tumour growth as the formation of new blood vessels provides a sufficient supply of nutrients for tumour survival and allows the cancer cells to spread to other areas (Sun *et al.*, 2015). Our results showed that the white dragon fruit exhibited high iron chelating activity. The transition metal ion Fe^{2+} is involved in the formation of free radicals and thus chelating can reduce ROS formation. ROS has been shown to promote angiogenesis via different pathways such as stimulation of the expression of vascular endothelial growth factor, an angiogenic factor engaged in tumour progression by promoting the growth of tumour vasculature (Kim and Byzova, 2014). In this study, WF H_2O and WF EtOH suppressed the veins in duck embryos with almost no vascularization at 2000 ppm (Figure 7). This anti-angiogenic activity may be attributed to the presence of indole derivatives which are effective ROS scavengers thus reducing the stimulators

of the induction of VEGF expression. Further investigations are required to elucidate the precise mechanisms and potential side effects.

5. Conclusion

This study showed that the peel of red dragon fruit, an underused resource, possesses consistently high antioxidant activity in all three assays: FRAP assay, DPPH assay, and FIC assay. It also inhibited tyrosinase, being more potent than rutin. These activities could be associated with the presence of tannins and high total phenolic content observed in the methanolic extract. These results suggest that the peel of the red dragon fruit can be utilized as a good source of functional components useful for enhancing the human defense system and as depigmenting agents. The flesh of the white dragon fruit, on the other hand, showed good anti-angiogenic activity, evidently suppressing the veins similar to that of quercetin. Thus, the white dragon fruit is a promising source and can be utilized for the discovery of angiogenesis inhibitors. Our study adds to the repertoire of health benefits of dragon fruit. This information could be utilized for the development of pharmaceutical and cosmetic products thereby increasing the commercial value of this plant.

Conflict of interest

The authors declare no conflict of interest.

References

Aguinaldo, A.M., Espeso, E.I., Guevara, B.Q. and Nonato, M.G. (2005). Phytochemistry Section. In Guevara B.Q. (Ed). A Guidebook to Plant Screening: Phytochemical and Biological, p. 23-62. Manila, Philippines: University of Santo Tomas Publishing House.

Amarowicz, R. (2007). Tannins: the new natural antioxidants? *European Journal of Lipid Science and Technology*, 109(6), 549-551. https://doi.org/10.1002/ejlt.200700145

Anand Swarup, K.R., Sattar, M.A., Abdullah, N.A., Abdulla, M.H., Salman, I.M., Rathore, H.A. and Johns, E.J. (2010). Effect of dragon fruit extract on oxidative stress and aortic stiffness in streptozotocin-induced diabetes in rats. *Pharmacognosy Research*, 2(1), 31-35. https://doi.org/10.4103/0974-8490.60582

Benzidia, B., Barbouchi, M., Hammouch, H., Belahbib, N., Zouarhi, M., Erramli, H., Daoud, N., Badrane, N. and Hajjaji, N. (2019). Chemical composition and antioxidant activity of tannins extract from green rind of *Aloe vera* (L.) Burm. F. *Journal of King Saud University – Science*, 31(4), 1175-1181. https://doi.org/10.1016/j.jksus.2018.05.022

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Carballo-Carbajal, I., Laguna, A., Romero-Giménez, J., Cuadros, T., Bové, J., Martinez-Vicente, M., Parent, A., Gonzalez-Sepulveda, M., Peñuelas, N., Torra, A., Rodríguez-Galván, B., Ballabio, A., Hasegawa, T., Bortolozzi, A., Gelpi, E. and Vila, M. (2019). Brain tyrosinase overexpression implicates age-dependent neuromelanin production in Parkinson's disease pathogenesis. *Nature Communications*, 10, 973. https://doi.org/10.1038/s41467-019-08858-y

Chang, T.-M. (2012). Tyrosinase and tyrosinase inhibitors. *Journal of Biocatalysis and Biotransformation*, 1, 2.

Chen, X.-X., Shi, Y., Chai, W.-M., Feng, H.-L., Zhuang, J.-X. and Chen, Q.-X. (2014). Condensed tannins from *Ficus virens* as tyrosinase inhibitors: structure, inhibitory activity and molecular mechanism. *PLoS ONE*, 9(3), e91809. https://doi.org/10.1371/journal.pone.0091809

Estevão, M.S., Carvalho, L.C., Ribeiro, D., Couto, D., Freitas, M., Gomes, A., Ferreira, L.M., Fernandes, E. and Marques, M.M. (2010). Antioxidant activity of unexplored indole derivatives: synthesis and screening. *European Journal of Medicinal Chemistry*, 45(11), 4869-4878. https://doi.org/10.1016/j.ejmech.2010.07.059

FFTC Agricultural Policy Platform (FFTC-AP). (2018). Current status of dragon fruit and its prospects in the Philippines. Retrieved on March 18, 2021 from FFTC-AP Website: https://ap.fftc.org.tw/article/1295.

Fawole, O.A., Makunga, N.P. and Opara, U.L. (2012). Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BioMed Central Complementary and Alternative Medicine*, 12, 200. https://doi.org/10.1186/1472-6882-12-200

Ferro, S., Certo, G., De Luca, L., Germanò, M.P., Rapisarda, A. and Gitto, R. (2016). Searching for indole derivatives as potential mushroom tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31(3), 398-403.

Gan, J., Feng, Y., He, Z., Li, X. and Zhang, H. (2017). Correlations between antioxidant activity and alkaloids and phenols of Maca (*Lepidium meyenii*).

Journal of Food Quality, 2017, 3185945. https://doi.org/10.1155/2017/3185945

Ghodke, P.A., Soni, N.N., Shah, D.B. and Maheshwari, D.G. (2017). Herbal facial cream from dragon fruit. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 2(6), 98-100.

Ghosh, D. (2015). Tannins from foods to combat diseases. *International Journal of Pharma Research and Review*, 4(5), 40-44.

Kim, Y.W. and Byzova, T.V. (2014). Oxidative stress in angiogenesis and vascular disease. *Blood*, 123(5), 625-631. https://doi.org/10.1182/blood-2013-09-512749

Luo, H., Cai, Y., Peng, Z., Liu, T. and Yang, S. (2014). Chemical composition and in vitro evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. *Chemistry Central Journal*, 8, 1. https://doi.org/10.1186/1752-153X-8-1

Makkar, H., Blümmel, M., Borowy, N. and Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture*, 61(2), 161-165. https://doi.org/10.1002/jsfa.2740610205

Healthline. (2018). 7 Great Reasons to Add Dragon Fruit to Your Diet. Retrieved on March 18, 2021 from Healthline Website: https://www.healthline.com/nutrition/dragon-fruit-benefits

Momtaz, S., Mapunya, B.M., Houghton, P.J., Edgerly, C., Hussein, A., Naidoo, S. and Lall, N. (2008). Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. *Journal of Ethnopharmacology*, 119(3), 507-512. https://doi.org/10.1055/s-0028-1083996

Paulpriya, K., Packia Lincy, M., Tresina, P.S. and Mohan, V.R. (2015). In vitro antioxidant activity, total phenolic and total flavonoid contents of aerial part extracts of *Daphniphyllum neilgherrense* (wt.) *rosenth*. *Journal of Bio Innovation*, 4(6), 257-268.

Singh, N. and Rahini, P.S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85(4), 611-616. https://doi.org/10.1016/j.foodchem.2003.07.003

Song, H., Chu, Q., Yan, F., Yang, Y., Han, W. and Zheng, X. (2016). Red pitaya betacyanins protects from diet-induced obesity, liver steatosis and insulin resistance in association with modulation of gut microbiota in mice. *Journal of Gastroenterology and Hepatology*, 31(8), 1462-1469. https://doi.org/10.1111/jgh.13278

Sun, Q., Heilmann, J. and König, B. (2015). Natural phenolic metabolites with anti-angiogenic properties - a review from the chemical point of view. *Beilstein Journal of Organic Chemistry*, 11, 249-264. https://doi.org/10.3762/bjoc.11.28

Tesoriere, L., Allegra, M., Butera, D. and Livrea, M. (2004). Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: potential health effects of betalains in humans. *The American Journal of Clinical Nutrition*, 80(4), 941-945. https://doi.org/10.1093/ajcn/80.4.941

Wichienchot, S., Jatupornpipat, M. and Rastall, R.A. (2010). Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. *Food Chemistry*, 120(3), 850-857. https://doi.org/10.1016/j.foodchem.2009.11.026

Xu, Y., Stokes, A.H., Freeman, W.M., Kumer, S.C., Vogt, B.A. and Vrana, K.E. (1997). Tyrosine mRNA is expressed in human substantia nigra. *Brain Research. Molecular Brain Research*, 45(1), 159-162. https://doi.org/10.1016/S0169-328X(96)00308-7

Yuliana, P., Laconi, E., Wina, E. and Jayanegara, A. (2014). Extraction of tannins and saponins from plant sources and their effects on In vitro methanogenesis and rumen fermentation. *Journal of the Indonesian Tropical Animal Agriculture*, 39(2), 91-97. https://doi.org/10.14710/jitaa.39.2.91-97

Zolghadri, S., Bahrami, A., Khan, M.T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F. and Saboury, A.A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 34(1), 279-309. https://doi.org/10.14710/jitaa.39.2.91-97

