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Characterization and assessment of phytochemical properties of dragon fruit (*Hylocereus undatus* and *Hylocereus polyrhizus)* peels

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Abstract Peels of two varieties of dragon fruit, white *(Hylocereus undatus*) and red *(H. polyrhizus*) from the Philippines, were investigated for their phytochemical properties for food waste utilization and development of value-adding products. A total of 9.53 kg red and 5.23 kg white dragon fruit peels were used as samples. After manually separating the peel from the flesh, the peel recovery was recorded as 26% and 35% for the red and white varieties, respectively. The fruit peels were dried using a cabinet drier set at 55^{o}C. The dried samples were soaked in 95% ethanol, and the extract was concentrated in vacuo at 56^{o}C. Preliminary phytochemical analysis for alkaloids, cardenolides, bufadienolides, anthraquinones, saponins, coumarins, tannins, phlobatannins, and flavonoids was done using standard methods. Thin layer chromatographic (TLC) analysis was performed to confirm the preliminary phytochemical analysis. Free radical scavenging assay using 2,2-diphenyl-1-picyrlhydzayl (DPPH) was used to evaluate the antioxidant property. Results showed that both peels were tested positive for alkaloids, cardenolides and bufadienolides, anthraquinones, and flavonoids. Coumarins, terpenoids, cardiac glycosides were also detected in the ethanolic extracts. Tannins and saponins were not present in the extracts during the preliminary phytochemical screening. TLC analysis revealed that the two peel samples have higher alcohols, phenols, steroids, and essential oils. The results suggested that the white dragon fruit variety has more flavonoids and anthraquinones and superior antioxidant properties than the red dragon fruit peels. The % free radical scavenging activity using DPPH assay revealed that the white variety has higher activity and is considered has a strong antioxidant while the red variety has a weak antioxidant. The results demonstrate that aside from the flesh of dragon fruit, the peels could be promoted as a potential source of antioxidants and other bioactive agents, which can be applied in food systems and cosmetic formulations.

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Introduction

Since the introduction of dragon fruit in the late '90s, various studies have been conducted about its nutrients and health benefits. The dragon fruit was proven to be a significant source of antioxidants and other phytochemicals. Because of the health benefit that can be derived from the fruit, production and global marketing increased. Dragon fruit production and technology development is now in the Southeast Asian countries like Malaysia, Vietnam, Taiwan, Thailand, the Philippines, and the Southeast Coast of China (Eusebio and Alaban, 2018). In 2018, Vietnam had the most significant planting area and dragon fruits in Asia, from 5,512 ha in 2000 to 55,419 ha in 2018, with about 1,074,242 tons of output (Hien, 2020).

Dragon fruit is a rich source of Vitamin C, calcium, and phosphorus. It offers a wide range of health benefits like reducing hypertension and diabetes because of its effect on carbohydrate metabolism and heart tissues formation. It is also rich in total ascorbic acid, total dietary fibers, pectin, and iron, which helps balance blood production (CABI, 2018; Kumar *et al.,* 2018).

Medical practitioners recommended having at least 25 grams of fiber every day. According to a dietician, high-fiber fruit like dragon fruit is likely to maintain a healthy weight (Dimou *et al.,* 2019) since fiber is also filling, which is helpful to individuals who are trying to lose weight.

In the Philippines, with the wide range of health benefits the dragon fruit offers, its popularity among the consumers led to increased demand, making farmers interested in continuing the dragon fruit production (Paull and Chem, 2019). Technologies on pest management (Balendres and Bengoa, 2019; Taguiam *et al.,* 2020a; Taguiam *et al.,* 2020b) and postharvest handling of fruits (Rodeo *et al.,* 2019) were conducted to improve dragon fruit production and handling.

Aside from dragon fruit flesh, studies also showed that the betalains in pulps of purple *Hylocereus* species have significant antioxidant capacity (Esquivel *et al.,* 2007). It is hypothesized that the peels also contain antioxidant properties due to their prominent color. Thus, both the pulps and the peels could be beneficial, especially in the food and pharmaceutical industry (Nurliyana *et al.,* 2010). Dragon fruit is also rich in pigment betalains comprising betacyanins and betaxanthins. Rebecca *et al.* (2008) reported the excellent tolerance of these pigments towards the factors causing color loss during processing. Rodriguez *et al.* (2016) disclosed that the antioxidant, anti-inflammatory, antiangiogenic, and glutathione S-transferases-inducing

activities of betalains from red dragon fruit peel were enhanced through carbohydrate encapsulation.

Product development with dragon fruit flesh in the country is enormous from ice cream, wine, soap, coffee, tea, and consumers accept these. The dragon fruit takes about 26-35% of waste from its peel. Thus, as the consumption increases, the waste also increases. Dzah *et al.* (2020) confirmed that fruit waste and by-products are high in antioxidant polyphenols and contain important chemical components beneficial to human health. Waste utilization can increase the economic benefits aside from it also decreasing environmental impact. This study aimed to characterize and assess the phytochemical properties of the peels of the Philippines' two varieties of dragon fruit, the red *(H. polyrhizus*) and white (*H. undatus*). This study further aimed to valorize the dragon fruit peels as a potential source of antioxidants and other bioactive agents, which can be applied in food systems and cosmetic formulations.

Materials and methods

Determination of phytochemical compounds in the dragon fruit peel and characterization of the phytochemical compounds of both varieties (*H. undatus and H. polyrhizus)* were performed at the Chemistry Laboratory at the Central Luzon State University from June 2020 to June 2021. Descriptive research was used to properly determine and characterize the presence of bioactive compounds in the two varieties. The scavenging properties of the extracts were also determined using nonlinear regression curve fitting function, sigmoidal dose-response of Graph Pad Prism 8. The program was used to estimate the median effective concentration (EC50) of sample, which was the concentration required to scavenge radical by 50%.

Preparation of dragon fruit peel samples

The fresh dragon fruits *(H. polyrhizus and H. undatus*) were obtained from REFMAD Farms, Burgos, Ilocos Norte. First, peels from the two varieties were manually separated from the flesh. Then, the percent recovery of the peels was computed using the following equation:

$$\%Recovery\ of\ the\ Peels = \frac{weight\ of\ peels}{weight\ of\ dragon\ fruit}\ x\ 100$$

The dragon fruit peels were then dried using a cabinet drier set at 55 ℃ for 11 hours or until the desired water activity (aw) was achieved. Next, the

peels were ground using a blender, and then the powdered sample was weighed and placed in separate sealed containers and stored in the refrigerator until further use.

Fruit peel extraction

The powdered peels were soaked in 95% ethanol for four days to solubilize the biologically active compounds present. The soaked samples were sonicated for 1 hour, then filtered. The residue was rinsed with fresh ethanol 6-7 times until the color of the liquid became clear. The combined filtrates were concentrated in vacuo using a rotary evaporator at 56℃ water bath, 120 rpm, and 9-11.5℃ condenser until the extract became 20-30 mL. The concentrated extract was placed in an Erlenmeyer flask, sealed, and kept refrigerated to prevent decomposition of the components that are sensitive to heat.

Phytochemical screening

Phytochemical screening of the dragon fruit peel extracts was based on the procedures recommended by Aguinaldo *et al.* (2005).

Thin layer chromatography

Thin layer chromatographic (TLC) analysis for each dragon fruit extract was carried out to detect the secondary metabolites present. The samples were labeled as A for the red variety and B for the white variety. The five solvent systems were the following: SS1- toluene-chloroform (9:11); SS2 - toluene-acetone-chloroform (40:25:35); SS3 - n-butanol-acetic acid-water (4:1:5); SS4 - chloroform-acetic acid-water (50:45:5); and SS5 - chloroform-methanol (5:1)
Each extract was spotted on the marked and labeled TLC plates (6 cm x 3 cm) and was developed in the different solvent systems in the developing chamber.
The spots for a specific metabolite were visualized on the TLC plates and were exposed under UV light to check the separation of the different compounds. Then, different spray reagents were used to visualize the spots, and the distinct spots were traced with a pencil, and the Rf values were computed (Aguinaldo *et al.,* 2005).

$$R_f = \frac{distance\ spot\ travelled}{distance\ mobile\ phase\ travelled}$$

Antioxidant analysis (DPPH radical scavenging activity)

The DPPH radical scavenging activity assay of Chan *et al.* (2007) was modified. A 1.5 mL of 1000, 300, 100, 10, 1, and 0.1 ppm of the crude ethanolic extracts were separately added to 2.5 mL DPPH solution (6.0mg DPPH/100 mL methanol). The control treatment was prepared by adding

methanol instead of the extract. Ascorbic acid was considered as the positive control. The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature before reading its absorbance at 517 nm in a visible spectrophotometer (T60 UV-VIS Spectrophotometer). The DPPH radical scavenging activity was computed using the formula:

$$\%\ scavenging\ activity = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ blank}\right) x\ 100$$

The test solutions were prepared in triplicates for three trials. The EC50 was estimated using nonlinear regression curve fitting function, sigmoidal dose-response of Graph Pad Prism 8.

Results

Percent recovery of the peels

After the pulp was separated from the peel, the peel recovery rate of red dragon fruit was 26%, whereas the peel recovery rate of white dragon fruit was 35%. The high recovery percentages in dragon fruit peels indicate that a significant amount of waste is produced throughout the process.

Phytochemical screening of red and white dragon fruit peels

The results of the qualitative analysis of phytochemicals presented on red and white dragon fruit peels are presented in Table 1. These tests were done using the standard methods by Aguinaldo *et al.* (2005).

Table 1. Secondary metabolites in the red dragon fruit peel (RDFP) and white dragon fruit peel (WDFP) ethanolic extracts

Secondary Metabolites	Test		RDFP	WDFP
Alkaloids	Preliminary	Mayer's Test	**	*
		Wagner's Test	***	**
	Confirmatory	Mayer's Test	**	*
		Wagner's Test	***	**
	Quaternary	Mayer's Test	****	-
		Wagner's test	****	****
Saponins	Froth test		-	-
Cardenolides and Bufadienolides	Keller-Kiliani Test		++	++
Anthraquinones	Borntrager's Test		-	-
Flavonoids	Bate-Smith and Metcalf Test		+	++
Phlobatannins	Hydrochloric Acid Test		-	-
Tannins	Ferric Chloride Test		-	-
Coumarins	Sodium Hydroxide Test		+	+
Terpenoids	Salowski Test		+	+

Legend: (*) - Primary alkaloid; (**) - secondary alkaloids; (***) - tertiary alkaloids; (****) - quaternary and amine oxide base; (+) -less abundant,(++) -much abundant; (–) - absent

The above results indicated that both red dragon fruit peel (RDFP) and white dragon fruit peel (WDFP) were tested positive for alkaloids, cardenolides and bufadienolides, flavonoids, coumarins, and terpenoids. Saponins, tannins, or phlobatannins were not found on both peels. The alkaloidal contents of the two varieties differed. The RDFP contains primary, secondary, and quaternary amine and oxide bases. In contrast, the WDFP contains secondary, tertiary, and quaternary amines and oxide bases as indicated in the precipitation reaction with the Mayer's and Wagner's reagents. Flavonoids of WDFP were more evident than the RDFP, as indicated by the more intense yellow precipitates.

Confirmatory test of phytochemicals present on red and white dragon fruit peel using thin- layer chromatography

Thin-layer chromatography (TLC) confirmed the presence of phytochemical constituents observed in the preliminary test. RDFP and WDFP samples were used. The results of the TLC confirmatory test are shown in Table 2.

Table 2. List of plant constituents, their visualizing agents, the indication of a positive test, and the TLC confirmatory test results

Component	Spray Reagent	Observation	Result	
			RDFP	WDFP
Flavonoids, Steroids	Antimony (III) chloride	Fluorescing spots in long-wave UV light	+	+
Phenols, Tannin Flavonoids	Potassium-ferricyanide-ferric chloride	Blue spots	+	+
Alkaloids	Dragendorff's Reagent	Orange spots	+	+
Cardenolides	3,5 Dinitrobenzoic acid Kedde Reagent	Red-blue violet-colored zones	-	-
Coumarins, Anthraquinones, Anthrones, Phenols	Methanolic potassium hydroxide (Bornträger reagent)	Anthraquinones give orange coloration; Anthrones give yellow and Coumarins react to form blue (UV 365nm) colored zones	+	+
Anthraquinones	Magnesium acetate in methanol	Orange-colored zones	+	+
Higher alcohols Phenols, Steroids, Essential oils	Vanillin-sulfuric Acid	Colorful zones	+	+

The TLC results showing the best solvent system and the number of spots and Rf values are presented in Table 3. Only the solvent system which gave the best separation of the spots. The spray reagents used to visualize the spots and observable colors are listed in Table 2. The results revealed that both samples were tested positive for flavonoids and steroids, consistent with the preliminary phytochemical analysis. TLC further revealed that RDFP extract was only four spots visualized while the WDFP was five. Separation of components observed in the solvent system n-butanol-acetic acid-water (4:1:5). The chromatograms developed using the different solvent systems and visualized using the spray reagents are shown in Figure 1.

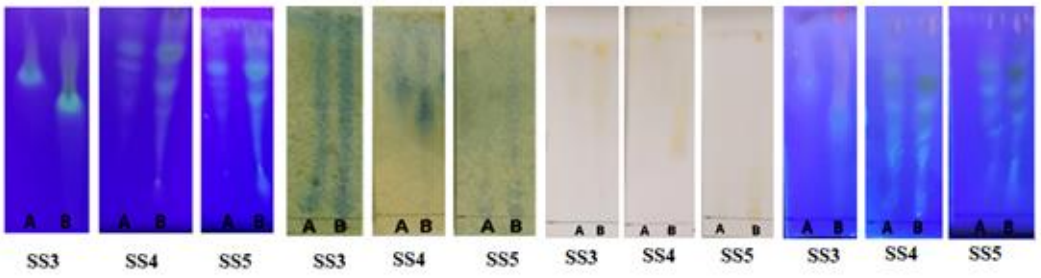

Figure 1. Chromatograms developed using the solvent systems (SS3-SS5) and the separation of spots using the different spray reagents

Table 3. R_f values of the different spots developed in the solvent systems which gave the highest number of spots visualized using the appropriate spray reagents

Secondary Metabolites	Solvent System	RDFP		WDFP	
		No. of Spots	R_f Value	No. of Spots	R_f Value
Falvonoids and steroids	n-Butanol-Acetic Acid-Water	4	0.90 0.77 0.68 0.55	5	0.90 0.72 0.59 0.46 0.13
Phenols, Tannins and Flavonoids	Chloroform-Acetic Acid-Water	1	0.98	1	0.96
Alkaloids	Chloroform-Methanol	1	0.96	1	0.96
Coumarins, Anthraquinones, Anthrones and Phenols	n-Butanol-Acetic Acid-Water	7	0.90 0.80 0.72 0.60 0.49 0.41 0.23	7	0.90 0.78 0.68 0.58 0.49 0.39 0.19
Anthraquinones	Chloroform-Methanol	1	0.98	1	0.98
Higher Alcohols, Phenols, Steroids and Essential Oils	n-Butanol-Acetic Acid-Water	1	0.96	3	0.96 0.71 0.28

The test for phenols, tannins, and flavonoids resulted in only one spot for both the samples using the different solvent systems, but the highest Rf value was observed in chloroform-acetic acid-water (50:45:5). Likewise, the alkaloids and anthraquinones test resulted in only one spot in both samples using chloroform-methanol (5:1). The test for coumarins, anthraquinones, anthrones, and phenols showed the separation of 7 spots in both samples to be almost similar Rf values. Lastly, the test for higher alcohols, phenols, steroids, and essential oils yielded only one spot for the RDFP while three spots for the WDFP using n-butanol-acetic acid-water (4:1:5).

DPPH free radical scavenging activity assay

The free radical scavenging activity of 100, 300, and 1000 ppm of the dragon fruit peel extract in comparison to ascorbic acid as the standard is shown in Figure 2. Ascorbic acid exhibited a free radical scavenging activity of 89.54, 94.98, and 95.05% at concentrations of 100, 300, and 1000 ppm, respectively. The red dragon fruit peel extract gave the lowest free radical scavenging activity than the white dragon fruit peel extract.

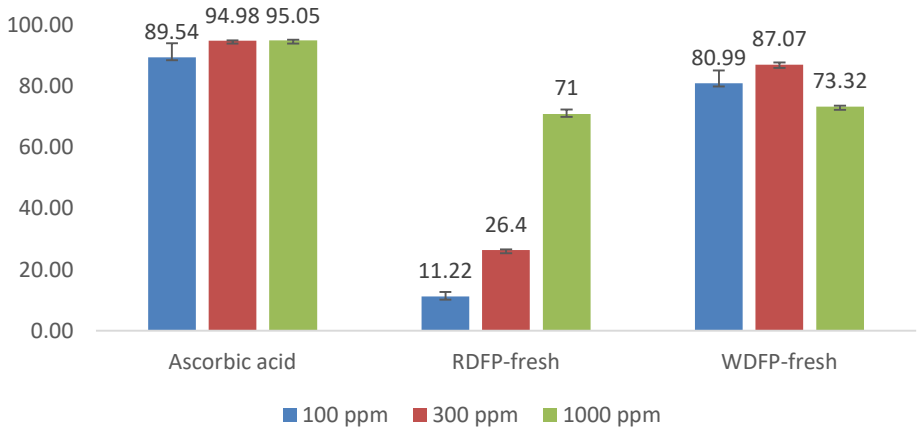

Figure 2. DPPH free radical scavenging activity of the RDFP and WDFP ethanolic extracts

The concentration-dependent response of the free radical scavenging activity of the ethanolic extracts using GraphPad Prism 8. According to Chen *et al.* (2013), GraphPad Prism showed the best performance giving minor variance with the actual EC50 (Figure 3). The antioxidant activity of the extracts increases with increased concentration.

Furthermore, the antioxidant activity was expressed in median effective concentration (EC50), the concentration needed to scavenge 50% of the free radicals (Table 4).

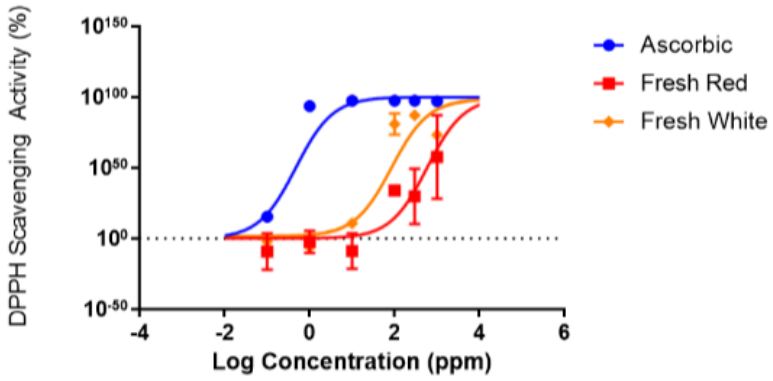

Figure 3. DPPH scavenging activity (%) of dragon fruit peel extracts and ascorbic acid as the standard against the log of concentration

Table 4. EC_{50} values of the extracts compared to ascorbic acid standard

Standard/Extract	EC_{50}	Classification* (Blois Method)
Ascorbic Acid (Standard)	0.47 ±0.80	Very strong antioxidant
RDFP Extract	596.50 ±0.63	Weak antioxidant
WDFP Extract	43.23 ±0.74	Strong antioxidant

* EC_{50}< 50 ppm = very strong antioxidant
EC_{50} = 50-100 ppm = strong antioxidant
EC_{50} = 101 – 150 ppm = medium antioxidant
EC_{50}> 150 ppm = weak antioxidant

Discussion

In this study, the recovery rate of red dragon fruit peels was 26%, whereas the recovery rate of white dragon fruit peels was 35%. The high recovery percentages in dragon fruit peels indicated that a significant amount of waste is produced throughout the process. Consequently, developing products that effectively use these wastes to promote a cleaner environment and additional income to dragon fruit farmers. Hence, all of the dragon fruit's parts and components can be utilized.

The results of the alkaloid test conformed with the study conducted by Lin *et al.* (2021), where ten alkaloids were found to be present in the green and red dragon fruit peels. These were N-benzyl methylene isomethylamine, choline, serotonin, spermine, trigonelline, gomphrenin I, 6-deoxyfagomine, dopamine hydrochloride, amaranthin, and N-cisferoloyltyramine. The red

variety did not contain tertiary amine, while the red variety did not contain primary amine. Based on the alkaloids identified by Lin *et al.* (2021), trigonelline was the only tertiary alkaloid presented in dragon fruit peels. The primary alkaloids are serotonin, spermine, and dopamine hydrochloride.

Tannins were not found in the red and white dragon fruit peels, contrasting with Manihuruk *et al.* (2017). The variations in the results can be attributed to the differences in the extraction procedure. In this study, the dragon fruit peels were extracted with 95% ethanol, while in their study, maceration using distilled water was acidified with citric acid to pH 5. Solvents and methods can affect the phytochemicals extracted from the samples (Truong *et al.,* 2019).

This study revealed that both peel samples contained flavonoids, coumarins, and terpenoids, among other substances. Despite these promising findings, the white dragon fruit peel consistently outperformed the red dragon fruit peel ineffectiveness. Susanti *et al.* (2012) published similar results, discovering that white dragon fruit is rich in flavonoids and has considerable antioxidant activity. This indicated that it may be used as a conveniently accessible source of naturally occurring antioxidants. Furthermore, it may be used for extensive study to evaluate white dragon fruit's potential to cure a wide variety of human diseases in the future (Wany *et al.*, 2020).

Additionally, thin layer chromatography (TLC) is a practical method for characterizing raw herbs, active constituent-enriched extracts, and formulations (Poole, 2003). Standardized TLC methods may be utilized successfully for screening analysis and quality assessment of a plant or its derived herbal items. These findings indicated that the dragon fruit peel extracts had antioxidant potential due to the polyphenolics visualized in the chromatograms.

Further analysis using the extract's DPPH free radical scavenging activities revealed that the white variety had higher antioxidant potential than the red variety. Nurliyana *et al.* (2010) reported the IC50 of the red and white dragon fruit peels to be 300 ppm and 400 ppm, and the antioxidant properties to be 83.48 ± 1.02%,87.02 ± 2.24%, respectively. The peel samples were extracted with 70% ethanol using Soxhlet for 12 hours, followed by lyophilisation. The EC50 values in this research were 596.5 ± 0.63 ppm for the red dragon fruit peel and 43.23 ± 0.74 ppm for the white variety. It can be inferred that the peels of the red variety in their study are both classified as "weak antioxidants." The use of Soxhlet in the extraction may affect the antioxidant property. According to Xu *et al.* (2007) stated that, heat has an effect on the phenolic compounds as well as on the flavone glycosides. The peels in this study were extracted with 95% ethanol using the soaking method. The extraction method may be the reason why the white variety showed a

"strong antioxidant" property. The red variety in this study was weak and conformed with that of Nurliyana *et al.* (2010).

In conclusion, this study revealed that the white variety (H. undatus) contained more phytochemical compounds in the two tested dragon fruit peel varieties, as confirmed by the different tests done and the TLC assays. Furthermore, white has higher % free radical scavenging activity than the red variety. Therefore, the white variety is considered a strong antioxidant, while the red variety weak antioxidant. Moreover, the white variety was superior in quality over the red variety. These findings suggested that both peel varieties could be used as a natural source of antioxidants which can be applied in the food system, pharmaceutical and cosmetic products.

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