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Assessment on the Physicochemical and Phytochemical Properties, Nutritional and Heavy Metal Contents, and Antioxidant Activities of *Hylocereus polyrhizus* Peel from Northern Philippines

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Abstract

Background/Objectives: In the Philippines, *Hylocereus polyrhizus* is gaining upsurge interest due to its potential health benefits. Aside from the edible fruit pulp, various products from its peel are being developed, and claimed as health boosters. Thus, this study was conducted to assess the physicochemical characteristics, phytochemical constituents, nutritional and heavy metal contents and antioxidant activity of the peel of *H. polyrhizus*. **Methods/Statistical analysis:** Physicochemical and phytochemical screening were conducted following established protocols. Nutritional value and trace metals were measured with food analytical standard methods. Antioxidant activity was estimated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing capacity (FRC) assays. Statistical analyses were carried out for DPPH and FRC assays using Graph-Pad Prism. **Findings:** Results showed that *H. polyrhizus* peel contains alkaloids, coumarins, flavonoids, phenols, and triterpenoids. Trace amounts of arsenic (0.022 mg/kg), lead (0.887 mg/kg), cadmium (0.068 mg/kg) and chromium (0.225 mg/kg) were detected which were all within the acceptable limit set by the WHO for herbal materials. *H. polyrhizus* peel contained 6.72% moisture, 14.7% ash, 0.526% total fat, 0.226% saturated fat, 3.73% total sugar, 4.28% crude protein, 73.8% total carbohydrate, and 63.2% total dietary fiber. Other nutrients detected are macronutrients such as K > Ca > Mg > Na, micronutrients Mn > Fe, and Vitamin C. Moreover, it exhibited remarkable antioxidant activity in terms of FRC assay and DPPH assay (IC₅₀ = 986.8 µg/mL). The present study results showed that *H. polyrhizus* peel could be used as a potentially rich source of nutrients and natural antioxidants. Thus, it can be developed as an ingredient for food and health products. **Novelty/Applications:** The preliminary data obtained provide information that could support the development of products from dragon fruit peel. The conversion of these wastes into utilizable natural health products would help in reducing environmental problems

associated with processing waste disposal.

Keywords: Antioxidant; Dragon Fruit Peel; *Hylocereus polyrhizus*;
Phytochemical Screening; Nutritional Content; Heavy Metals

1 Introduction

Hylocereus polyrhizus, also known as pitaya or dragon fruit, is an introduced crop in the Philippines that have gained popularity in research and development as an alternative crop in addressing economic growth in the country⁽¹⁾. Morphologically, the fruit of *H. polyrhizus* is oblong with a pink to red peel and large green scales and its pulp is colored red to intense purple⁽²⁾. Aside from being a rich source of phytochemicals and nutrients that could provide many beneficial effects when consumed fresh, it is also utilized to develop and formulate food and health products⁽²⁾. The cultivation of *H. polyrhizus* is increasing dramatically especially in the Philippines' northern region for the past few years owing to the overwhelming demand from local markets⁽³⁾. Besides, *H. polyrhizus* is believed to be the favorite of local consumers due to its sweet taste and attractive color compared to other species or varieties⁽³⁾. However, the consumption of *H. polyrhizus* gained significant increase in waste material, especially the peels. As a result, this waste material can cause detrimental effects in the environment. Annually, the Philippines generate approximately 6.53 million tons of fruit and vegetable waste⁽⁴⁾. Therefore, to keep pace with the zero-waste management system, it is essential to utilize the *H. polyrhizus* peel for food and natural health-based products including nutraceuticals and cosmeceuticals.

Since there is a growing consumer awareness on the safety, and health issues concerning the use of food and natural health products which includes nutraceuticals, food supplements, cosmeceuticals, and among others, there is a need to establish the qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility of raw materials in the product development. Notably, literature suggests that geographical locations have considerable impact on the levels of phytochemicals, proximate nutrients, selected minerals and conversely their biological activities⁽⁵⁾. This study is an exerted effort in the research and development to facilitate the further expansion of the emerging industry of dragon fruit in the northern Philippines which is constrained by the lack of standardization in fruit quality and product development⁽¹⁾. Therefore, the aim of the present work is to determine the physicochemical characteristics, phytochemical constituents, traces of heavy metals, proximate composition, minerals and vitamin content, and antioxidant activities of *H. polyrhizus* peel obtained in Ilocos Norte, Philippines.

2 Materials and Methods

2.1 Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 1,10-phenanthroline monohydrate, ferric chloride and ascorbic acid were obtained from Sigma-Aldrich (Germany/USA). All other chemicals were of analytical grade and supplied from other commercial sources.

2.2 Plant material

An approximate of 30 kilograms of *H. polyrhizus* fruits were purchased in Batac, Ilocos Norte. Fruit peels were separated from the edible pulps. The peels were oven-dried at 35°C for 24 hours, pulverized using Cyclotec mill then kept in an air-tight container and stored at 5°C until further analysis. A 500 g of the resulting powder was extracted through maceration with methanol for 72 hours then concentrated using a rotary evaporator at 40°C. The peel crude extract was obtained and stored at 5°C until further

analysis.

2.3 Physicochemical evaluation

The physicochemical characteristics of powdered peel and extract were determined as per WHO guidelines⁽⁶⁾. Parameters evaluated include the color, odor, taste, appearance, pH, specific gravity, moisture content, ash content and aqueous solubility.

2.4 Phytochemical screening

The peel crude extract was qualitatively tested to reveal the presence of phytochemical constituents such as alkaloids, coumarins, flavonoids, phenols and triterpenoids. These were identified by characteristic color changes (chemical-based) using previously reported procedure⁽⁷⁾.

2.5 Determination of proximate, heavy metal, mineral and vitamin content

Analyses on proximate, heavy metal, mineral and vitamin composition of *H. polyrhizus* peel were performed at the Standards and Testing Division, Department of Science and Technology – Industrial Technology Development Institute (DOST-ITDI), Bicutan, Taguig, Metro Manila, Philippines using AOAC official method⁽⁸⁾. Briefly, the AOAC official method numbers 923.09 and 985.29 were used to analyze total sugar and total dietary fiber, respectively. Analyses were conducted by block digestion-Kjeldahl for crude protein, acid hydrolysis for total fat, gas chromatography for saturated fat, and by calculation for total carbohydrate and for food energy. Trace amounts of lead, cadmium, and chromium were determined according to the AOAC official method number 999.11. The hydride vapor generator atomic absorption spectrometry was used for detection of arsenic content. The AOAC official method number 969.32 was used in the detection of calcium, magnesium, sodium, iron, potassium, manganese and high-performance liquid chromatography for vitamin C analysis. The results were expressed by calculating the mean values obtained in each analysis.

2.6 DPPH radical scavenging assay

Antioxidant radical scavenging activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The assay was performed as previously described⁽⁹⁾ with minor modification. DPPH 0.1 mM was prepared with methanol. Then, 1.5 ml of various concentration of peel extract in methanol (50 µg/mL – 500 µg/mL) were pipetted into 1.5 ml DPPH solution and mixed. After incubation for 30 minutes in a dark place at room temperature, the absorbance was read against a control at 517 nm. Inhibition of free radical by DPPH in percent (%) is calculated using the following formula: Inhibition (%) = $[A_{control} - A_{sample} / A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the test sample. The concentration that provides 50% inhibition (IC_{50}) expressed in µg/mL was calculated against test sample concentration. Ascorbic acid was used as the positive control.

2.7 Ferric reducing capacity assay

The ferric reducing capacity (FRC) assay is based on the formation of $Fe^{III}-(Phen)_3$ complex and its disruption in the presence of reducing agents⁽¹⁰⁾. It is similar to the FRAP assay except the use of 1, 10-phenanthroline instead of TPTZ (2,4,6-tri-pyridyl-s-triazine)⁽¹¹⁾. The FRC of *H. polyrhizus* peel extract as antioxidant was determined according to the method described with slight modifications⁽¹²⁾. The reaction mixture containing 0.6 mL 0.05% O-phenanthroline in methanol, 1.2 mL 200 mM ferric chloride, and 1.2 mL extract at various concentrations ranging from 50 µg/mL to 500 µg/mL in a final volume of 3 ml was incubated for 10 minutes at room temperature. Then, the absorbance of the reaction mixture was read at 510 nm. An increase in the absorbance of the reaction mixture indicated increased reducing capacity. Ascorbic acid was used as positive control.

2.8 Data analysis

All analyses for antioxidant activities (DPPH and FRC assays) were carried out in triplicates and data reported are mean \pm standard deviation (SD). All statistical analyses including computation of IC_{50} values were performed using GraphPad Prism 9.0.

3 Results and Discussion

3.1 Physicochemical evaluation

Table 1. Physicochemical Characteristics of *H. polyrhizus*

Parameter	Peel Powder	Methanol Peel Extract
Color	Dark pink	Red purple
Appearance	Fibrous powder	Gummy
Odor	Indistinct	Indistinct
Taste	Acidic	Acidic
pH	5	4.8
Specific gravity	—	1.1361
Moisture content	67.2 mg/g	—
Ash content	147 mg/g	—
Aqueous solubility	Very slightly soluble	Freely soluble

The peel powder's water solubility is a consideration in establishment of a suitable formulation strategy in product development. Peel powder was very slightly soluble (1:1000-10000) to water while the peel crude extract was freely soluble (1:1). The peel powder's low moisture content implies its stability for a longer period and less susceptibility to microbial contamination⁽¹³⁾. The peel powder's ash content represents 14.7% of the total sample is greater than the 14% maximum acceptable limit recommended by European Pharmacopoeia⁽¹⁴⁾. The relatively high ash content value can be attributed to the presence of certain minerals⁽¹⁵⁾. The pH and specific gravity of the extract were also recorded as shown in Table 1.

3.2 Phytochemical screening

The presence of constituents such as alkaloids, coumarins, flavonoids, phenols, and triterpenoids in *H. polyrhizus* peel (Table 2), suggests its potential medicinal and nutritional applications. Alkaloids showed wide variety of biological activities including antihypertensive, antiarrhythmic, antimalarial activity, anticancer actions and among others⁽¹⁶⁾. Coumarin derivatives from other plants have shown potential as a skin whitening agent⁽¹⁷⁾ while p-coumaric acid could also attenuate skin hyperpigmentation⁽¹⁸⁾. Besides the antioxidant effect of flavonoids, there is also a growing evidence of the versatile health benefits which includes anti-inflammatory⁽¹⁹⁾, anti-proliferative and anticancer activity⁽²⁰⁾, antihypertensive effects, coronary heart disease prevention⁽²¹⁾ and anti-human immunodeficiency virus functions⁽²²⁾. Similarly, phenolic compounds are known for their chemo-preventive properties such as antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory effects⁽²³⁾. These constituents in the peel extracts may act synergistically or antagonistically to produce biological activities and health benefits.

3.3 Heavy Metals, Nutritional, Minerals, and Vitamin Composition

In parallel with the increasing interest in the health benefits of *H. polyrhizus* peel, safety concerns are also recognized. The concentrations of arsenic, lead, cadmium, and chromium for the tested peel powder were found to be 0.22 mg/kg, 0.068 mg/kg, 0.0225 mg/kg, and 0.887 mg/kg respectively, which were all within the maximum permissible limit set by the WHO for herbal medicines⁽⁶⁾.

On the other hand, the crude protein content of the dragon fruit peel which is 42.8 mg/g may be useful in food formulation systems⁽²⁴⁾. Generally, fruits contain low amounts of fat. Total fat includes all types of dietary fat, which is equivalent to 5.26 mg/g in the fruit peel and a saturated fat of 2.26 mg/g. The total carbohydrate is the combined amount of all three types of carbohydrate namely, starch, sugar and fiber. From the total carbohydrate content, 5.05 % is the total sugar which is lower than the result obtained by other study which is 8.4% and were identified mainly as glucose, fructose and maltose⁽²⁵⁾. The high content in the peel powder's total dietary fiber may help in the maintenance of human health. Fiber decreases the risks of constipation by increasing stool bulk⁽²⁶⁾ and known to reduce cholesterol level of the body⁽²⁷⁾. Generally, the fruit peel had relatively high calories of nutritive value (317 kCal/100g) that could provide health benefits.

H. polyrhizus peel powder contained certain concentrations of minerals (Table 3) which may support a wide range of potential health benefits. Among the tested macro elements, potassium was highest followed by Ca, Mg and Na while Mn was higher than Fe in microelements. Potassium and sodium play roles in the maintenance of human normal physiology^(28,29).

Table 2. Phytochemical Components of *H. polyrhizus*

Phytochemical	Test	Result
Alkaloid	Wagner	+
Phytosterols	Liebermann-Burchard	-
Tannins	Ferric Chloride	-
Phenols	Lead acetate	+
Flavonoids	Alkaline reagent	+
Coumarins	10% Sodium hydroxide	+
Saponins	Froth	-
Quinones	Sulphuric acid	-
Cardiac Glycosides	Keller-Killani	-
Triterpenoids	Salkowski	+
Anthraquinones	2% HCl	-
Steroids and Phytosteroids	Chloroform + sulphuric acid	-

(+) presence

(-) absence

However, too much sodium in the diet causes an increase in blood pressure⁽³⁰⁾. In contrast, reduced blood pressure is linked to increased potassium intakes which may be due to potassium's ability to increase sodium excretion and the vasoactive effects of potassium on blood vessels⁽²⁹⁾.

Table 3. Proximate composition of *H. polyrhizus* peel powder

Composition	Amount per 1 gram
Crude Protein (Nx6.25)	42.8 mg
Total Fat	5.26 mg
Saturated Fat	2.26 mg
Total Carbohydrate	738 mg
Total Sugar	37.3 mg
Total Dietary Fiber	632 mg
Food Energy	317 Kcal/100g

The low sodium and relatively high potassium concentrations obtained from this study suggest that the consumption of dragon fruit peel is tolerable to hypertensive patients requiring low sodium diet. The presence of 49 mg/kg Vitamin C in the dragon fruit peel contributed for its acidic taste (Table 4). Numerous researches have shown that an adequate intake of vitamin C assumes a beneficial role in lowering the risk of developing cancers⁽³¹⁾ and prevention of diseases⁽³²⁾.

Table 4. Mineral content per 1 gram of *H. polyrhizus* peel powder

Minerals	Amount per 1 gram
Calcium	9.56 mg
Magnesium	3.79 mg
Sodium	0.83 mg
Iron	0.023 mg
Potassium	54.4 mg
Manganese	0.11 mg
Vitamin C	49 mg/kg

3.4 DPPH Antioxidant Activity

H. polyrhizus peel extract and the positive control ascorbic acid exhibited a concentration dependent DPPH radical inhibition (Figure 1). This represents that an increased amount of antioxidant in a given volume of peel extract is responsible for the increased reduction of the DPPH solution⁽³³⁾. In this study, *H. polyrhizus* peel extract exhibited the minimum activity of 11.77% at 50 $\mu\text{g/mL}$ and the maximum activity of 39.24% at 500 $\mu\text{g/mL}$. The IC_{50} value obtained by interpolation from linear regression analysis was 986.8 $\mu\text{g/mL}$ which is comparative with the $\text{IC}_{50} = 994.25 \pm 29.63 \mu\text{g/mL}$ of 95% aqueous ethanol dragon fruit peel extract in Taiwan⁽³⁴⁾. These values were higher in comparison with the other peel extracts using 70% ethanol ($0.30 \pm 0.01 \text{ mg/mL}$) in Malaysia⁽³⁵⁾ and both ethanol HCl (159.6 $\mu\text{g/mL}$) and ethyl acetate (648.9 $\mu\text{g/mL}$) in Indonesia following DPPH assay⁽³⁶⁾. The data suggest the considerable impact of geographical distributions, solvents and extraction procedures on the antioxidant activity of the *H. polyrhizus* peel.

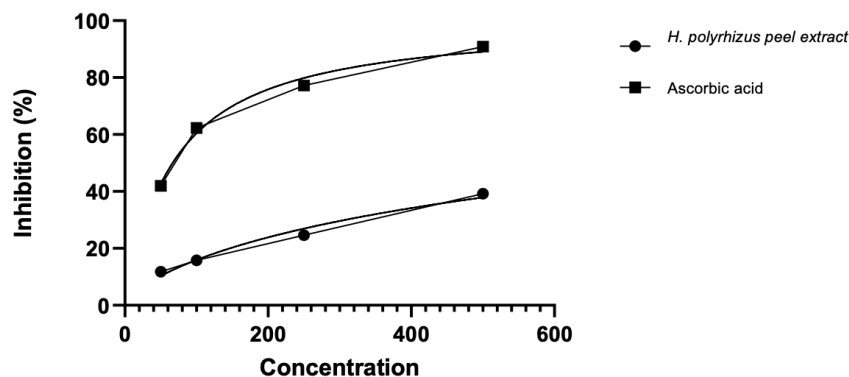


Fig 1. DPPH radical scavenging activities of *H. polyrhizus* peel extract and ascorbic acid at different concentrations (50, 100, 250, 500 $\mu\text{g/mL}$). Values are expressed as the mean \pm standard deviation ($n=3$) of the inhibition percentage.

3.5 Ferric Reducing Capacity

As secondary antioxidant, *H. polyrhizus* peel extract and ascorbic acid showed dose dependent ferric reducing capacity (Figure 2). A gradual increase in the absorbance of *H. polyrhizus* peel extract at 510 nm ranging from 0.032 ± 0.002 to 0.079 ± 0.002 was observed as the concentration increased from 50 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$. Similarly, higher absorbance of ascorbic acid ranged from 0.073 ± 0.001 to 0.124 ± 0.001 which indicated reduction of ferric ions to ferrous ions^(37,38). Hence, the peel extract of dragon fruit may act as electron donors⁽³⁹⁾ in a sufficient amount and could react with free radicals to convert them into more stable products thus decreasing the extent of Fenton reaction which is implicated in many diseases⁽³³⁾.

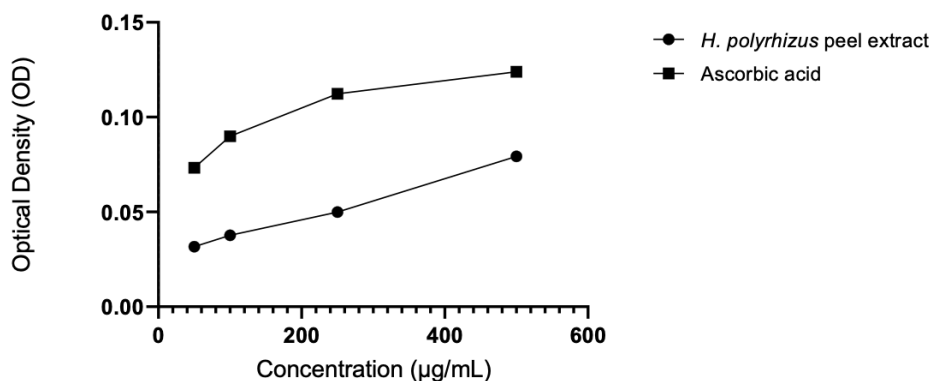


Fig 2. FRC of *H. polyrhizus* peel extract and ascorbic acid at different concentrations (50, 100, 250, 500 $\mu\text{g/mL}$). Values are expressed as the mean \pm standard deviation ($n=3$) of the OD.

4 Conclusion

The *H. polyrhizus* peel contained important phytochemical constituents that can exhibit biological activities. The quantitative and qualitative values detected in different analyses were all within acceptable limits and therefore, it is suggested as a potential source of micro and macronutrients, vitamin C and antioxidants that could be of nutritional and health benefits. Thus, *H. polyrhizus* peel can be considered in the development and formulation of natural health products.

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