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Antioxidant Potential and Active Compound Identification of *Hylocereus costaricensis* and *Hylocereus undatus* Peel Extracts using LC-MS/MS

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Abstract: The object of this research was to determine the antioxidant potential and identify active compounds in the peel extracts of red dragon fruit *(Hylocereus costaricensis*) and white dragon fruit (*Hylocereus undatus*). Phytochemical tests were conducted to identify flavonoids, alkaloids, tannins, coumarins, and saponins. The antioxidant activity was assessed using the DPPH assay, while vitamin C content was quantified using UV-Vis spectrophotometry, and bioactive antioxidant compounds were identified through LC-MS/MS spectrophotometry. The results demonstrated that phytochemical screening revealed the presence of flavonoids and alkaloids in both extracts. Antioxidant activity assays indicated that the red dragon fruit peel extract exhibited an IC_{50} of 32.7 ppm (very active), while the white dragon fruit peel extract had an IC_{50} of 50.2 ppm (active), with the vitamin C control showing an IC_{50} of 11.90 ppm. The red dragon fruit peel extract had the highest vitamin C content (1.45%) compared to the white dragon fruit peel (1.04%). LC-MS/MS analysis identified several organic compounds, including the flavonoid isoliquiritigenin and alkaloid compounds such as aporphine, melosmine, 3-carbamoyl-1-(2-deoxy-beta-*D*-erythro-pentofuranosyl)-4,5-dimethylpyridinium, and alkaloid group (6-Amino-5-{[2(diethylamino) ethyl]amino}-1-propyl -2,4(1H,3H)-pyrimidinedione). These findings suggest that both red and white dragon fruit peel extracts possess significant antioxidant potential, with red dragon fruit peel demonstrating superior activity. This study can be used for further research on food technology in producing food products that are rich in nutrients.

Keywords: antioxidant, *Hylocereus costaricensis*, *Hylocereus undatus*, LC-MS

Abstrak: *Tujuan penelitian ini adalah untuk mengevaluasi potensi antioksidan dan mengidentifikasi senyawa aktif dalam ekstrak kulit buah naga merah (Hylocereus costaricensis) dan buah naga putih (Hylocereus undatus). Uji fitokimia dilakukan untuk mengidentifikasi flavonoid, alkaloid, tanin, kumarin, dan saponin. Aktivitas antioksidan ditentukan menggunakan uji DPPH, sedangkan kandungan vitamin C diukur menggunakan spektrofotometri UV-Vis, dan senyawa antioksidan bioaktif diidentifikasi melalui LC-MS/MS spektofotometri. Hasil penelitian mengungkapkan bahwa skrining fitokimia menunjukkan adanya flavonoid dan alkaloid pada kedua ekstrak. Uji aktivitas antioksidan menunjukkan bahwa ekstrak kulit buah naga merah menunjukkan IC50 sebesar 32,7 ppm (sangat aktif), sedangkan ekstrak kulit buah naga putih memiliki IC50 sebesar 50,2 ppm (aktif), dengan kontrol vitamin C menunjukkan IC50 sebesar 11,90 ppm. Ekstrak kulit buah naga merah memiliki kadar vitamin C tertinggi (1,45%) dibandingkan dengan kulit buah naga putih (1,04%). Analisis LC-MS/MS mengidentifikasi beberapa senyawa organik, termasuk flavonoid isoliquiritigenin dan senyawa alkaloid seperti aporfin, melosmin, 3-karbamoil-1-(2-deoksi-beta-D-eritro-pentofuranosil)-4,5-dimetilpiridinium, dan grup alkaloid (6-Amino-5-{[2(dietilamino) etil]amino}-1-propil-2,4(1H,3H)-pirimidindion). Penemuan ini menyatakan baik ekstrak kulit buah naga merah ataupun putih keduanya berpotensi sebagai antioksidan yang cukup signifikan, dimana kulit buah naga merah memperlihatkan potensi aktivitas yang lebih unggul. Penelitian ini dapat digunakan untuk penelitian lebih lanjut terhadap teknologi pangan dalam menghasilkan produk pangan yang kaya nutrisi.*

Kata kunci: *antioxidant, Hylocereus costaricensis, Hylocereus undatus, LC-MS*

INTRODUCTION

Natural product compounds are well-known in their bioactivities such as antimicrobial, anti-cancer, anti-viral, anti-inflammatory, and antioxidant (Sinaga *et al*. 2023; Mayanti *et al*. 2022). Natural antioxidants are considered the best source of protection against

oxidative damage, and many studies have been conducted to discover antioxidants derived from natural sources (Mulyani *et al*. 2023; Ballester *et al*. 2023; Mulyati *et al*. 2024). The results of these studies have led to the development of food products or supplements that can be consumed by the general publics. One plant that holds potential as a source of antioxidants is the red dragon fruit (*Hylocereus polyrhizus*) (Nishikito *et al*. 2023) While the flesh of the dragon fruit is widely known for its health benefits, the peel also contains bioactive compounds with medicinal potential, such as cyanidin 3-rhamnosyl glucoside 5-glucoside, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, polyphenols, carotenoids, phytoalbumin, and betalains (Bhadauria *et al*. 2024; Kiranmai 2022). Betalains are polar pigments, consisting of betacyanins and betaxanthins (Figure 1) (Rebecca *et al*. 2010).

According to Nishikito (2023), the red dragon fruit peel has a stronger ability to inhibit cancer cell growth compared to its flesh and does not contain any toxins (Nishikito *et al*. 2023). Furthermore, given the growing emphasis on sustainable utilization of agricultural waste, further investigation into the bioactive potential of dragon fruit peels is essential. Previous studies have explored the antioxidant activity and phytochemical composition of different parts of *Hylocereus* species. Suh *et al*. (2014) investigated metabolite profiling and betalain biosynthesis in *H. polyrhizus* and *H. undatus*, highlighting the role of betalains in antioxidant activity. Similarly, Som *et al*. (2019) compared the antioxidant properties of H. undatus peel and foliage, focusing on total phenolic content and radical scavenging assays. Choo & Yong (2011) assessed free radical scavenging and ferrous ion chelating activities in dragon fruit, attributing its antioxidant effects to phenolic compounds and ascorbic acid. However, while these studies confirm the antioxidant potential of dragon fruit, they primarily focus on betalains, phenolics, and vitamin C, with limited exploration of other bioactive compounds such as alkaloids and flavonoids.

To address these gaps, this project aims to determine the antioxidant potential of *Hylocereus costaricensis* and *Hylocereus undatus* peel extracts and identify bioactive compounds using LC-MS/MS analysis. Unlike previous studies, which predominantly focus on betalains and phenolics, this research expands the scope by investigating the existence of alkaloids and flavonoids in dragon fruit peels, identifying compounds such as isoliquiritigenin, melosmine, and aporphine derivatives for the first time. Alkaloids are the most abundant organic compounds found in nature, primarily derived from plants. All alkaloids contain at least one nitrogen atom, typically in a basic form, and this nitrogen atom is often part of a heterocyclic ring (Zhao *et al*. 2024). Based on the nitrogenous heterocyclic ring, alkaloids can be categorized into several groups, such as pyrrolidine, piperidine, isoquinoline, quinoline, and indole alkaloid (Mulyani *et al*. 2023; Safriansyah *et al*. 2022). Flavonoids, on the other hand, are the largest group of phenolic compounds found in nature (Zhao *et al*. 2024). These compounds are responsible for the red, purple, blue, and yellow pigments in plants. In higher plants, flavonoids are present in both vegetative parts and flowers. Terpenoids, often characterized by their

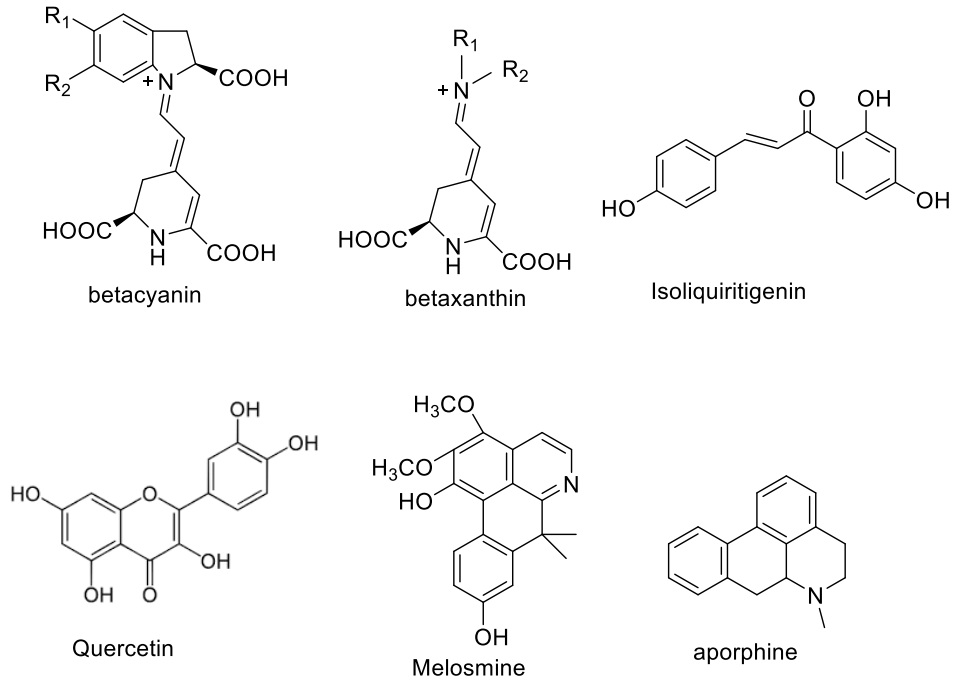

Figure 1. The isolated chemical structures of the most common found in red and white dragon extracts.

fragrance, are isolated from plant materials through distillation and are known as essential oils (Sinaga *et al*. 2022; Sinaga *et al*. 2023; Widiastuti *et al*. 2023)

Additionally, while most previous studies focus on *H. polyrhizus* and *H. undatus*, this study includes *H. costaricensis* (red-flesh dragon fruit), which has been less studied in terms of its bioactive compound profile and antioxidant activity. The findings of this project also demonstrate that the red dragon fruit peel extract exhibits superior antioxidant potential (IC_{50}: 32.7 ppm) compared to the white dragon fruit peel extract (IC_{50}: 50.2 ppm), reinforcing its potential application in nutraceuticals and functional foods. By providing a more comprehensive phytochemical profile of dragon fruit peels, this study contributes new insights into the antioxidant mechanisms beyond betalains and phenolics.

MATERIALS AND METHOD

Materials

The instruments used in this study consist of a water bath, glassware, porcelain crucibles, an oven, an evaporator, a UV-Vis spectrophotometer, which has a maximum wavelength of 270 nm, and an LC-MS/MS with an LC component that séparates intricate mixtures using a C18 reverse-phase column under a gradient elution system with a mobile phase consisting of water with 0.1% formic acid and acetonitrile with 0.1% formic acid. The tandem mass spectrometer, operating in tandem (LC-MS/MS), employs the electrospray ionization (ESI) method in both positive and negative modes to detect compounds within a mass range of m/z 100–1000. The materials used in this research include red dragon fruit peel, white dragon fruit peel, 70% methanol solution, magnesium powder, concentrated HCl solution, amyl alcohol solution, chloroform-ammonia solution, distilled water (aquades), Mayer's reagent, Dragendorff’s reagent, and Wagner’s reagent (Widiastuti *et al*. 2023).

Determination of Moisture Content

A total of 3.00 grams of the simplicia sample was weighed into a pre-dried and pre-weighed petri dish. The total weight was recorded, and the sample was dried in an oven at 100-105°C for 3 hours. The petri dish was carefully placed to avoid contact with the oven walls. The petri dish containing the sample was then transferred into a desiccator, covered, cooled, and reweighed. The dish was returned to the oven until a constant weight was achieved. The moisture content was calculated using the formula (Nielsen 2009):

$$\% \text{ Moisture content} = (W_0+W_s)-W_i / W_s \times 100\%$$

Where:
W_0 = Weight of the empty petri dish (g)
Wi = Weight of the petri dish and sample after drying (g)
W_s = Initial weight of the sample (g).

Determination of ash content

Approximately 2-3 grams of the sample was weighed accurately and placed into a porcelain crucible that had been preheated to obtain a constant weight. The sample in the crucible was incinerated, cooled, and reweighed. The ash content was calculated based on the dried material using the formula (Ismail 2017):

$$\% \text{ Ash content} = (W_2-W_0)/(W_1-W_0) \times 100\%$$

Where:
W_0 = Weight of the empty crucible (g) : (g) :
W_1 = Weight of the crucible and sample before incineration
W_2 = Weight of the crucible and sample after incineration (g)

Extraction and Fractionation

The extraction process began by peeling red and white dragon fruits to collect the peels. The peels were dried until their moisture content was below 10% to prevent fungal growth, then stored as simplicia. The simplicia was extracted using maceration with 70% methanol in a ratio of 1:10 for 3 x 24 hours. The process was repeated until complete extraction was achieved. The extract was separated from methanol through evaporation using a rotary evaporator (Widiastuti *et al*. 2024).

Phytochemical Screening

Phytochemical screening was conducted on the dragon fruit peel extract to identify secondary metabolites through qualitative analysis. The tests performed included the detection of flavonoids, alkaloids, tannins, coumarins, and saponins (Mulyati *et al*. 2024) :

Flavonoid Test: The test solution was constituted by first dissolving approximately 0.1 milliliters (mL) of the extract in 100 milliliters (mL) of hot water. The mixture was subsequently boiled for five minutes. A total of 5 mL of the solution was poured into a test tube, followed by the addition of 0.10 mg of Mg powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol. The solution was vigorously shaken. The occurrence of flavonoids was indicated by the appearance of red, yellow, or orange coloration in the amyl alcohol layer.

Alkaloid Test: Approximately 0.3 mL of the extract was dissolved in 10 mL of chloroform-ammonia, and then mixed with a few drops of 2 M H_2SO_4. Following a thorough shaking process, the mixture exhibited a division into two distinct layers. The layer that was found to be acidic (and colorless) was subsequently transferred into a test tube via pipette. Subsequently, Mayer's, Dragendorff's, and Wagner's reagents were introduced to the mixture. The appearance of a white precipitate (Mayer's), an

orange-red precipitate (Dragendorff's), or a brown precipitation (Wagner's) confirmed the presence of alkaloids.

Tannin Test: Approximately 0.1 mL of the extract was dissolved in 1 mL methanol, filtered, and mixed with a few drops of 1% $FeCl_3$. The presence of tannins was indicated by the formation of green, blue, or purple coloration.

Coumarin Test: A total of 2 mL of the extract was mixed with 10 mL chloroform, heated for 10 minutes in a water bath, and cooled. Then, 0.5 mL of 10% ammonia was added. The presence of coumarins was indicated by green or blue fluorescence under UV light at 366 nm.

Saponin Test: A total of 100 mL of the extract was diluted with distilled water in a 100 mL volumetric flask. A 10 mL aliquot was transferred to a test tube, heated for 5 minutes, cooled, and vigorously shaken. Persistent foam for 15 minutes indicated the presence of saponins.

Antioxidant Activity using DPPH Assay

The antioxidant activity of the dragon fruit peel extract was determined using DPPH method. A DPPH solution was prepared by dissolving 19.716 mg of DPPH (molecular weight 394.32) in methanol in a 50 mL volumetric flask, which was then covered with aluminum foil. To determine the optimal incubation time, a 1 mL aliquot of 1 mM DPPH solution was diluted to 5 mL with methanol, homogenized, and measured at maximum wavelength intervals of 10, 20, 30, 40, 50, and 60 minutes. A blank solution was prepared by diluting 1.0 mL of 0.2 mM DPPH solution to 5 mL with methanol, incubated at 37°C for the optimum incubation time, and measured at the maximum wavelength using a UV-Vis spectrophotometer (517 nm). For the test solution, 50 mg of dragon fruit peel extract was dissolved in methanol in a 50 mL volumetric flask to prepare a 1000 ppm stock solution. The solubility of the extract in methanol was visually assessed to ensure complete dissolution. The solution was vortexed and sonicated for 5 minutes to enhance solubility. No visible precipitate was observed, confirming complete solubility. Working solutions at concentrations of 100, 200, 300, 400, and 500 ppm were made by diluting appropriate volumes of stock solution to 5 mL with methanol and homogenizing each with 1 mL of 1 mM DPPH solution. The preparation of the standard vitamin C curve involved dissolving 50 mg of vitamin C in methanol to create a 1000 ppm stock solution, followed by the preparation of working solutions at 5, 10, 15, 20, and 25 ppm. Both test solutions and standards were incubated at 37°C for the optimal time and their absorbance measured at 517 nm to evaluate antioxidant activity. The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{blank absorbance-sample absorbance}}{\text{Sample absorbance}} x100\%$$

The IC_{50} value was determined by plotting concentration against inhibition percentage. The concentration that inhibited 50% of the DPPH radical was calculated using the equation $y = a\,x + b$,

where $y = 50$.

x = concentration

a = intercept

b = slope

Vitamin C Content Analysis

The vitamin C content in the dragon fruit peel extract was analyzed using UV-Vis spectrophotometry by preparing a standard vitamin C solution at 100 ppm. This was achieved by dissolving 50 mg of ascorbic acid in distilled water in a 500 mL volumetric flask. The maximum absorbance wavelength (at 270 nm) was determined by scanning a 2 ppm vitamin C solution (prepared by diluting 1 mL of 100 ppm solution to 50 mL) within 200–400 nm. A calibration curve was constructed using solutions of 4, 8, 12, and 16 ppm, prepared by diluting appropriate volumes of the 100 ppm standard solution. For the sample analysis, concentrated extracts of red and white dragon fruit peel, weighing 1.000 g and 0.995 g, respectively, were dissolved in distilled water in 25 mL volumetric flasks. These were further diluted to 20 ppm and measured for absorbance at the maximum wavelength (Rahman *et al*. 2007).

Identification of Active Compounds in Both Extracts Using LC-MS/MS

The analysis of both extracts was conducted using a method known as liquid chromatography tandem mass spectrometry (LC-MS/MS), a sophisticated analytical technique that was employed to identify bioactive compounds. The identification of bioactive compounds was confirmed by comparing retention times, exact masses (m/z), and MS/MS fragmentation patterns with authentic standards, published literature, and mass spectral databases such as METLIN, MassBank, and HMDB (Wishart *et al*. 2007). Compounds were considered tentatively identified if the molecular ion and fragmentation patterns matched within a mass accuracy of ≤5 ppmA total of 2 mL of the extract was filtered using a 0.2-micron syringe filter. A 200 µL aliquot of filtrate was mixed with 800 µL methanol and placed into a 2 mL vial for injection. The injection volume was set at 20 µL. The liquid chromatography (LC) system employed an Agilent Zorbax XDB column (2.1 mm x 50 mm, particle size 1.8 µm) with a solvent gradient of formic acid (0.1%) in water (Solvent A) and acetonitrile as Solvent B (0.1% formic acid). The flow rate was set at 0.3 mL/min with a maximum pressure of 400 bar. The needle wash solution comprised a 3:1 methanol:water mixture. In the mass

spectrometry analysis, the column compartment temperature was maintained at 30°C, and nitrogen gas was utilized at 350°C with a flow rate of 6 L/min and a nebulizer pressure of 25 psi. The capillary voltage was set at 3000 volts. The mass scan ranged from 50 to 600 with a scan time of 250 ms. Fragmentor voltages of 20, 40, 80, 120, and 160 were optimized, while the accelerator voltage was set at 7, with positive polarity.

RESULT AND DISSCUSION

The average moisture content of white dragon fruit peel was found to be 4.70%, while that of red dragon fruit peel was 4.82%. These values comply with the standard moisture content for simplicia, which must not exceed 10% (Abidin *et al*. 2025). A low moisture content is essential to prevent microbial growth, maintain stability during storage, and preserve the active compounds for extended use. The slightly higher moisture content in red dragon fruit peel may indicate differences in structural or compositional factors, such as fiber content or water-binding capacity, which merit further investigation.

The ash content of white dragon fruit peel was recorded at 1.81%, and that of red dragon fruit peel was 1.68%. These values fall within the acceptable limits defined by the Materia Medica Indonesia V Edition, which stipulates ash content should not exceed 4% (Depkes RI 1989). The low ash content not only confirms the purity of the samples but also suggests minimal contamination by inorganic materials, which is crucial for the quality of herbal products (Salgueiro *et al*. 2010). The maceration process yielded 5.0388 g of concentrated extract from white dragon fruit peel and 5.4759 g from red dragon fruit peel. The extraction yields were 27.37% for red dragon fruit peel and 25.10% for white dragon fruit peel, indicating that red dragon fruit peel contains a higher proportion of methanol-soluble compounds. This difference underscores the compositional variation between the two types of dragon fruit peels, potentially influenced by pigment concentration and secondary metabolite diversity in red dragon fruit.

Phytochemical analysis revealed the presence of flavonoids and alkaloids in both red and white dragon fruit peel extracts (can be seen in Table 1). The orange coloration in the flavonoid test highlights the abundance of bioactive flavonoid compounds, which are widely recognized for their antioxidative and anti-inflammatory properties (Manzoor *et al*. 2021). Alkaloid testing confirmed the presence of nitrogen-containing compounds with positive results across Mayer's, Dragendorff's, and Wagner's reagents. These compounds are known for their diverse pharmacological effects, including neuroprotective and anticancer activities. The absence of tannins, coumarins, and saponins simplifies the chemical complexity of the extracts, potentially focusing the antioxidant activity on flavonoid and alkaloid constituents (Kaliyaperumal *et al*. 2020).

Results of Antioxidant Activity using DPPH Assay

The antioxidant activity of the extracts was evaluated using the DPPH assay, a reliable method for assessing free radical scavenging potential. The antioxidant activity was classified based on IC_{50} values as proposed by (Molyneux 2004), where an IC_{50} below 50 ppm indicates very strong antioxidant activity, 50–100 ppm strong activity, 100–200 ppm moderate activity, and above 200 ppm weak activity. The results showed that higher concentrations of the extract corresponded to a more intense yellow color, indicative of stronger antioxidant activity.

The percentage of DPPH radical inhibition was plotted against extract concentration to generate a dose-response curve. A linear regression equation ($y = ax + b$) was derived, where y represents the percentage inhibition, and the concentration (x) corresponding to y = 50% inhibition was calculated as the IC_{50} value. Figure 2 presents the IC_{50} determination graph for both H. costaricensis and H. undatus peel extracts. The results indicate that the IC_{50} of *H. costaricensis* peel extract was 32.7 ppm, classifying it as a very strong antioxidant, while H. undatus peel extract exhibited an IC_{50} of 50.2 ppm, indicating strong antioxidant activity. In comparison, the IC_{50} of vitamin C (positive control) was 11.90 ppm. These findings demonstrate that the red dragon fruit peel extract exhibits higher antioxidant potential than the white dragon fruit peel extract. The stronger antioxidant activity of red dragon fruit peel can be attributed to its higher concentration of flavonoids

Table 1. The result of phytochemical test of *Hylocereus costaricensis* and *Hylocereus undatus* skin extracts

Phytochemical identification	*Hylocereus costaricensis* Skin Extract	*Hylocereus undatus* Skin Extract
Flavonoid	+ (orange)	+ (Orange)
Alkaloid	+ (white)	+ (white)
o Meyer	+ (brown)	+ (brown)
o Dragendorf	+ (Red)	+ (red)
o Wagner		
Tannin	-	-
Coumarin	-	-
Saponin	-	-

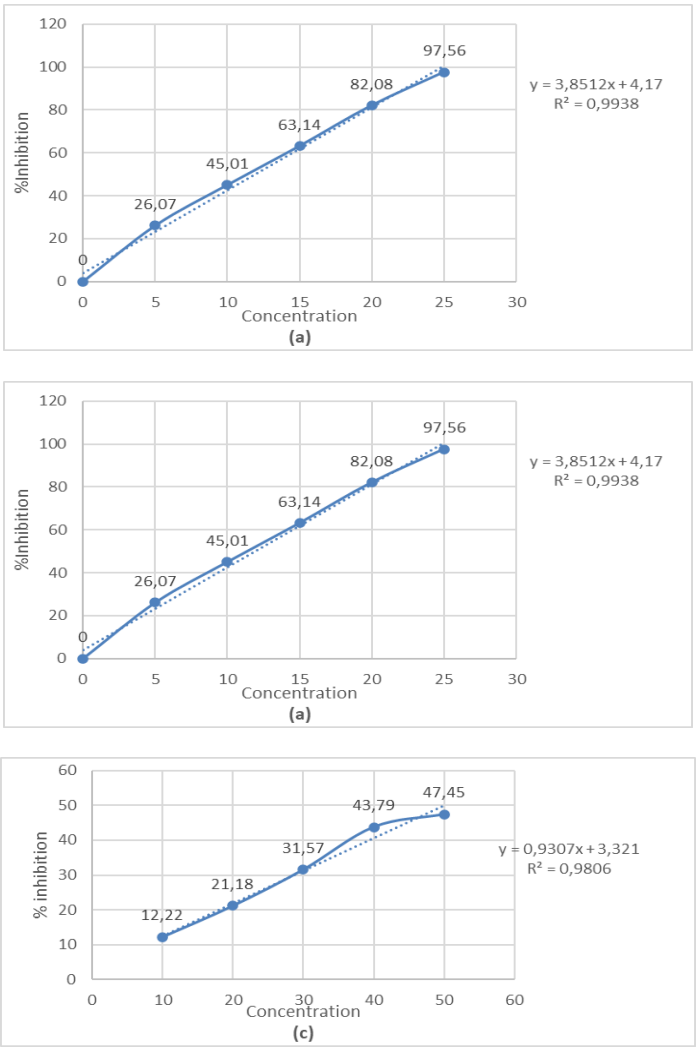

Figure 2. The IC_{50} determination graph of vitamin C (c) as positive control, H. costaricensis (b) and H. undatus (c) peel extracts.

Table 2. Vitamin C content of red and white dragon fruit peel extracts.

Sample	Absorbance	Concentration (ppm)	% Proportion
Red dragon fruit peel extracts	0.312	7.26	1.45
White dragon fruit peel extracts	0.223	5.19	1.04

and alkaloids, as well as its elevated vitamin C content. These components act synergistically to neutralize free radicals, reduce oxidative stress, and potentially protect against chronic diseases such as cardiovascular disorders and cancer.

The vitamin C content in the extracts was determined using UV-Vis spectrophotometry at a maximum wavelength of 270 nm. The vitamin C content analysis revealed that *Hylocereus costaricensis* (red dragon fruit peel) contained 1.45% vitamin C, whereas *Hylocereus undatus* (white dragon fruit peel) had 1.04% vitamin C. This indicates that the red dragon fruit peel has a higher vitamin C concentration compared to the white variety. The percentage increase was calculated using the formula: [(1.45 - 1.04) / 1.04] × 100, yielding an approximate 39% higher vitamin C content in red dragon fruit peel. This significant difference suggests that the red dragon fruit peel may contribute more to antioxidant activity due to its greater vitamin C concentration, which is a well-known natural antioxidant. However, since other bioactive compounds such as flavonoids and alkaloids also play a role in antioxidant mechanisms, further analysis is needed to determine the specific contribution of vitamin C to the overall antioxidant potential of each extract.

Identification of Compounds in both extracts with LC-MS/MS

Identification of compounds with LC-MS/MS was made to determine the peak areas, molecular

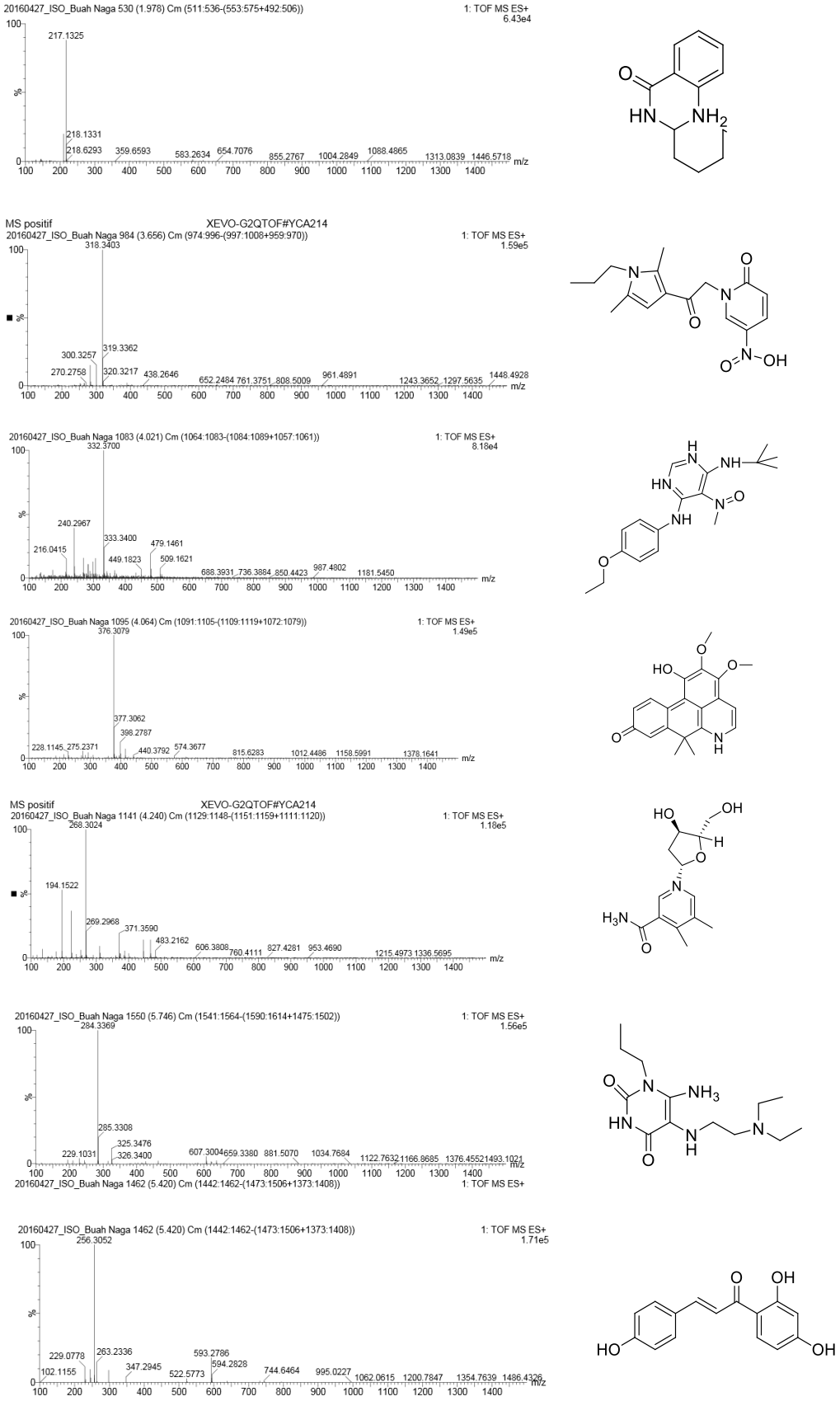

Figure 3. LCMS/MS chromatograms of compounds (1–7) isolated from the combination extracts (*Hylocereus costaricensis* and *Hylocereus undatus*).

Table 3. The results of identification of organic compounds of *Hylocereus costaricensis* and *Hylocereus undatus* fruit peel extract using LC-MS/MS.

RT (Minute)	Chemical compounds	Molecular formula	Mr (m/z)	Compound Group
1,978	6-oxo-2-phenyl-1H-pyrimidine-5-carboxylic Acid	$C_{13}H_{18}N_{2O}$	217.1325	Alkaloid
3,656	1-[2-(2,5-Dimethyl-1-propyl-1H-pyrrol-3-yl)-2-oxoethyl]-5-nitro-2(1H)-pyridinone	$C_{16}H_{19}N_3O_4$	318.3403	Alkaloid
4,021	N-(4-Ethoxyphenyl)-N'-(2-methyl-2-propanyl)-5-nitro-4,6-pyrimidinediamine	$C_{16}H_{21}N_5O_3$	332.3700	Alkaloid
4,064	1-Hydroxy-2,3-dimethoxy-7,7-dimethyl-6H-dibenzo[de,g]quinolin-9(7H)-one/Aporphine alkaloid/ Melosmine	C20H19NO4	376.3079	Alkaloid
4,240	3-Carbamoyl-1-(2-deoxy-beta-D-erythro-pentofuranosyl)-4,5-dimethylpyridinium	$C_{13}H_{19}N_2O_4$	268.3024	Alkaloid
5,746	6-Amino-5-{[2-(diethylamino)ethyl]amino}-1-propyl-2,4(1H,3H)-pyrimidinedione	$C_{13}H_{25}N_5O_2$	284.3369	Alkaloid
5,420	(2E)-1-(2,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one / Isoliquiritigenin	$C_{15}H_{12}O$	256.3052	Flavonoid

weights, and possible structures of compounds present in the fractions. LC-MS/MS analysis (Figure 3 and Table 3) identified several bioactive compounds in the combination extracts (*Hylocereus costaricensis* and *Hylocereus undatus*), including isoliquiritigenin, a potent flavonoid known for its antioxidative and anti-inflammatory properties. The analysis also detected multiple alkaloids, such as 6-oxo-2-phenyl-1H-pyrimidine-5-carboxylic acid, aporphine alkaloids like melosmine, and pyrimidine derivatives. These compounds are associated with various therapeutic effects, including neuroprotection, anti-cancer activity, and anti-inflammatory responses. Notably, the higher concentration of these compounds in red dragon fruit peel aligns with its enhanced bioactivity.

The findings of this study showed, although vitamin C content was measured using UV-Vis spectrophotometry, the LC-MS/MS analysis did not detect ascorbic acid in the peel extracts. This suggests that vitamin C may be present at levels below the detection limit or is not the primary contributor to antioxidant activity. Instead, the antioxidant potential observed in *H. costaricensis* and *H. undatus* peel extracts is likely attributed to their high flavonoid and alkaloid content, particularly isoliquiritigenin and melosmine, which have been previously reported to exhibit strong free radical scavenging properties." the significant potential of red dragon fruit peel as a natural antioxidant source. Its higher vitamin C content and the presence of bioactive flavonoids and alkaloids contribute to its superior antioxidative properties (Sharopov *et al*. 2018; Tang *et al*. 2024; Zhang *et al.* 2023) compared to white dragon fruit peel . Isoliquiritigenin, identified in LC-MS analysis, is particularly notable for its capacity to scavenge free radicals and reduce oxidative damage, making it a promising candidate for therapeutic applications. The variations in antioxidant activity and phytochemical composition between red and white dragon fruit peels are likely influenced by genetic factors, environmental conditions, and ripening stages. These differences suggest that red dragon fruit peel may be better suited for developing high-value nutraceuticals and functional foods targeting oxidative stress-related conditions. To enhance the utilization of dragon fruit peel extracts, further research should focus on optimizing extraction methods, such as ultrasound-assisted or supercritical fluid extraction, to maximize the yield and purity of bioactive compounds. Additionally, isolating and characterizing individual compounds will provide deeper insights into their specific biological activities and potential synergies. The practical applications of these findings extend to the development of natural antioxidants for food preservation, cosmetics, and pharmaceuticals. Given the growing demand for sustainable and plant-based health solutions, red dragon fruit peel extracts represent an eco-friendly and economically viable option for combating oxidative stress and promoting health.

CONCLUSION

This study demonstrated that red and white dragon fruit peels contain bioactive compounds, primarily flavonoids and alkaloids, that contribute to their antioxidant activity. The LC-MS analysis revealed that both extracts contain isoliquiritigenin and multiple alkaloids, but no detectable ascorbic acid. These findings confirm that the antioxidant potential of the extracts is not solely due to vitamin C but rather the synergistic effect of flavonoids and

alkaloids. The higher antioxidant activity of red dragon fruit peel correlates with its greater flavonoid and alkaloid content. These results suggest that dragon fruit peels could serve as valuable sources of natural antioxidants for nutraceutical and pharmaceutical applications. Advanced analysis should also be conducted to isolate and identify individual active compounds present in red and white dragon fruit peels. This will further elucidate their potential applications in nutraceuticals and pharmaceuticals, enhancing their economic and therapeutic value.

REFERENCES

Abidin, A.Z., Steven, S., Syahriani, W.F., Satyodriyani, K.R., Yemensia, E.V., Graha, H.P.R., Soekotjo, E.S.A., Putra, R.P. & Rzeznicka, I. (2025). Performance of simplicia herbs drying in a dryer assisted with superabsorbent polymer. *Indian Chemical Engineer*, advance online publication. 1–18.

Ballester, P., Cerdá, B., Arcusa, R., García-Muñoz, A.M., Marhuenda, J. & Zafrilla, P. (2023). Antioxidant activity in extracts from Zingiberaceae family: Cardamom, turmeric, and ginger. *Molecules*. **28(10)**: 1-21.

Bhadauria, S., Mansi, T.C. & Chaurasia, S. (2024). Exploring the therapeutic potential of dragon fruit: An insightful review. *International Journal of Pharmacognosy*. **11(6)**: 222-234.

Choo, W.S. & Yong, W. K. (2011). Antioxidant properties of two species of Hylocereus fruits. *Advances in Applied Science Research*. **2(3)**: 418-425.

Departemen Kesehatan Republik Indonesia (1989) *Materia Medika Indonesia Jilid V*. Depkes Republik Indonesia. Jakarta.

Ismail, B.P. (2017). Ash content determination. In Nielsen, S.S. (Eds). *Food Analysis Laboratory Manual*. 3rd ed. Pp. 117–119. Springer. Cham.

Kaliyaperumal, S., Periyasamy, K., Balakrishnan, U., Palanivel, P. & Egbuna, C. (2020). Antiviral phytocompounds for drug development: a data mining study. In *Phytochemicals as Lead Compounds for New Drug Discovery*. Pp. 239 - 244. Elsevier. Amsterdam.

Kiranmai, M. (2022). Review of exotic fruit: Nutritional composition, nutraceutical properties and food application of Dragon fruit (Hylocereus spp.). *The Pharma Innovation Journal*. **11(6)**: 613-622.

Manzoor, M., Singh, J., Gani, A. & Noor, N. (2021). Valorization of natural colors as health-promoting bioactive compounds: Phytochemical profile, extraction techniques, and pharmacological perspectives. *Food Chemistry*. **362**: 130141.

Mayanti, T., Sinaga, S. E. & Supratman, U. (2022). Phytochemistry and biological activity of Lansium domesticum Corr. species: A review. *Journal of Pharmacy and Pharmacology*. **74(11)**: 1568-1587.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*. **26(2)**: 211–219.

Mulyani, Y., Sinaga, S.E. & Supratman, U. (2023). Phytochemistry and biological activities of endophytic fungi from the meliaceae family. *Molecules*. **28(2)**: 1-28.

Mulyati, A.H., Alawiyah, H., Marom, M.F. & Warnasih, S. (2024) Antioxidant Testing and identification of bioactive compounds in ethanol extract of propolis from various locations in indonesia using LCMS-QTOF. *Chimica et Natura Acta*. **12(3)**: 193–203.

Nielsen, S.S. (2009). Determination of moisture content. *In Food Analysis Laboratory Manual.* Pp. 17–27. Springer US. Boston, MA.

Nishikito, D.F., Borges, A.C.A., Laurindo, L.F., Otoboni, A.M.M.B., Direito, R., Goulart, R. de A., Nicolau, C.C.T., Fiorini, A.M.R., Sinatora, R.V. & Barbalho, S.M. (2023) Anti-Inflammatory, antioxidant, and other health effects of dragon fruit and potential delivery systems for its bioactive compounds. *Pharmaceutics*. **15(1)**: 1-38.

Rahman, M.M., Khan, M.M.R. & Hosain, M.M. (2007). Analysis of vitamin C (ascorbic acid) contents in various fruits and vegetables by UV-spectrophotometry. *Bangladesh Journal of Scientific and Industrial Research*. **42(4)**: 417-424.

Rebecca, O.P.S., Boyce, A.N. & Chandran, S. (2010). Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *African Journal of Biotechnology*. **9(10)**: 1450-1454.

Safriansyah, W., Sinaga, S.E., Supratman, U. & Harneti, D. (2022). Phytochemistry and biological activities of Guarea genus (Meliaceae). *Molecules*. **27(24)**: 1-33.

Salgueiro, L., Martins, A.P. & Correia, H. (2010). Raw materials: the importance of quality and safety. A review. *Flavour and Fragrance Journal*. **25(5)**: 253-271.

Sharopov, F., Valiev, A., Gulmurodov, I., Sobeh, M., Satyal, P. & Wink, M. (2018). Alkaloid content, antioxidant and cytotoxic activities of various parts of *Papaver somniferum*. *Pharmaceutical Chemistry Journal*. **52**: 459-463.

Sinaga, S.E., Abdullah, F.F., Supratman, U., Mayanti, T. & Maharani, R. (2022). Isolation and structure determination of stigmaterol from the stem bark of *Lansium domesticum* Corr. Cv. Kokossan. *Chimica et Natura Acta*. **10(3)**: 106-111.

Sinaga, S.E., Fajar, M., Mayanti, T. & Supratman, U. (2023). Bioactivities screening and elucidation

of terpenoid from the stembark extracts of *Lansium domesticum* Corr. cv. Kokosan (Meliaceae). *Sustainability*. **15(3)**: 1-10.

Som, A.M., Ahmat, N., Hamid, H.A.A. & Azizuddin, N. (2019). A comparative study on foliage and peels of Hylocereus undatus (white dragon fruit) regarding their antioxidant activity and phenolic content. *Heliyon*. **5(2)**: 1-13.

Suh, D.H., Lee, S., Heo, D.Y., Kim, Y.S., Cho, S.K., Lee, S. & Lee, C.H. (2014). Metabolite profiling of red and white pitayas (*Hylocereus polyrhizus* and *Hylocereus undatus*) for comparing betalain biosynthesis and antioxidant activity. *Journal of Agricultural and Food Chemistry*. **62(34)**: 8764-8771.

Tang, X., Zhang, Y., Wang, L., Sun, C., Wang, L. & Huang, Z. (2024). Excited-state antioxidant capacity of Flavonoids based on solvent effect: A TD-DFT study. *Journal of Molecular Liquids.* **404**: 124973.

Widiastuti, D., Sinaga, S.E., Warnasih, S., Pujiyawati, E., Salam, S. & Putra, W.E. (2023). Identification of active compounds from *Averrhoa bilimbi* L.(Belimbing Wuluh) flower using LC-MS and antidiabetic activity test using in vitro and in silico approaches. *Trends in Sciences*. **20(8)**: 6761-6761.

Widiastuti, D., Warnasih, S., Mulyati, A.H., Sutanto, L., Herlina, E., Triastinurmiatiningsih, T., Sinaga, S.E., Salam, S., Harneti, D., Lesmana, R., Supratman, U., Susianti, S., Agustine, D. & Mulyani, R. (2024). Steroid compounds of *Manihot esculenta* Crantz var. Sao Pedro Petro (tuber) and their cytotoxic effects on melanoma cancer cells (B16-F10). *Trends in Sciences*. **21(4)**: 1-11.

Wishart, D.S., Tzur, D., Knox, C., Eisner, R., Guo, A.C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M.A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K., Stothard, P., Amegbey, G., Block, D., Hau, D.D., Wagner, J., Miniaci, J., Clements, M., Gebremedhin, M., Guo, N., Zhang, Y., Duggan, G.E., MacInnis, G.D., Weljie, A.M., Dowlatabadi, R., Bamforth, F., Clive, D., Greiner, R., Li, L., Marrie, T., Sykes, B.D., Vogel, H.J. & Querengesser, L. (2007). HMDB: The human metabolome database. *Nucleic Acids Research***. 35(Suppl. 1)**: 521–526.

Zhang, X., Tang, Y., Lu, G. & Gu, J. (2023). Pharmacological activity of flavonoid quercetin and its therapeutic potential in testicular injury. *Nutrients*. **15(9)**: 1-21.

Zhao, Z., Wang, L., Chen, J., Zhang, N., Zhou, W., & Song, Y. (2024). Altitudinal variation of dragon fruit metabolite profiles as revealed by UPLC-MS/MS-based widely targeted metabolomics analysis. *BMC Plant Biology*. **24(1)**: 1-12.