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RESEARCH ARTICLE

Antioxidant Potency of Red Dragon Fruit Flesh and Peel Prepared by Different Methods

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Abstract: ***Background*:** Red dragon fruit (*Hylocereus polyrhizus*, (F.A.C. Weber) Britton & Rose) is widely consumed all over the world nowadays. The peel and flesh of red dragon fruit contain many bioactive compounds with high antioxidant activity. The preparation process is critical to maximizing the yield of the antioxidant content.

***Objective*:** The objectives of this research were to evaluate total phenolic content (TPC), total flavonoid content (TFC), as well as the antioxidant activity of peel and flesh of red dragon fruit prepared by various methods.

***Methods*:** The fresh and dried samples of peel and flesh of red dragon fruit were prepared via maceration and non-maceration process. Ethanol (96%) was used as the solvent in maceration. In the non-maceration process, the samples were ground using a blender and pressed using a juicer. TPC was analyzed by Folin-Ciocalteau methods, while TFC was determined by spectrophotometry UV-Vis with $AlCl_3$. Antioxidant activity was analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching (BCB) tests.

***Results*:** TPC from all of the measured samples varied from 22.43±0.27 to 80.54±0.43 mg GAE/g dry extract. The highest TPC concentration was found in the blended peel via maceration and the lowest concentration was found in the blended flesh without maceration. The dried peel via maceration treatment had the highest TFC (51.96±0.084 mg of QE/g dry extract). Regarding to the antioxidant activity, the blended flesh ethanolic extract and blended peel ethanolic extract had the highest DPPH radical scavenging, IC50=966.83±11.62 and 973.81±3.571ppm, respectively. While the blended peel ethanolic extract had the highest BCB antioxidant activity (IC_{50}= 45.48±6,79 mg/mL).

***Conclusion*:** Preparation methods affect the antioxidant activity of red dragon fruit peel and flesh. The highest TPC and antioxidant activity (BCB test) can be found in the ethanolic extract of the blended peel. The highest TFC can be found in the ethanolic extract of dried-peel. Both the ethanolic extracts, blended peel and blended flesh, had the same DPPH radical scavenging activity.

Keywords: Antioxidant activity, flavonoid, natural health, phenol, preparation method, red dragon fruit.

1. INTRODUCTION

Red dragon fruit is one of the tropical fruits that are widely consumed all over the world. This fruit contains many bioactive compounds, such as polyphenols, beta-carotene, flavonoids, and vitamins (B1, B2, B3, and C). According to a research [1], the primary pigment in red dragon fruit is betacyanin, which produces the purple color.

The high content of bioactive compounds in this fruit has driven researchers to explore its potency as a natural health supplement. The study showed the effectiveness of dragon fruit to decrease some metabolic syndrome symptoms [2].

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Some authors investigated the efficacy of this fruit against diabetes [3]. In another study [4], The study reported the benefit of red dragon fruit's flesh to prevent inflammation and colitis in a murine model. Some authors studied the utilization of red dragon fruit peels and found they can reduce cholesterol, triglycerides, and Low-Density Lipid (LDL) levels in hyperlipidemic mice [5]. Various benefits of red dragon fruit may be derived from their nutritional content, both in the peel and the flesh.

There is a variation in the findings of the antioxidant content in the peel and flesh of red dragon fruit [6]. Another study reported that flesh of dragon fruit had higher antioxidant activity compared to the peel [7]. Different results were obtained by researchers [8] who observed that the antioxidant capacity in the peel of red dragon fruit which was higher than the flesh. Both studies [9, 10] showed that various extraction methods might influence the antioxidant capacity of the fruit.

Despite its high potency of nutritious, healthy chemical content, this fruit is seasonal. Therefore the processing of the fruit parts by a suitable method of preparation may give extra value to the fruit. Additionally, the preparation method plays a critical role in the fruit's phytochemical content In a study, it has been shown that conventional and ultrasonic-assisted extraction methods could give different results in Total Flavonoid Content (TFC), Total Phenolic Content (TPC), and scavenging activity of the flesh and peel of red dragon fruit [11]. The objectives of this research were to determine antioxidant content (both TPC and TFC) and antioxidant activity of the peel and the flesh of red dragon fruit prepared by different methods. Finding the best method of preparation may give valuable information for the fruit's utilization as one of the natural health supplement products.

2. MATERIALS AND METHODS

2.1. Plant Material

The red dragon fruits used in this research were obtained from a local farmer in Bantul, Yogyakarta, Indonesia. The specimen of the plant was identified in the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta by Abdul Razaq Chasani, S. Si., M. Si., Ph.D. Certificate number 01037/S.Yb/III/2017.

2.2. Chemicals and Reagents

Ethanol (96%), quercetin, gallic acid, DPPH, β-carotene, chloroform, sodium carbonate, aluminum chloride ($AlCl_3$), potassium acetate, and linoleic acid were purchased from Merck (Germany). Folin ciocalteu's reagent was purchased from Sigma Aldrich (USA).

2.3. Sample Preparation

Red dragon fruits were firstly washed under running tap water and air-dried. Then, the fruits were peeled in order to separate the flesh from the peel. In this research, we used two preparation methods, *i.e.*, maceration and non-maceration.

2.4. Sample Preparation *via* Maceration

In this preparation, both peel and flesh were used as samples. The peel and the flesh were separately ground using an electric blender (Philips HR2115, Indonesia) before maceration in ethanol. The maceration processes for each sample used ethanol (96%) as the solvent for 48 h at room temperature (20°C), then the extract was evaporated using a vacuum rotary evaporator (Heidolph, Germany). Meanwhile, some peel was dried at 50°C for four days using a laboratory drying oven. The dried peel was subsequently pulverized into powder before it was macerated in ethanol. The maceration processes for this sample also used ethanol (96%) as the solvent for 48 h at room temperature, then the extract was evaporated using a vacuum rotary evaporator (Heidolph, Germany). After ethanol was evaporated, the extract of macerated samples (peel and flesh) was freeze-dried with a freeze dryer (Alpha, Germany) at -20°C for 14 days.

2.5. Sample Preparation *via* Non-maceration

In the non-maceration preparation, only the flesh of the fruits was used. The flesh was first divided into three equal parts by weight. The first part of the flesh was minced and pressed using a juice extractor (Kirin KJE-598, Indonesia) to produce filtrates and pomaces. The filtrates and pomaces of the fruit were later freeze-dried at -20°C for 14 days. The second part was blended using an electric blender (Philips HR 2115, Indonesia) prior to freeze-drying at -20°C for 14 days. The third part was dried in a laboratory drying oven at 50°C for approximately nine days and followed by freeze-dried at -20°C for 14 days.

2.6. Determination of Total Phenolic Content (TPC)

TPC was determined according to the study [12] using the Folin-Ciocalteau reagent. The amount of freeze-dried extract (50 mg) was dissolved in 5 mL of distilled water. This diluted extract was sonicated for 15 min then filtered. Distilled water was added into the diluted extract until 10 mL. Folin-Ciocalteau reagent (1.5 mL) was thoroughly added into 300 μL of this diluted extract and to gallic acid for 3 minutes. Sodium carbonate 7.5% (1.2 mL) was added into the mixture. After incubation at room temperature for 1 hour, the absorbance of the mixture was determined at 761 nm using an ultraviolet-visible (UV-vis) spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid was used as a standard. TPC was expressed as milligram of Gallic acid equivalents per gram of dried extract (mg GAE/g DE).

2.7. Determination of Total Flavonoid Content (TFC)

TFC was determined according to a study [13] using quercetin as the standard. The peel and the flesh extracts (250 mg) were dissolved in 2.5 mL 96% ethanol, sonicated for 15 minutes, then filtered. The mixture was then dissolved using ethanol (96%) in a 5 mL measuring flask. Subsequently, ethanol 96% (1.5 mL), aluminum chloride 10% (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (2.8 mL) were added into 0.5 mL of those sample solution before incubation for 30 min at room temperature. The absorbance

was determined at 435.40 nm using an ultraviolet-visible (UV-vis) spectrophotometer (Shimadzu UV-1800, Japan). Quercetin was used as a standard. TFC was expressed as milligram of quercetin equivalents per gram of dried extract (mg QE/g dry extract).

2.8. Determination of Antioxidant Activity by DPPH Radical Scavenging Test

The DPPH radical scavenging of the samples was measured by using a modified method described by [8]. Different concentrations of extract (250, 500, 750,1000, 1250, 1500 and 1750 ppm) were prepared. One mL of DPPH 0,15 mM solution was added to each concentration of extract (1 mL). This mixture was then incubated in a range operating time at room temperature. The absorbance was measured at 516 nm. DPPH radical scavenging activity was calculated using a formula as follows:

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Note:

A control = Absorbance of control (DPPH solution)

A sample = Absorbance of the sample with DPPH solution

2.9. Determination of Antioxidant Activity by β- carotene Bleaching (BCB) Test

BCB test was determined according to a study [14]. β-carotene (6 mg) was dissolved in 20 mL chloroform. Three mL of the solution was added to 40 μL linoleic acid and 400 µL tween 20. As chloroform was removed, the β-carotene emulsion was obtained. 100 mL of distilled water was added into the emulsion. The fruit extracts in the range of 1000-2500 μg/mL were later added into the tube and incubated on a water bath at 50°C. The β-carotene emulsion was monitored by measuring absorbance at 460 nm within 0, 60, 120 minutes after incubation. The mixture of ethanol with distilled water (1:1) was used as a control. Alpha-tocopherol and quercetin were used as standards.

Percentage of inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{A_{A(120)} - A_{C(120)}}{A_{C(0)} - A_{C(120)}} \times 100$$

Note

$A_{A(120)}$ = absorbance of the antioxidant at t=120 min,

$A_{C(120)}$ = absorbance of the control at t =120 min,

$A_{C(0)}$ = absorbance of the control at t = 0 min,

2.10. Statistical Analysis

All experiments were performed in triplicate. The data were presented as a mean ± standard deviation. Statistical significance was set at p=0.05. The differences in TPC, TFC, and antioxidant activity between seven treatments were analyzed using one-way Anova and LSD test. Correlation between TPC and TFC with the antioxidant activity was tested using Pearson correlation coefficient. Data and graphs were subjected to analysis using SPSS Version 23.

3. RESULTS AND DISCUSSION

Antioxidant activity of natural plant material, such as fruit depends on various factors, one of which is the preparation procedure. In this study, the potential of antioxidant content, both phenolic and flavonoid, as well as the antioxidant activity of the peel and the flesh of red dragon fruit that prepared by different methods, were analyzed. There were two preparation methods analyzed, *via* maceration and non-maceration. In this study, the traditional use of this fruit, which is simply crushing the flesh into a pulp by a blender, was also observed. This method was included in order to evaluate the traditional preparation and consumption method of the red dragon fruit.

3.1. Total Phenolic Content (TPC)

TPC was measured by using the Folin-Ciocalteau method according to the study [12]. The results are shown in Table **1**. The values were obtained from calibration curve y = 0.00931x + 0.00385 with R^2 = 0.99958, where x is the concentration of Gallic acid solution (mg/L) and y is the absorbance expressed as mg GAE/g.

TPC from all of the measured samples varied from 22.43 ± 0.27 to 80.54 ± 0.43 mg GAE/g. Overall, the preparation *via* maceration produced higher TPC than the non-maceration process. TPC yields in the red dragon fruit's peel (dried peel and blended peel) with the maceration process were two-fold higher than TPC in the flesh (Table **1**). The highest concentration was found in the blended peel *via* maceration (80.54 ± 0.43 mg GAE/g dry extract), and the lowest concentration was found in the blended flesh without maceration (22.43 ± 0.27 mg GAE/g dry extract). Maceration treatment had a significant effect on the TPC of red dragon fruit. According toa study [15], extraction is a critical process, which determines the release of analyte (such as phenol compound) from the matrix into the medium. In this study, the maceration process was carried out successfully, releasing more phenolic compounds from the peel and the pulp of the red dragon fruit rather than the non-maceration process.

The phenolic content of ethanolic extract between the peel and the flesh of dragon fruit was significantly different. The authors of a study[6, 8, 16] reported that phenolic compounds in red dragon fruit peel were higher than the flesh. Another study reported that TPC in the unedible parts of a plant (like peel and seed of a fruit, leave, and stem) was higher than the edible part, such as the fruit's flesh [8].

3.2. Total Flavonoid Content (TFC)

Spectrophotometry methods analyzed the total flavonoid content of samples. The results showed that the peel of red dragon fruit, both dried and blended using maceration treatment, had the higher flavonoid content, 51.959 ± 0.084 and 39.386 ± 0.077 mg of QE/g dry extract, respectively (Table **1**). The TFC of the peel was significantly different from the flesh, both *via* maceration and non-maceration processes. These results were consistent with in a study of the authors.

Table 1. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and radical scavenging activity (DPPH and BCB assay) of red dragon fruit peel and flesh prepared by vary methods.

No.	Treatments	TPC (mg of GAE*/g Dry Extract)	TFC (mg of QE**/g Dry Extract)	DPPH IC_{50} (ppm)	BCB IC_{50} (ppm)
	Via maceration***				
1	Dried peel	78.86 ± 0.43^{e}	51.96 ± 0.08^{f}	1064.93 ± 0.78^{b}	73.17 ± 7,46^{a}
2	Blended peel	80.54 ± 0.43^{f}	39.39 ± 0.08^{e}	973.91 ± 3.57^{a}	45.48 ± 6,79^{a}
3	Blended flesh	35.00 ± 0.27^{d}	14.24 ± 0.14^{b}	966.83 ± 11.62^{a}	790.27 ± 151.29^{b}
	Non-maceration				
4	Dried flesh	35.53 ± 0.16^{d}	13.37 ± 0.09^{a}	1092.33 ± 8.90^{c}	72.66 ± 8.27^{a}
5	Blended flesh	22.43 ± 0.27^{a}	36.07 ± 0.11^{d}	1319.32 ± 4.44^{d}	283.71 ± 57,94^{a}
6	The pomace of pressed flesh	26.83 ± 0.38^{b}	29.70 ± 0.10^{c}	2783.27 ± 24.38^{f}	106.39 ± 25.40^{a}
7	The filtrate of pressed flesh	30.99 ± 0.27^{c}	14.05 ± 0.11^{b}	1551.88 ± 14.07^{e}	888.13 ± 190.78^{b}

TPC, TFC, DPPH, and BCB data were presented as mean ± standard deviation. The difference between the superscript letters in the same column shows a significant difference with $p < 0.05$ (n=3).
*mg gallic acid equivalent/g dry extract **Quercetin equivalent.
***Maceration using ethanol as the solvent

[16, 17], who reported that the peel of red dragon fruit contained higher flavonoid content than the flesh [14]. Kulistic T, Radonic A, Katalinic V, Milos M showed that flavonoid content in the peel was 3-fold higher than the flesh.

Interestingly, we found in this study the TFC of the flesh without the maceration process was higher than the dried and blended flesh processing *via* maceration. Extraction *via* maceration using ethanol (96%) gave no effect to flavonoid yield. It is estimated that ethanol as the solvent in the maceration process was not optimal for extracting flavonoid from the peel and the pulp of the red dragon fruit.

The maceration process was not optimal for extracting flavonoid from the peel and the pulp of the red dragon fruit.

3.3. Antioxidant Activity by DPPH Assay

The DPPH radical scavenging method was used to evaluate the antioxidant activity of different red dragon fruit extracts in this study. DPPH is a stable free radical easily scavenged by an antioxidant. The IC_{50} values from all of the samples, as shown in Table **1** was the concentration of the sample required to scavenge 50% of the DPPH free radical.

The pomace of the blended flesh yielded the lowest DPPH radical scavenging (IC_{50}=2783.27 ppm). Whereas, the blended flesh ethanolic extract (IC50= 966.83 ± 11.62 ppm) was the highest. Considering the antioxidant activity, according to a study [18], the higher the IC_{50} value, the lower will be the antioxidant activity. The blended flesh ethanolic extract had the highest antioxidant activity but did not show a significant difference with the blended peel ethanolic extract (IC50= 973.91 ± 3.57 ppm).

3.4. Antioxidant Activity by BCB Assay

The principle of the BCB test is the decolorization of yellow color in the β-carotene solution which canrelated to the breaking of the C=C double bond by lipid peroxyl radicals generated from autooxidation of linoleic acid [19]. This test showed that the blended peel ethanolic extract had the highest antioxidant activity (IC_{50}= 45.48 ± 6,79 mg/mL) while filtrate of pressed flesh had the lowest antioxidant activity (IC_{50}= 888.13 ± 190.78 mg/mL).

We used two different assays to analyze the antioxidant potency of red dragon fruit due to the diversity of antioxidant content in red dragon fruit. The authors of this study discussed that the DPPH test is generally used to assess antioxidant potency for both hydrophilic and lipophilic subtances [14]. DPPH assay is preferable to get preliminary information about the antioxidant capacity of new substances. On the other hand, the BCB test is appropriate for lipophilic antioxidant assay only.

3.5. Correlation between TPC and TFC with the Antioxidant Activity

The amount of antioxidant activity of blended peel ethanolic extract is in accordance with its total phenol compounds. In this study, we analyzed the correlation between phenol and flavonoid content with antioxidant activity (IC_{50} of DPPH and BCB).

We found that DPPH scavenging activity had strong correlation to TPC, but weak correlation to TFC (r = -0,704 and r = -0.262, respectively). A negative value means higher TPC/TFC correlates to lower IC_{50} of DPPH activity. As explained above, a lower IC_{50} value indicates higher antioxidant activity. This result confirmed that the total phenolic content of the samples had a significant effect on the DPPH scavenging activity.

β-carotene bleaching assay depicted the weak correlation between phenol and flavonoid content with antioxidant activity, but the correlation value of phenolic content was slightly

higher than flavonoid content (r = -0.410 and r = 0.394, respectively). This result demonstrated that phenols and flavonoids did not give significant support to the antioxidant power of red dragon fruit samples based on this assay.

Overall, this study implies that the peel of red dragon fruit contains higher antioxidant properties compared to the flesh. The maceration process to the peel using dried and fresh fruit relatively resulted in slight differences in antioxidant capacity. Azwanida N reported that pre-extraction preparation of plant samples, as grinding and drying, influence the preservation of phytochemicals in the final extract [9]. The fresh or dried samples give different result in extraction yield. Matsusaka Y, Kawabata J reported that the inedible parts of a plant such as peel and seed had higher antioxidant capacity compared to the edible ones [20]

CONCLUSION

Preparation methods affect the antioxidant activity of red dragon fruit peel and flesh. The highest TPC and antioxidant activity (BCB test) can be found in the ethanolic extract of the blended peel. Both the ethanolic extracts, blended peel and blended flesh, had the same DPPH radical scavenging activity. The highest TFC can be found in the ethanolic extract of dried-peel.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the Repository UAD at http://eprints.uad.ac.id/15740/, [21].

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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