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ANALYSIS AND OPTIMIZATION EXTRACTION BETANIN FROM JUICE OF PEEL AND FLESH OF RED DRAGON FRUIT (*Hylocereus Costaricencis*)

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Abstract: Currently, health issues are no longer related to how to treat diseases but rather prioritize the prevention of various diseases by eating foods that contain high levels of nutrients, known as functional foods. Red dragon fruit contains betanin dye, which has various health benefits and can be grouped into functional foods. Some studies have been conducted to obtain betanin from red dragon fruit, but it is not yet known how much betanin content is present in the juice of the flesh and peel of red dragon fruit (Hylocereus costaricencis) and how to determine the optimum extraction conditions. This study aims to analyze the betanin content in the skin and flesh of red dragon fruit and the optimal extraction conditions so that betanin can be obtained in maximum quantities. The extraction method used was the maceration method with three solvents, namely, water, ethanol, and methanol. Analysis of the betanin content in the skin and flesh of dragon fruit was carried out using UV–Vis spectrophotometry. The results of the study revealed that water solvents can extract betanin well. In addition, the ratios of dragon fruit to water were 2:1 (flesh and water) and 1:1 (skin and water), respectively, while the optimum extraction temperature was 4–8°C. The optimum extraction time was 24 hours. Betanin was obtained from the skin and flesh of red dragon fruit at a sample:solvent ratio of 1:1, with values of 21.71 mg/mL and 17.8 mg/mL, respectively.

Keywords: Betanin, Red Dragon Fruit, Extraction

Abstrak: Saat ini permasalahan kesehatan tidak lagi berkaitan dengan cara pengobatan bila terkena penyakit, melainkan mengutamakan pencegahan berbagai penyakit dengan mengonsumsi makanan yang mengandung gizi tinggi yang dikenal dengan pangan fungsional. Buah naga merah mengandung zat warna betanin yang memiliki berbagai manfaat bagi kesehatan sehingga dapat dikelompokkan ke dalam pangan fungsional. Beberapa penelitian dilakukan untuk mendapatkan betanin dari buah naga merah, namun belum diketahui berapa kandungan betanin pada jus daging dan kulit buah naga (Hylocereus costaricencis) serta menetapkan kondisi optimum ekstraksi. Penelitian ini bertujuan untuk menganalisis kandungan betanin yang terdapat pada kulit dan daging buah naga merah serta

kondisi ekstraksi yang optimal seringas dapat diperoleh betanin dalam jumlah yang maksimal. Metode ekstraksi yang digunakan adalah metode maserasi dengan menggunakan tiga pelarut yaitu air, etanol, dan metanol. Analisis kandungan betanin pada kulit dan daging buah naga dilakukan dengan metode spektrofotometri UV–Vis. Hasil penelitian menunjukkan bahwa pelarut air dapat mengekstrak betanin dengan baik, selain itu perbandingan buah naga dan pelarut masing-masing adalah 2:1 (daging dan air) dan 1:1 (kulit dan air), sedangkan suhu ekstraksi optimum diperoleh pada suhu 4–8°C. Waktu ekstraksi optimum adalah 24 jam. Telah diperoleh betanin dari kulit dan daging buah naga merah pada komposisi sampel: pelarut 1:1 berturut-turut 21.71 mg/mL and 17,8 mg/mL.

Kata kunci: Betanin, Buah Naga Merah, Ekstraksi

INTRODUCTION

Currently, people's concern for health is increasing. Efforts to maintain health are starting to shift from the use of synthetic drugs to the use of functional foods (Shashirekha et al. 2015). Vegetables and fruits are examples of functional foods that are rich in health benefits for the body. One fruit whose availability is not dependent on the season and is relatively affordable is red dragon fruit (*Hylocereus costaricencis*). In addition to having a delicious taste and being quite popular with the public, some studies state that dragon fruit has properties that are beneficial for human health.

Dragon fruit contains bioactive substances that are beneficial to the body, including antioxidants (ascorbic acid, beta-carotene, and anthocyanins), and contains dietary fiber in the form of pectin. In addition, dragon fruit contains several minerals, such as calcium and iron. The vitamins present in dragon fruit include vitamin B_1, vitamin B_2, vitamin B_3, and vitamin C (Marlina et al. 2019).. In addition, dragon fruit also contains a coloring agent known as betalain, which has many health benefits, one of which is antiviral activity (Jun Chang et al. 2020).

Currently, dragon fruit is distinguished by the color of its flesh, which is red, white, or yellow. The type of dragon fruit that is widely available on the market and attracts consumers is red dragon fruit (Li et al. 2022). Red dragon fruit consists of two types, namely, red dragon fruit with red skin and red fruit flesh (*Hylocereus Polyrhizus*) and red skin dragon fruit with purplish red flesh (*Hylocerus costaricencis*)(Tarte et al. 2023). The red color of dragon fruit comes from its natural pigment, betalain (Cheok et al. 2022) (Hendra et al. 2020). Betalain is a group of natural water-soluble nitrogen-containing pigments that originate from tyrosine in fruits and vegetables and are divided into two

groups, betacyanin and betaxanthin (Huang et al. 2021).

Betanin/betacyanin (betanidin 5-O-b-D-glucosid) is the main phytochemical of betalain, a water-soluble nitrogenous heterocyclic compound that is red–violet in color. In addition, betanin is described as a bioactive compound capable of inhibiting membrane lipid peroxidation and low-density lipoprotein (LDL), modulating ROS generation and deep gene expression to reduce cytokine release and increase antioxidant enzyme activity (Esatbeyoglu et al. 2015). Other health benefits of betanin include its antimicrobial, antioxidant, antihypercholesterolemic and various other properties (Hendra et al. 2020; Panjaitan et al. 2022; Wijesinghe and Choo, 2022).

Various studies have been conducted to extract betanin or betacyanin from the peel of red dragon fruit, but the extraction of betanin has focused mostly on red dragon fruit with red peel and flesh (*Hyloceureus Polyrhizus*) and dragon fruit with red peel and white flesh (Hylocereus udantus), but little research has been conducted related to betanin extraction in dragon fruit with red peel and purplish red flesh (Hylocereus costaricencis) (Khoo et al. 2022; Thi and Chi, 2021).

In addition, in previous studies, there were differences in the extraction methods used, one of which was the composition of the sample (fruit or peel) and the solvent used. Sri Priatni's (2015) research used a sample composition (fruit/peel):solvent ratio of 1:4, whereas Hook Eng Khoo's (2022) research extracted betanin with a sample:solvent composition of 1:10. In addition, the extraction conditions used also differ (Khoo et al. 2022; Priatni and Pradita, 2015). Thus, it is necessary to conduct research related to the analysis of betanin content in dragon fruit peel and flesh and the optimization of extraction conditions via the maceration method on red dragon fruit (*Hylocereus costaricencis*). Therefore, this study aims to analyze the betanin content in the peel and flesh of red dragon fruit (*Hylocereus costaricense*) and determine the optimum extraction conditions, including the type of solvent, the ratio of samples to solvents and the optimum extraction temperature.

METHOD

Materials

Dragon fruit (*Hylocereus costaricencis*) was purchased from the local market of the Bandung area and was produced by Brin and the MIPA

Laboratory, IAIN Syekh Nurjati Cirebon. The dragon fruits (peel and flesh) are separated, and each is mashed with a blender. Standard Betanin from Sigma Aldrich, methanol and ethanol from Merck, aquades, a set of glassware, Whatmann no. 1, Thermo Fisher Scientific GENESYS 10S spectrophotometer

Extraction with various solvents

A total of 50 grams of dragon fruit that had been mashed was weighed and placed in 3 200 mL beakers. Solvents (water, ethanol, and methanol) were added to each beaker to a volume of 250 mL. Extraction was carried out via the maceration method during an extraction time of 3 × 24 hours at room temperature and 1 × 24 hours in a refrigerator. The extract was filtered using Whatman 1. After that, the betanin content of the extract was analyzed via a UV–vis spectrophotometer and compared with that of the standard (Priatni and Pradita, 2015).

Curve calibration

Standard betanin powder (250 mg) was weighed and then placed into a 50 mL measuring flask such that a betanin concentration of 5 mg/mL was obtained. Next, a series of betanin concentrations of 1 mg/mL were prepared: 2 mg/mL, 3 mg/mL, 4 mg/mL, and 5 mg/mL in a 10 mL measuring flask. Next, the absorption at a wavelength of 530 nm was measured, and a standard curve was generated to determine the concentration of betanin in the dragon fruit extract (Khoo et al. 2022).

Optimization of extraction with various ratios of substances (peel and flesh) that drag on fruit and solvents

The peel and flesh of the dragon fruit were weighed to 50 grams each, and then, 50 mL, 100 mL, 150 mL, and 200 mL were added to the water. A ratio of substances: solvents was obtained (2:1% b/v; 1:1% b/v; 1:2% b/v; 1:3% b/v; and 1:4% b/v), and the mixture was allowed to stand for 24 hours. The mixture was then filtered, and the absorption was measured at a wavelength of 530 nm with a UV–vis spectrophotometer (Li et al. 2022; Priatni and Pradita, 2015).

Optimization of extraction at chiller and freezer temperatures

The peel and flesh dragon fruit with a ratio of aquades of successively 1:1% b/v and 2:1% b/v are put into the Chiller and Freezer, allowed to stand for 24 hours, and then filtered, and the absorption at a wavelength of 530 nm is measured with a UV–vis spectrophotometer (Priatni and Pradita, 2015).

RESULTS AND DISCUSSION

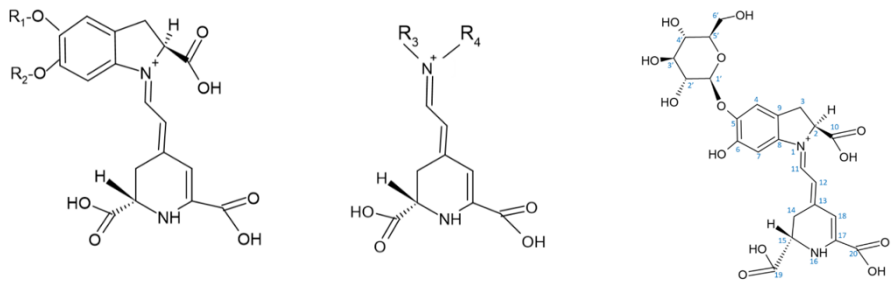
This study was conducted to analyze the betanin content in the flesh and skin of red dragon fruit (*Hylocereus costaricencis*) and to determine the optimum conditions for betanin extraction via the maceration method. Many studies have been conducted on the betanin content in the skin of red dragon fruit of the Hylocereus Polyrhizus type, but few studies have been conducted on the *Hylocereus costaricencis* type of red dragon fruit. Many red dragon fruits of the *Hylocereus costaricencis* type are also sold on the Bandung market. The type of dragon fruit, in addition to being determined through laboratory determination, can also be seen from the color of the fruit flesh, where the *Hylocereus costaricencis* type of dragon fruit has a more purplish red flesh (Luu et al. 2021).

Research has shown that the health benefits of dragon fruit are due to the content of coloring substances, including betanin. The health benefits of betanin have been studied in recent years, starting from its effects as an antioxidant (Hendra et al. 2020), antibacterial (Wijesinghe & Choo, 2022), and antihypercholesterol (Panjaitan et al. 2022), and many other benefits have been studied. The many health benefits of betanin in red dragon fruit allow the fruit to be used as a functional food (Susmita & Safina, 2022). This study was conducted to analyze the betanin content of the peel and flesh of red dragon fruit (*Hylocereus costaricenc*is) obtained through extraction via the maceration method and to obtain optimum extraction conditions so that betanin can be obtained in maximum quantities.

Determination of Dragon Fruit

This study began by determining the dragon fruit studied, which was obtained from one of the markets in the Bandung area. On the basis of the results of the determination of dragon fruit carried out by Brin and the MIPA Laboratory, IAIN Syekh Nurjati Cirebon obtained the type of dragon fruit studied, namely, red dragon fruit (*Hylocereus costaricencis*). The dragon fruit is obtained from fruit shops in the Bandung area.

Dragon fruit contains color pigments known as betalain compounds (Khoo et al. 2022). Betalain consists of the red pigment betactyanin and the yellow pigment betaxanthin. Betanin is a major component of betacyanins (Sadowska-Bartosz and Bartosz, 2021).



(a) (b) (c)

Figure 1. (a0 Structure of Betacyanin; (b) Structure of Betaxanthin; (c) Structure of Betanin

Figure 1 shows the structure of the dye in plants, namely, betalain, where a is the structure of betacyanin, b is the structure of betaxanthin, and c is the structure of betanin, which is the largest part of betacyanin.

In this study, extraction was carried out via the maceration method. At the beginning of the maceration, research was carried out for 3 × 24 hours. After extraction, the color of the extract was observed in ethanol, water, and methanol. The results of the effects of betanin extract on the peel and flesh of dragon fruit with various solvents are given in Figure 2. Figure 2 shows the results of betanin extraction in 3 different solvents, namely, water, methanol, and ethanol, after 3x24 h of maceration. The betaine was well extracted in water, while in methanol and ethanol, betanin color degradation occurred. In dragon fruit extracts with various solvents, the results were obtained if the color of betanin was in water extracts/aquades, whereas methanol and ethanol extracts showed the color of betaxanthin, yellow color in dragon fruit (Khoo et al. 2022).

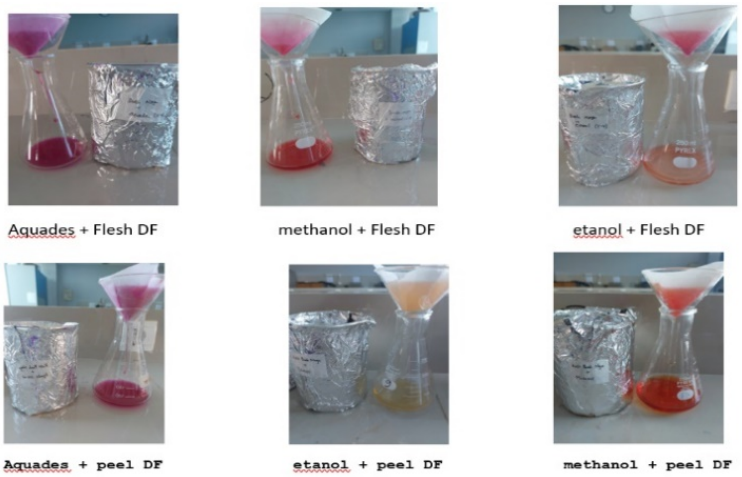
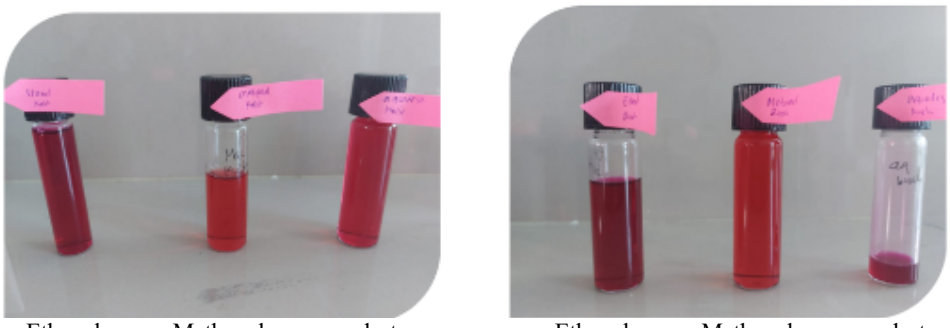


Figure 2. Peel and Flesh of Dragon Fruit Extraction Results with Various Solvents in 3 × 24 hours at room temperature



Ethanol Methanol aquadest
(a) Peel of Dragon Fruit

Ethanol Methanol aquadest
(b) Flesh of Dragon Fruit

Figure 3. Peel (a) and Flesh (b) of Dragon Fruit Extraction Results with Various Solvents in a 1 × 24 h refrigerator

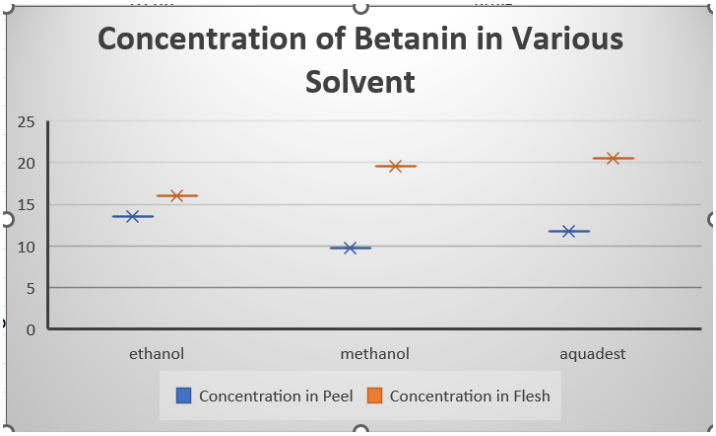


Figure 4: Concentration of Betanin in Various Solvents

Priatini et al. (2015) conducted maceration for 24 hours at refrigerator temperature, after which the next maceration process was carried out at refrigerator temperature for 24 hours. Figure 3 shows the results of betanin extraction, which was macerated in the refrigerator for 1 × 24 hours. The results of betanin extraction with various solvents for 1 × 24 h at refrigerator temperature show that betanin is extracted and that there is no degradation of betanin pigments, but betanin stability remains better in the aquadest. This can be seen in Figure 4. On the basis of the results of the extraction, water/aquadest was chosen as the solvent for isolating betanin from the peel and flesh of dragon fruit.

The determination of betanin levels in dragon fruit was performed via a UV–vis spectrophotometer, and a standard curve of betanin obtained from Sigma Aldrich was generated previously. The results of the standard curve analysis of betanin at concentrations ranging from 1–5 mg/mL are shown in Table 1 and Figure 5.

Table 1. Standard Curve Betanin

Concentration (mg/mL)	I	II	III	Mean
5	0,954	0,954	0,996	0,9680
4	0,872	0,87	0,804	0,8486
3	0,568	0,568	0,58	0,5720
2	0,424	0,424	0,405	0,4176
1	0,24	0,244	0,209	0,2310

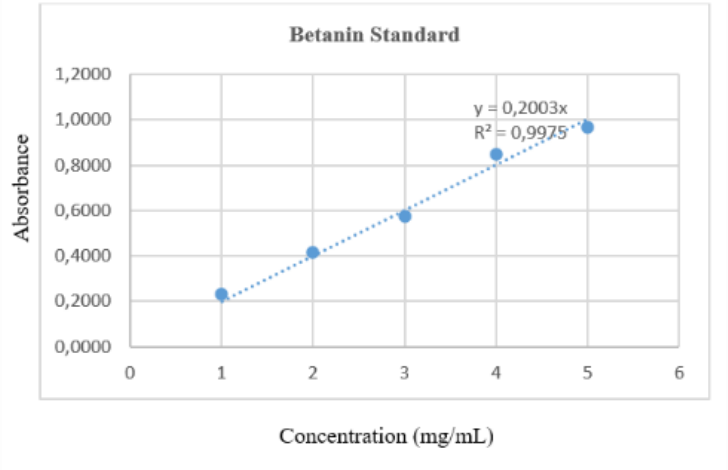


Figure 5. Standard Curve of Betanin

Table 1 shows the results of standard betanin absorbance measurements at concentrations ranging from 1–5 mg/mL on a Thermo Fisher Scientific GENESYS 10S UV–Vis spectrophotometer. The higher the concentration of betanin is, the greater the absorption produced, which is in line with Lambert Beer's Law. From the data, a calibration curve is then created, as shown in Figure 5.

A calibration curve is tripled (three repetitions) so that the result of the equation y = 0.2003 x with a correlation coefficient R^2 = 0.9975. The betanin absorbances extracted from the peel and flesh of dragon fruit were 2.585 and 4.435 mg/g, respectively, such that betanin concentrations of 12.9 mg/mL and 22.14 mg/mL were obtained so that betanin was obtained in the peel of dragon fruit at 51 mg/g fruit and in the flesh of dragon fruit at 88.56 mg/g fruit.

The results of this study revealed that the betanin content in the peel and flesh of red dragon fruit (*Hylocereus costaricencis*) was greater than that reported by Hock Eng Khoo et al. (2022), who extracted 7.44 ± 0.01 mg/g and 9.44 ± 0.01 mg/g, respectively, of betanin from red dragon fruit (*Hylocereus polyrhizus* Chi, 2021) and obtained a betacyanin content of 3.42 mg/100 g.

The next stage of research implementation is the extraction of various solvent ratios. The best results are obtained when the ratio of fruit to solvent is 2:1 (24.34 mg/dL) and the peel solvent ratio is 1:1. For the peel of dragon fruit, a 2:1 extraction ratio cannot be obtained because the solution is very concentrated; thus, filtering cannot be performed. Table 2 shows the results of betanin extraction at various solvent concentrations.

The extraction of betanin from various natural sources, such as roselle flowers (Anggistia et al. 2016), binahong seeds (Cahyani and Sanjaya, 2021), *Rivina humilis L*. plants (Sinaga, 2021), and dragon fruit (Asra et al. 2019), has been widely researched. In previous studies, the betanin extraction process was carried out at various concentrations between simplicia and the solvent.

Research (Priatni and Pradita, 2015) has conducted betacyanin extraction from dragon fruit skin at a ratio between the skin and various solvents of 1:4, whereas research (Asra et al. 2019) has used a ratio of dragon fruit skin to water (2:1). The ratio of dragon fruit peel/pulp with solvent can also be 1:10 (Khoo et al. 2022).

In this study, various comparisons of simplicia and solvent compositions were carried out to determine the optimum conditions for obtaining betanin. The results of the present study were obtained if the differences in the compositions of simplicia and solvents among various comparisons were not significant. This is presented in Table 2.

Table 2. Results of Betanin Concentration Measurement at Various Ratios of Fruit and Peel: Aquadest

Sample: Aquadest	**Concentration (mg/mL)**		**Anova One Way**	
	Flesh	**Peel**	**P value of Flesh**	**P Value of Peel**
1 : 1	21,71±0,60	17,8±3,12	0,528	0,89
1 : 2	21,65±3,5	10,37±2,5		
1 : 3	20,25±0,5	8,62±4,5		
1 : 4	20,94±1,67	7,51±0,8		

Table 3. Two-way Analysis of Betanin Concentrations via Temperature Extraction

Temperature	**Part of Dragon Fruits**		**P Value**
	Flesh (mg/mL)	**Peel (mg/mL)**	
Chiller	20,78	1,98	0,69
	19,18	2,01	
Freezer	19,71	2,57	
	19,89	2,49	

On the basis of the betanin concentration data obtained through various variations in the ratio of dragon fruit peel/flesh with aquades processed via anova one way using SPSS, there is no significant difference, indicating that betanin extraction from dragon fruit can be performed with any comparison. This can be seen from the p value obtained for dragon fruit flesh of 0.528 and the p value for dragon fruit skin of 0.89. Nevertheless, the highest concentration of betanin from dragon fruit flesh was obtained at a ratio of simple:solvent (2:1), whereas that from dragon fruit skin was obtained at a ratio of simple:solvent (1:1).

The results of the study related to the comparison of samples and solvents are not in line with the research conducted by Mai Thi Phuong Chi (2021) on the extraction of betacyanin from the skin of white-fleshed dragon fruit (Hylocereus udantus), which revealed that the highest extraction results were obtained with a fairly dilute sample and solvent composition, namely, 20 parts of aquades and 1 part of dragon fruit peel (Thi and Chi, 2021)..

The optimization process of extracting betanin from the peel and flesh of dragon fruit is then carried out at the extraction temperature. The effect of temperature was initially carried out at room temperature, in a chiller, and in a freezer, but for extraction at room temperature, there was degradation of betanin compounds so that absorbance measurements could not be made; this was accomplished by Davi in 2019, who conducted betanin stability tests at temperatures of 4–8°C and -30°C(da Silva et al. 2019)..

The results revealed that when both fruit and dragon fruit skin were extracted at a temperature of 4–8°C and frozen, there was no significant difference. Table 3 shows the results of the data analysis via two-way ANOVA, which yielded a p value of 0.69 (<0.05), indicating that there was no significant difference in the effect of temperature on the betanin extraction yield.

CONCLUSION

Betanin was successfully extracted from the peel and flesh of red dragon fruit (*Hylocereus constaricencis*) via aquades, and the optimum concentration of betanin from dragon fruit flesh was determined at a ratio of fruit flesh:solvent of 2:1, whereas in the peel of dragon fruit, the optimal ratio of peel:solvent was 1:1, and the temperature had no effect on the yield of extraction. The concentration of betanin in the flesh of dragon fruit juice was greater than that in the peel of dragon fruit with the same solvent

ratio (1:1% w/v), with values of 21.71 mg/mL and 17.8 mg/mL, respectively.

This research is not completely perfect because the pH has not yet been optimized, and the extraction temperature needs to be studied with more variation. Thus, it is expected that the amount of betanin obtained from dragon fruit juice can be maximized.

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