

# The Radical Scavenging Activity Of 2-2' Diphenyl -1- Picrylhydrazil (Dpph) On The Methanol Extracts And Ethyl Acetate Fractions Of Red Dragon Fruit Peel (*Hylocereus Polyrhizus* (F.A.C.Weber) Britton Dan Rose)

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## Abstract

Red dragon fruit (*H. Polyrhizus*) is one of the plants that has a great potential as natural antioxidant. This study tested the activity of radical scavenging of 2-2' diphenyl -1- picryl hidrazil (DPPH) in the methanol extract, as well as in the soluble and insoluble fractions of ethyl acetate of red dragon fruit peel. This research is carried out through various stages, such as: extraction and fractionation to obtain both insoluble fraction and soluble fractions of ethyl acetate. Antioxidant activity test is conducted by the method of thin layer chromatography and spectrophotometry. Antioxidant activity test, IC<sub>50</sub> values of methanol extract, ethyl acetate soluble fraction, and insoluble fraction of ethyl acetate had been obtained consecutively as much as 241.19 µg /mL, 8.34 µg/mL, 46.84 µg/mL. The soluble fraction of ethyl acetate had greater antioxidant activity compared to the methanol extract and the insoluble fractions of ethyl acetate.

**Keywords:** Red dragon fruit, Antioxidant activity.

## Introduction

Red dragon fruit (*Hylocereus polyrhizus*) is one of the Cactacea family members that comes from Latin America and has been considerably fostered in Indonesia. The red dragon fruit contains *lycopene* that functions as natural antioxidant and well-known to prevent cancer and heart disease, as well as to lower blood pressure. Not only the red dragon fruit pulp that is beneficial, but its peel is also highly potential, for it contains high concentration of β-amirin, -amirin, oktacosane, γ-sitosterol, octadecane, 1-tetracosanol, heptacosane, campesterol, and betalain [1]. The red dragon fruit peel is efficacious as antioxidant, antibacterial, and source of natural pigment [2]. Based on a research it is found that antioxidant activity in the red dragon fruit peel is higher than that of the red dragon fruit pulp [3]. The red dragon fruit peel contains betalain color pigment; a compound that has antioxidant activity. Antioxidant activity testing employs DPPH radical scavenging method interpreted through IC<sub>50</sub> values. DPPH method is the simplest method. DPPH as free hydrogen scavenger will react with a compound that contains OH structural units (polyphenol category) and another compound that can release Hydrogen ion (H<sup>+</sup>).

## Research Methodology

## Materials

The main component used in this research is red dragon fruit (*Hylocereus polyrhizus*) peel that is obtained from Bantul regency, Yogyakarta, Indonesia. The used solvent is methanol, pro-analysis quality ethyl acetate (Merck), Vitamin C, and 2-2' diphenyl-1-picrylhydrazil (Sigma)

## Extract Preparation

As much as 500 grams of red dragon fruit peel is macerated with methanol and stirred repetitively, its filtrate is vaped by rotavapor until thick extract is formed. 8.33 grams of the thick extract is then partitioned with 50 mL ethyl acetate. Partition is performed repetitively until clear ethyl acetate fraction is obtained. The ethyl acetate fraction and insoluble fraction of ethyl acetate is vaped until thickened.

## Phytochemical Screening

The extract and fraction was tasted for the presence of bioactive compounds by using following standard method.

Test for triterpenoids: to extract and fraction solution. 10 drops of acetic anhydride was added and mixed well. To this a concentrated sulphuric acid was added from the sides of the test tube appearance of greenish blue colour indicates the presence of triterpenoids [4].

Test for flavonoids: the extract and fraction was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids [5].

Test for saponins : the extract and fraction was mixed with 5 mL of water and vigorously shaken. The formation of stable foam indicated the presence of saponins [4] [5] [6].

Test for tannins : the diluted ferric chloride solution was added to extract in a test tube and observation made. The appearance of a dark green color or blue indicates the presence of tannins [5].

Test for alkaloids : extract and fraction was dissolved in dilute hydrochloric acid and filtered. Filtrate was treated with dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloid [5].

Test for steroids : extract and fraction was mixed with a chloroform and concentrated sulphuric acid was added. A red colour produced in the lower chloroform layer indicated the presence of steroids [4].

### Radical Scavenging Activity through DPPH method

Determining the antioxidant activity by employing free radical scavenging through DPPH method as has been stated, the test is conducted using some methods as follows: a certain amount of

sample is added with 1.0 mL DPPH 0.4 mM and 3.950 mL methanol. This mixture is then vortexed and let alone for approximately 30 minutes. Subsequently, the mixture's absorbance is measured with 515 nm wave length on methanol as a blank. The absorbance measurement is also performed on the controls that consist of 1.0 mL DPPH and 4.0 mL methanol. Vitamin C is used as comparative compound [7][8]. The percentage of free radical scavenging by the sample can be calculated using the formula as follows:

$$\text{Percent (\%)} \text{ of DPPH radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where  $A_0$ : control absorbance (does not contain sample).

$A_1$ : test sample or comparative compound absorbance.

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## Result And Discussion

### Phytochemical Screening

Preliminary phytochemical screening show in table 1.

Table 1 : Results of phytochemical test

Test	Triterpenoids	Flavonoids	Saponins	phenolic	Alkaloids	steroids
Methanol Extract	+	+	-	+	+	-
Ethyl acetate soluble fraction	+	+	-	+	+	-
Ethyl acetate insoluble fraction	-	+	-	+	+	+

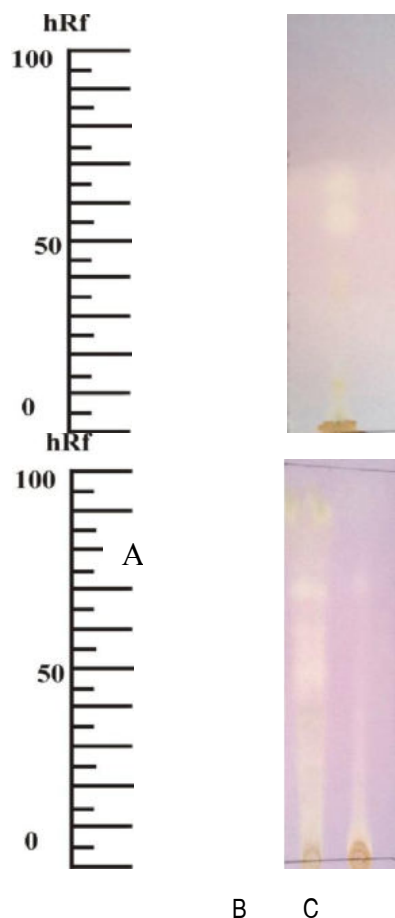
### Antioxidant Activity Testing

Antioxidant activity testing on test compound of red dragon fruit peel fraction and extract can be conducted through DPPH method (2,2'-diphenyl-1-picrylhydrazyl). DPPH is a stable free radical and often used in order to evaluate the antioxidant activities of several compounds or natural ingredients extracts.

The antioxidant interaction with DPPH, either as electron transfer or hydrogen radical on DPPH, will neutralize the character of free

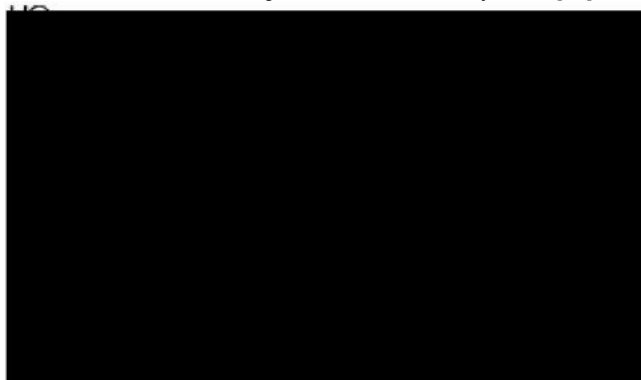
radical of DPPH and form reduced DPPH. DPPH that dissolves in polar soluble is, for example, methanol and ethanol [9].

Chromatogram of the red dragon fruit peel, soluble fraction of ethyl acetate, and insoluble fraction of ethyl acetate after sprayed with DPPH within was benzene movement phase: ethyl acetate (4:1) and silicate still phase  $F_{254}$  can be seen in Figure 1. And the test result of antioxidant activity ( $IC_{50}$ ) of the extract and fraction result can be seen in Table 2.



**Figure 1. The result of chromatogram DPPH**  
 A: methanol extract of the red dragon fruit peel  
 B: ethyl acetate soluble fraction  
 C: ethyl acetate insoluble fraction

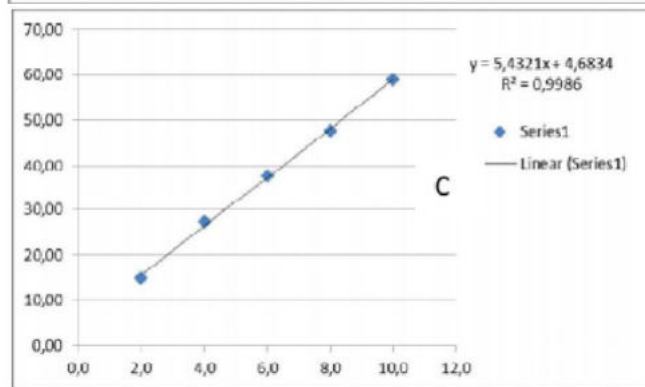
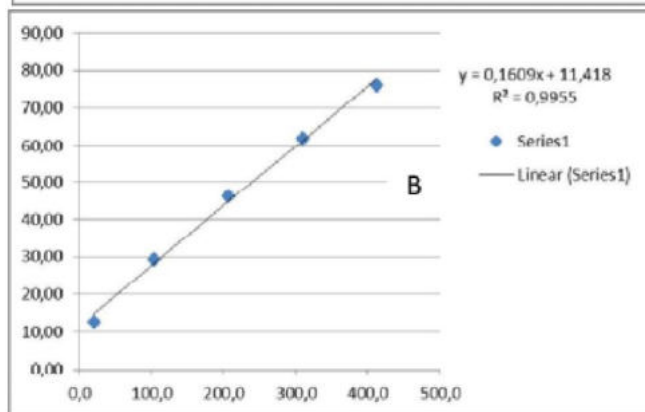
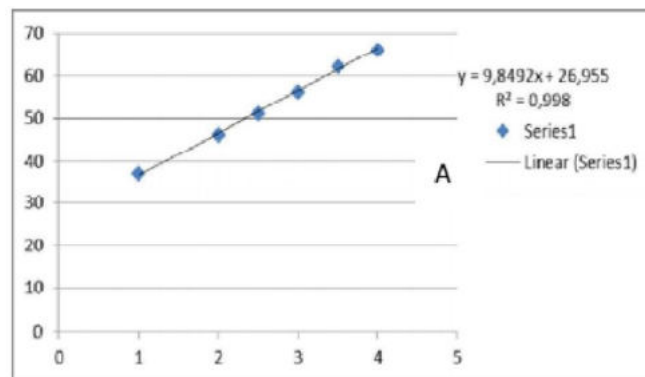
Phenolic compound may act as antioxidant by scavenging the radical [10]. Betasianin in the red dragon fruit peel belongs to phenolic compound [11]. Hence, there are chances that betasianin compound in this fruit significantly acts as antioxidant. The higher content of betasianin, the higher antioxidant activity it has [12].

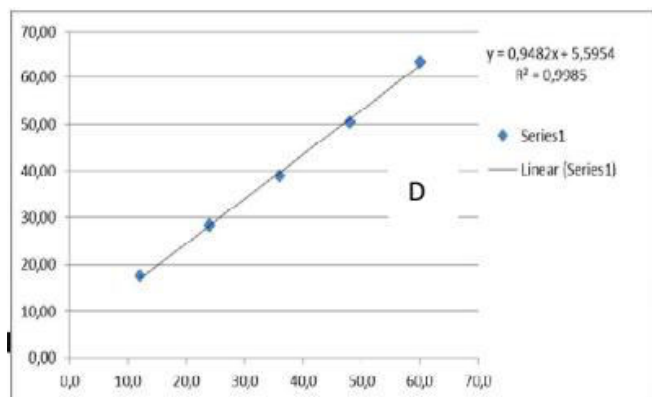


**Figure 2. Betasianin structure**

**Table 2. IC<sub>50</sub> values of test compound with DPPH method.**

No.	Sample	IC <sub>50</sub>
1	Vitamin C	2,34 µg/mL
2	Methanol extract	241,19 µg/mL
3	Ethyl acetate soluble fraction	8,34 µg/mL
4	Ethyl acetate insoluble fraction	46,84 µg/mL





**Figure 3.** The relationship between test compound level and percentage value of DPPH radical scavenging; A) Vitamin C, B) Methanol extract, C) Ethyl acetate insoluble fraction, and D) Ethyl acetate soluble fraction.

Table 1 indicates that ethyl acetate soluble fraction has the greatest antioxidant potential, compared to methanol extract and ethyl acetate insoluble fraction. But it is still smaller than Vitamin C control. The result of extraction and antioxidant activity is highly related to the solvent polarity which determines the success of antioxidant compound extracting.

## Conclusion

IC<sub>50</sub> values of methanol extract, ethyl acetate soluble fraction, and ethyl acetate insoluble fractions obtained from the result of this antioxidant activity are 241.19 µg/mL, 8.34 µg/mL, 46,84 µg/mL. The soluble fraction of ethyl acetate has greater antioxidant activity compared to the methanol extract and the ethyl acetate insoluble fraction

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