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Antioxidant Testing Using DPPH for Dadap Leaves Through Without Extraction Stage

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Abstract

Antioxidant testing is widely needed in health aspects. Knowledge of antioxidant values can help in the selection and development of food products. The DPPH method is widely used because of its ease and efficiency. For plant samples, initial treatment is needed to ensure the accuracy of the solution concentration. Many plant samples must be extracted first. This process is time consuming and has mass validity issues. This study aims to: to evaluate a DPPH antioxidant test method without extraction that is efficient, effective, practical, and environmentally friendly, (2) to analyze the validity of the developed method. The result revealed that the IC50 value was obtained about 1028.85 ppm. The linearity and precision were 0.98 and 0.02% respectively. This method proved that without extraction stage, the IC50 can be determined.

Keywords: DPPH; antioxidant; testing; dadap leaves

Introduction

Food plays an important role in body health. Body health can be attacked by free radicals in the body that can trigger serious diseases [1]. Meanwhile, free radicals can be stopped by antioxidant compounds. These compounds are contained in food at certain levels. These levels are analyzed by antioxidant test results [2]. Quantitative analysis of antioxidants aims to obtain a value of the content that states how much its concentration is. Antioxidant testing methods include = DPPH, FRAP, ABS, etc. Many of these test applications are for health, food, and beverage products [3]. Antioxidant test using the DPPH method. DPPH has a chemical formula, namely 2,2-diphenyl-1-picrylhydrazyl. DPPH is used as a model of free radical compounds. The interaction of DPPH (a free radical model) with antioxidants produces neutral and safe products. This method has been widely used in previous studies [4].

Many studies before the DPPH test must prepare samples in the form of extraction [5]. The extraction carried out also varies in methods from the use of appropriate solvents, time, tools used, and other technical parameters (pressure, temperature, humidity). Then, sample preparation is made with a concentration variation with a minimum of 4-5 concentration variants to create a linear regression equation. The equation is important for determining the IC50 [6]. This study offers a DPPH test without the extraction stage for sample preparation. Without any extraction stage, this DPPH test will be analyzed for its validation. The development of this test will save a lot of time, materials, tools, and support the reduction of environmental impacts. In this study, Dadap leave sample were taken as a model of materials containing antioxidant compounds. The objectives of this study are: (1) to evaluate a DPPH antioxidant test method without extraction that is efficient, effective, practical, and environmentally friendly, (2) to analyze the validity of the developed method.

Materials and Methods

The tools used were a 10 mL measuring flask, stirrer, analytical balance (Kern), incubator (memmert), test tube (iwaki), test tube rack, dropper, 5 mL-10 mL measuring cup, Erlenmeyer flask, weighing paper, and label paper. The materials used were ascorbic acid (p.a.), DPPH (p.a., Himedia), methanol (Smartlab), Dadap leave from Malang. The instrument used was a UV-Vis spectrophotometer (Thermo Scientific, Evolution 201).

Antioxidant test with Dadap leaves without extraction stage

There are two types of sample preparation, namely sample preparation from extraction results and sample preparation without extraction. Both will be compared and become the subject of discussion. Sample preparation is made 1000 ppm by: mixing 10 mg of sample with methanol solvent in a 10 mL measuring flask. Specifically, for samples without extraction, the sample mass is obtained from the following formula:

$$m = \frac{w\ x\ 100}{\%\ yield}$$

Note: m = mass of sample required to equivalent 10 mg of extracted sample, w = mass is expected to be 1000 ppm from sample preparation, 100 = multiplying factor, %yield = percent yield of samples obtained from literature/extraction results. After making a 1000 ppm solution, concentration variations were made by dilution to 30, 60, 90, 120, 150 ppm.

Next, the preparation of the positive control solution (ascorbic acid) was made 100 ppm by: mixing 1 mg with methanol solvent in a 10 mL measuring flask. Then, the positive control solution was made with concentration variations of 4, 8, 12, 16, 20 ppm through dilution. Then, the preparation of the DPPH solution was prepared with a concentration of 0.76 mM (300 ppm) which was made by: 3 mg of powder with methanol solvent in a 10 mL measuring flask. The mixture and incubation were carried out at room temperature, and the absorbance reading was carried out with a UV-Vis spectrophotometer instrument at a wavelength of 517 nm. Furthermore, the results of the UV-Vis data were in the form of absorbance values. Absorbance data from various concentration variations were used to calculate the % inhibition with the formula:

$$\%\ Inhibition = \frac{(A_{negative\ control} - A_{blank}) - (A_{sample\ or\ positive\ control} - A_{sample\ control})}{(A_{negative\ control} - A_{blank})} \times 100\% \quad \text{(Equation 1)}$$

Note: Abs = absorbance of instrument

Then, a graph is made with the x-axis (concentration) and the y-axis (% inhibition). Finally, the IC50 value is calculated using the linear regression equation obtained from the graph.

Results and Discussion

Antioxidant testing was carried out in a laboratory. After absorbance measurement, the analysis is used to result the % inhibition value. The results can be seen in Table 1.

Table 1: Results of the absorbance and the % inhibition

Concentration (ppm)	Abs. (1)	Abs. (2)	Abs. (3)	Average	Abs, negative control	Abs, sample control	Average of % Inhibition
250	1.705	1.707	1.708	1.707	1.956	0.103	18.00
200	1.747	1.749	1.752	1.749	1.956	0.103	15.85
150	1.779	1.771	1.774	1.775	1.956	0.103	14.52
100	1.838	1.837	1.826	1.834	1.956	0.103	11.50
50	1.86	1.863	1.862	1.862	1.956	0.103	10.07

Note: Abs = absorbance

Subsequently, the linear regression curve between % inhibition and concentration can be seen in Figure 1.

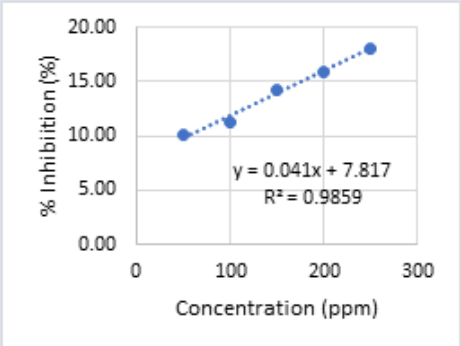

Figure 1: Linear regression curve of first antioxidant testing of Dadap leaves

Based on Figure 1, the equation is y = 0.041x + 7.817 with R^2 equals to 0.9859. the R^2 value shows linearity because of the acceptable linearity is $R^2 \geq 0.98$ (7). Therefore, the IC50 of Dadap leaves is 1028.85 ppm. The value was categorized as low level of antioxidant activity. Furthermore, the precision of this method by RSD (Relative Standard Deviation) is 0.02% based on Table 2. the precision is acceptable because of less than 2% limit [8]. Therefore, antioxidant of a sample without extraction stage can be tested by DPPH method.

Table 2: Precision calculation of the developed method

IC50	Value
first IC50	1028.85
second IC50	1069.57
Third IC50	1030.49
Standard deviation	23.05
Average	1042.97
Relative standard deviation	0.02

The previous study revealed that antioxidant activity of dadap serep leaves were very strong with an IC50 value of 8.32 ppm [9]. The testing using the DPPH (1.1-diphenyl-2picrylhydrazyl) with 70% ethanol as solvent. In this study, the IC50 value was very lower than the previous study. The possible reasons are difference in concentration of DPPH, difference in solvent, and difference in the mixture of DPPH and sample [10].

Conclusion

Based on the results, the method of this study was efficient and effective. The IC50 obtained based on the calculation is 1028.85 ppm. This method was acceptable since the linearity and precision were acceptable. Therefore, without extraction stage, the antioxidant testing can be carried out. The future challenge is how to evaluate the free-extraction sample using other methods such as FIC, FRAP, and ABTS.

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