Phytochemical Test, Vitamin C Content and Antioxidant Activities Beet Root (Beta vulgaris Linn.) Extracts as Food Coloring Agent from Some Areas in Java Island

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Abstract. The use of coloring agent as food additives has received attention. This is due to the frequent abuse of synthetic dyes that are not for food. Beet root are usually used as natural dyes. This is because beet roots, especially the tubers, are rich in betalain pigments. The purpose of this study was to determine secondary metabolites, vitamin C content and antioxidant activity beet root extracts in several regions of Java. Phytochemical test results show that all of beet root extracts positive contain phenolic, tannin, flavonoids and saponins. The content of vitamin C extract of beet roots originating from the regions of Bogor, West Java, Central Java and East Java respectively is 57,1714; 63,6470; 54.9943; and 65.9868 mg / 100 g of ingredients. The antioxidant activities all of samples included very weak categories, namely for extracting red beet roots from Bogor 1,276.28 ppm; West Java 1,497.18 ppm; Central Java 1,316.21 ppm; and East Java 1,759.08 ppm.

1. Introduction

The use of coloring agent as food additives has received attention. This is due to the frequent abuse of synthetic dyes that are not for food. This abuse occurs because the price of dyes for food tends to be more expensive than synthetic dyes that are not for food. Synthetic dyes generally have carcinogenic properties that can be harmful to human health. Therefore it is necessary to look for sources of natural dyes that are safe and have low prices.

Beet root (Beta vulgaris L.) is a root-shaped plant that resembles tubers, including those of the Chenopodiaceae family. Beet root is a potential source of dietary fiber, vitamins and minerals. The potential vitamin content in red beet is folic acid and vitamin C, while the mineral content is manganese, potassium, magnesium, iron, copper, and phosphorus. Other contents contained in red beet include zinc, bioflavonoids, pure sugar, and betain [1]. Beet root has a physical characteristic that is in the form of grass with a very short and almost invisible stem, has a taproot that grows into tubers, its tubers are round like potatoes and are red to dark purple which when the fruit is cut it will look white stripes in red young.

Beet roots are usually used as natural dyes. This is because the beet roots, especially the tubers, are rich in betalain pigments. According to [2] and [3], betalain is a pigment of a group of water-soluble
alkaloids, hydrogen pigments, and an anthocyanin substitute in most families of the Caryophyllales order, including Amaranthaceae. Betalain was initially categorized as an anthocyanin with nitrogen because there is nitrogen in the structure of the ring. Along with the development of science and knowledge, betalain is no longer classified as an anthocyanin part, but stands alone as a type of pigment. Betalain is a parent of the betasianin group that is violet red and betaxanthin yellow. The concentration ratio of betasianins and betaxanthines varies depending on the beetroot varieties. The difference in the ratio of the two pigments causes red color variations in bits [4].

Betasianin is one of the pigments that can be used as natural dyes and can be extracted from plants. Betasianin has a soluble nature in water solvents, so betacanins are very well developed as natural dyes. In plants, betasianin is found in parts of flowers, fruits and leaves that have purplish red color [5]. Betasianin is very sensitive to several factors. The factors that affect the stability of betasianins are temperature, pH, light, oxygen and metal ions [6].

Betasianin can be used as a natural coloring agent in the form of extracts. Betasianin can be extracted using aquadest, methanol and ethanol [7]. [8] conducted a research to extract betacyanin from Opuntia fruit using distilled water solvent and distilled water mixture: ethanol. From the results of the study it was found that betasianin extraction using aquadest solvents gave a higher total betasianin than the distilled water mixture: ethanol. Maia in [7] reported that betalain extraction using ethanol-hydrochloric acid (99: 1) solvents gave a higher total betasianin than aquadest solvents. Betasianin extraction using aquadest solvents and slightly acidic conditions gave stability to betasianin compounds [5].

Besides being used as a dye, betasianin also has a function as a natural antioxidant [9]. Beet is one of the most useful food ingredients. One of the benefits is providing natural colors in the manufacture of food products. The pigment found in red beets is betanine. Betanin compounds in different bits with anthocyanin pigments in other plants because these pigments also contain nitrogen compounds which have a positive effect on free radical activity and cancer so that beet roots are also being developed as an alternative to coloring sausage products [10].

2. Materials and Methods

The ingredients of this study are beet root from some areas in Java Island, ethanol, and citric acid. The equipment used in this research are spectrophotometer, rotary evaporator, magnetic stirrer, chromatography, glassware. The stages of this study were begun by extracting betasianin pigments from red beetroot tubers. Extraction was carried out by maceration using 2% ethanol + citric acid solvent for 24 hours at room temperature, airtight, and light resistant. Comparison of ingredients with solvents is 1:10 (b / v). The extraction results are then filtered using filter paper and obtained a filtrate that has been separated from the pulp. This filtrate is concentrated using a rotary evaporator at 40° C. The concentrated extract is stored in a sealed dark bottle in the refrigerator.

The phytochemical test of red beet extract using ethanol plus 2% citric acid solvent aims to determine the bioactive compounds contained in beet root extracts and be able to act as antioxidants. Tests carried out consisted of: Testing of flavonoids [11], Testing of saponins [12], Testing of tannins [12], Testing of phenolics [13].

The content of vitamin C is determined based on the reduction of oxidation titration by titrating the sample in a mixture solution of acetic acid and meta phosphate using 2,6 dichlorophenol indophenol. The titration is stopped if the sample solution that was originally clear becomes pink. Antioxidant activity was determined based on the reduction percentage of DPPH absorption using equations, namely as follows :

\[
\text{Percentage of reduction of DPPH absorption: } \frac{A_{DPPH}(t) - A_{sample}(t)}{A_{DPPH}(t)} \times 100\% \tag{1}
\]

\(A_{DPPH}(t)\) is absorption from DPPH in time t
\(A_{sample}(t)\) is absorption from sample at time t

The method of data analysis used in this study is descriptive method that is in the form of qualitative and quantitative data. Quantitative data in the form of graphs and tables.
3. Results and Discussions

3.1. Phytochemical test

The testing of phytochemical compounds in red beet extract aims to determine the content of the active compounds contained in the extract. The results of this test are used to identify groups of compounds that act as antimicrobial components of red beet extract. The tests carried out consisted of qualitative testing to determine the presence of a compound in extracts and quantitative testing to determine the total content of a phytochemical compound. The results of phytochemical content testing are presented in Table 1.

| Phytochemical  | Result | Picture |
|----------------|--------|---------|
| Phenolic       | +      | ![Image](image1.png) |
| Tannin         | +      | ![Image](image2.png) |
| Flavonoid      | +      | ![Image](image3.png) |
| Saponin        | +      | ![Image](image4.png) |

The results of flavonoid testing of all beet root extract samples showed positive. This is indicated by the occurrence of extract color changes from pink to brownish orange. Reagents used are concentrated HCl and Mg powder. The addition of concentrated metal Mg and HCl aims to reduce the benzopirone nucleus found in the structure of flavonoids so that red or orange flavillium salts are formed [14].

Saponin testing is done by adding hot water to the sample and shaking it until the foam is formed. When adding 1% (dilute) hydrochloric acid the foam remains stable, indicating a positive sample containing saponins [15]. Saponin tests show positive all of beet root extracts containing saponin
compounds which are characterized by the formation of foam. This is in line with the results of research by [16] which stated that beet root extracts contained saponin compounds.

In testing to determine the presence of phenolic compounds in beet root extracts, FeCl$_3$ 5% (b / v) was used. However, the addition of 5% FeCl$_3$ can only show the presence of phenolic compounds in general and cannot distinguish the type of group. The positive results of this test are indicated by the formation of strong green, red, blue, purple, or black complexes [17]. The following are the reactions that occur in the phenolic test:

$$6 \text{ArOH} + \text{FeCl}_3 \rightarrow [\text{Fe(OAr)}_6]^3+ + 3 \text{HCl} + 3 \text{H}^+$$

(2)

The color complexes (green, black, dark blue)

The test results showed that all samples of beet root extracts positively contained phenolic compounds. This is characterized by changes in the color of the extract from pink to blackish green.

Tannin testing was carried out using 1% FeCl$_3$ reagent. Positive test results are marked by changes in color to blackish green. [11] states that color changes occur because tannins react with Fe$^{3+}$ ions to form complex compounds that have covalent bonds of coordination between metal ions and non-metals. The results of testing the tannin beet root extracts showed a positive result which was marked by changes in the color of the extract from pink to blackish green.

3.2. Antioxidant test

Testing of antioxidant activity was carried out using the DPPH method. The interaction of antioxidants with DPPH either electron transfer or hydrogen radicals on DPPH will neutralize free radicals from DPPH is the principle of the DPPH method [18]. The color change that occurs in DPPH solution in methanol which is originally concentrated purple becomes pale yellow indicating antioxidant activity [19]. This method was chosen because the testing is simple, easy, fast and requires only a small sample [20].

![Figure 1. Relationship Between Beet Root Extract from Bogor with % Antioxidant Inhibition](image)

The antioxidant activity of beet root extracts was expressed in percent (%) inhibition of DPPH radicals. To obtain the percentage of inhibition seen from the difference in absorption between the sample Absorban measured by a UV-Vis spectrophotometer at a wavelength of 517 nm. Then from the graph of the relationship between the concentration of the sample and the percent inhibition of DPPH, linear regression was used to find the IC50 value. The acquisition of IC50 value was used to determine the magnitude of antioxidant activity where IC50 is the concentration of sample solution needed to inhibit 50% of DPPH free radicals [21]. Based on [22], the smaller the IC50 value indicates the greater
antioxidant activity in the material tested. The results of testing the percent inhibition of beet root extracts from some regions in Java are presented in Figures 1 to 4.

Based on the results of linear regression analysis the relationship between the concentration of beet root extract from Bogor with the percent inhibition of DPPH absorbance obtained a linear regression equation: $y = 0.0275x + 14.631$ as presented in Figure 1. The linear regression equation obtained can determine IC50 value, namely extract concentration beet root that can reduce 50% DPPH absorbance. From the calculation results obtained the IC50 value averages 1276.28 ppm. This shows that the beet root extract from Bogor has very weak antioxidant activity because it has an IC50 value of more than 200 ppm [23].

![Figure 2](image-url). Relationship Between Beet Root Extract from West Java with % Antioxidant Inhibition

![Figure 3](image-url). Relationship Between Beet Root Extract from Central Java with % Antioxidant Inhibition
Figure 4. Relationship Between Beet Root Extract from East Java with % Antioxidant Inhibition

Based on the results of linear regression analysis the relationship between the concentration of beet root extract from West Java with the percent inhibition of DPPH absorbance obtained a linear regression equation: 0.0191x + 21.735 as presented in Figure 2. The linear regression equation obtained can determine IC50 value, namely extract concentration beet root that can reduce 50% DPPH absorbance. From the calculation results obtained the IC50 value averages 1497.18 ppm. This shows that the beet root extract from West Java has very weak antioxidant activity because it has an IC50 value of more than 200 ppm [23].

Based on the results of linear regression analysis the relationship between the concentration of beet root extract from Center Java with the percent inhibition of DPPH absorbance obtained a linear regression equation: 0.0239x + 19.321 as presented in Figure 3. The linear regression equation obtained can determine IC50 value, namely extract concentration beet root that can reduce 50% DPPH absorbance. From the calculation results obtained the IC50 value averages 1316.21 ppm. This shows that the beet root extract from Center Java has very weak antioxidant activity because it has an IC50 value of more than 200 ppm [23].

Based on the results of linear regression analysis the relationship between the concentration of beet root extract from East Java with the percent inhibition of DPPH absorbance obtained a linear regression equation: 0.0175x + 19.691 as presented in Figure 4. The linear regression equation obtained can determine IC50 value, namely extract concentration beet root that can reduce 50% DPPH absorbance. From the calculation results obtained the IC50 value averages 1759.08 ppm. This shows that the beet root extract from East Java has very weak antioxidant activity because it has an IC50 value of more than 200 ppm [23].

4. Conclusions

Conclusion. Phytochemical test results of positive that all of beet root extract contain phenolic, tannin, flavonoids and saponins. The content of vitamin C extracts of beet root originating from the regions of Bogor, West Java, Central Java and East Java respectively is 57.1714; 63.6470; 54.9943; and 65.9868 mg / 100 g of ingredients. The antioxidant activities of the four samples included very weak categories, namely for extracting red beet roots from Bogor 1276.28 ppm; West Java 1497.18 ppm; Central Java 1316.21 ppm; and East Java 1759.08 ppm.
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