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Determination of Total Flavonoid Contents of The Antioxidant Herbal Beverage *Caesalpinia sappan* L. Quantitatively Using The Calorimetry Methods of The $AlCl_3$ Reaction That Forms Complex Compounds

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ABSTRACT

Body health is an important thing, so many choices of methods for maintaining body fitness are offered. One way is to consume herbal plants that contain antioxidant compounds. Antioxidant compounds can neutralize and prevent damage caused by free radicals in the body. The plant that contains high antioxidant compounds is secang (*Caesalpinia sappan* L.). In previous research, a test of the antioxidant activity of this plant with an IC_{50} value of 11.37 ppm was reported, but a test to determine the total flavonoid content of the compound had never been carried out. So, this research is interesting to carry out with the aim of finding out the total flavonoid levels in a serving size of 0.05 grams of secang powder. The method for determining total flavonoid content used is the calorimetry method with $AlCl_3$ reagent as a complex compound forming and quantitative analysis using an Ultraviolet Visible (UV-Vis) spectrophotometer. The flavonoid content value obtained was 0.2370% b/b for a serving size of 0.05 grams of sample. These results indicate that the secang sample contains flavonoid compounds which can be useful for increasing the body's immunity.

Keywords: *Caesalpinia sappan* L., flavonoid, calorimetry, antioxidant

I. INTRODUCTION

Dirty environmental conditions and unhealthy lifestyles will cause various kinds of degenerative diseases. This is due to increased levels of free radical formation in the body due to oxidation reactions. Free radicals are compounds that have unpaired electrons and are also by-products in metabolic processes which are also known as Reactive Oxygen Species (ROS) and reactive nitrogen compounds (SNR) which are integrated with each other (Rahman et al., 2021). According to Simanjuntak (2020), excessive formation of ROS and SNR in the body can be prevented and neutralized by antioxidant compounds through the process of inhibiting chain oxidation reactions. Antioxidants consist of two types based on their source, namely endogenous antioxidants (produced by the body) and exogenous antioxidants (from outside the body) which can be obtained from the intake of additional supplements such as plants that are rich in antioxidants (Langgori & Betty Elok Kristiani, 2021).

The sappan plant (*Caesalpinia sappan* L.) is known to have potential as a plant that contains high antioxidant compounds and has been used by the community as a herbal medicinal plant to treat various diseases, such as tetanus, blood clots and pain. *C. sappan* L. is a plant belonging to the genus *Caesalpiniaceae* (Ma et al., 2020). Based on the phytochemical screening test, *C. sappan* L. contains secondary metabolite compounds in the flavonoid group (Kurniati et al., 2012).

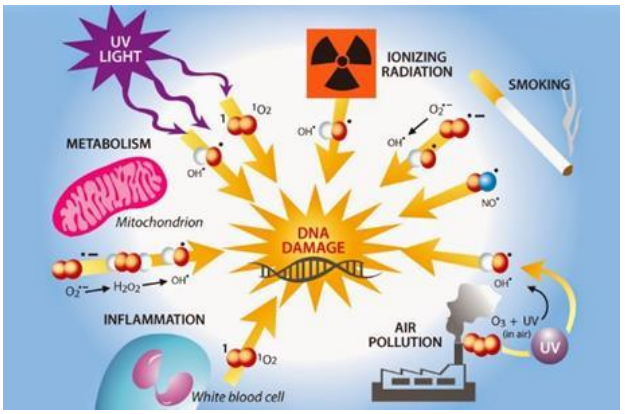

Figure 1. Source of free radicals that attack DNA (Parwata, 2016)

The *C. sappan* L. plant has been widely processed into products in the form of natural dyes, drinks and food (Asfar et al., 2019; Kurniati et al., 2012; Utari, 2017). Processing in the form of drinks or food must of course pay attention to several standards for whether the product is safe and suitable for consumption by consumers and can be commercialized. However, if we look at previous studies, quantitative testing has never been carried out to determine total flavonoid levels with certain serving sizes using the $AlCl_3$ reagent calorimetry method as a complex compound formation.

(a) (b)

Figure 2. (a) Stem (b) Flower (Sari dan Suhartati et al., 2016)

Based on the description above, it is interesting to carry out this research to determine the total flavonoid content in 0.05 grams of sample weight which is equivalent to the number of servings of one tea bag product using a quantitative calorimetry test method using $AlCl_3$ reagent as a complex compound formation followed by testing using an Ultraviolet Spectrophotometer Visible (UV-Vis). This method has the basic principle of a color formation reaction, from the reaction of $AlCl_3$ with flavonoid compounds to form a yellow color (Lindawati et al., 2022). The advantage of using this method is that it is easier to do, the process is faster and simpler compared to other methods.

II. METHODS

Equipment used includes: UV-Vis spectrophotometer (PGI T60, United Kingdom), oven, analytical balance (Osuka, PRC), electric stove (Maspion S300, Indonesia), blender (Miyako, Indonesia), desiccator, plastic press (Impulse sealer, PFS-200, 300 watts), iron spatula, dropper pipette, test tube, test tube rack, and glassware (Pyrex). ***The materials used include:*** secang wood, distilled water (H_2O), ethanol (C_2H_5OH), methanol (CH_3OH), Aluminum chloride ($AlCl_3$) 10%, Potassium Acetate (CH_3COOK) 1 M, positive control (quarcetin), and tea bags.

Sample Preparation

The secang wood used comes from Seppang Village, Gantarang District, Bulukumba Regency, South Sulawesi. This research was carried out through several process stages, namely the first stage of sample preparation including collecting, drying and refining the sample. Next, the second stage of the test to determine total flavonoid levels includes determining the maximum wavelength of quarcetin, determining the standard curve for quarcetin and determining the flavonoid levels of secang samples (Larasati et al., 2023).

Quantitative Test of Total Flavonoid Content

In testing total flavonoid levels, quarcetin was used as a positive control or standard. The maximum wavelength range of 400-450 nm can be obtained by running an Ultra Violet Visible (UV-Vis) spectrophotometer. This wavelength will be the standard for measuring the absorbance of a cup sample. The next stage is determining the quarcetin standard curve.

A stock solution was made by weighing 25 mg of quarcetin, then placing it in a volumetric flask and adding 25 mL of ethanol. Then a solution was made with a concentration of 100 ppm by taking 1 mL of the stock solution and diluting it to a volume of 10 mL using ethanol solution. Next, solutions were made with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. 1 mL of each solution of this concentration was taken and then 2% $AlCl_3$, 1 mL and CH_3COOK 1 mL were added. This mixed solution was incubated for one hour. Then the absorbance of all mixed solutions was measured at the maximum wavelength according to the standard quarcetin solution. The final stage is determining the total flavonoid content of the secang sample.

The sample was weighed as much as 0.05 g, then dissolved in 10 mL of ethanol solution. Next, 0.5 mL of the test sample solution was taken and 3 mL of methanol was added, then 0.2 mL of $AlCl_3$ and 0.2 mL of 1 M CH_3COOK were added. Then the volume was increased to 10 mL by adding 6.2 mL of distilled water. After that, the absorbance of the mixture was measured at the maximum wavelength (400-500 nm) using a UV-Vis spectrophotometer.

III. RESULTS AND DISCUSSION

Sample Preparation

The secang wood that has been collected is then cut into smaller sizes, so that the process of evaporation of the water content can easily occur. Then enter the drying stage, small pieces of secang wood are dried by air-drying. The drying process aims to avoid damage to the sample due to microbial activity (enzymatic reactions) and inhibit fungal growth so that the sample can last longer. This drying method is used to maintain the chemical composition of the sample so that it does not change (Handayani et al., 2014).

Figure 3. Sappan wood samples

The dried secang wood samples were then smoothed using a blender. However, at this stage the sample is not made completely fine, but takes the form of a coarse powder. This is so that when put into a tea bag, the sample does not leak out. The secang sample which is made

into a coarse powder also aims to expand the contact surface of the sample so that the active ingredient can easily move from the sample to the solvent or there is direct contact between the sample and the solvent (Tedju et al., 2018).

Quantitative Test of Total Flavonoid Content

Penentuan kadar flavonoid menggunakan baku standar senyawa dari golongan flavonoid, yaitu kuersetin. Struktur senyawa kuersetin memiliki gugus karbonil pada atao C4 dan lima gugus hidroksil (-OH) yang masing-masing terletak pada atom C3, C5, C7, C3' dan C4' (Cahyono et al., 2020). Senyawa kuersetin digunakan sebagai pembanding karena memiliki aktivitas antioksidan yang tinggi, yaitu, IC_{50} sebesar 5,35 µg/mL (Handayani et al., 2014).

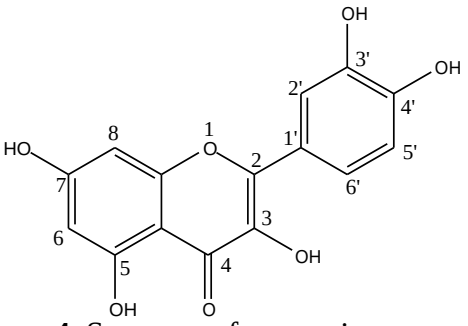

Figure 4. Structure of quarcetin compounds

1. Determination of the maximum wave length of quarcetin

The quarcetin wavelength was measured using a UV-Vis spectrophotometer at wavelengths between 400 nm to 450 nm and the maximum wavelength was 439 nm. This data was then used as a comparison for absorbance measurements from secang wood extract which was the research sample.

Table 1. Absorbance of quarcetin,λ=439nm

Concentration (ppm)	Absorbance (nm)
2	0,015
4	0,028
8	0,062
16	0,129
32	0,258

The results of measuring the absorbance of quarcetin shown in table 1 show that there is an increase in absorbance as the concentration increases. In Larasati (2023), research, the data has a similar form, namely that the higher the concentration, the higher the absorbance.

2. Determination of the standard curve for quarcetin

Kurva baku kuersetin dapat ditentukan dengan cara data pengukuran absorbansinya dimasukkan dalam microsoft exel lalu data konsentrasi dan absorbansi diplotkan, sehingga diperoleh persamaan regresi linier, yaitu y = 0.0082x - 0.0028 dengan data R2 = 0.9998.

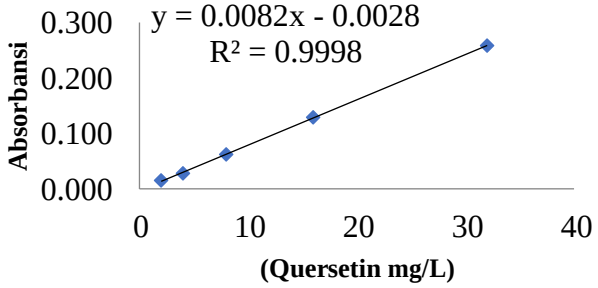

Figure 5. Quarcetin standard curve

3. Determination of total flavonoid levels

Determination of total flavonoid content was carried out by forming an Al(III)-flavonoid complex compound (**Figure 6**) between the keto group on the C-4 atom and the hydroxy group on the C-5 atom with Aluminum chloride after adding $AlCl_3$ solution, this was indicated by a change in color intensity. the solution turns yellow as a result of the shift in wavelength towards the visible (Azizah et al., 2014; Nurmila et al., 2019). So that the wavelength can be maintained at the visible wavelength, potassium acetate solution (CH_3COOK) is added. Next, the incubation process lasts for one hour which aims to ensure that the mixture is homogeneous and the reaction is complete (Larasati et al., 2023). Then the absorbance was measured at the maximum wavelength (400-500 nm). The results of determining the flavonoid levels of the secang samples can be seen in **table 2**.

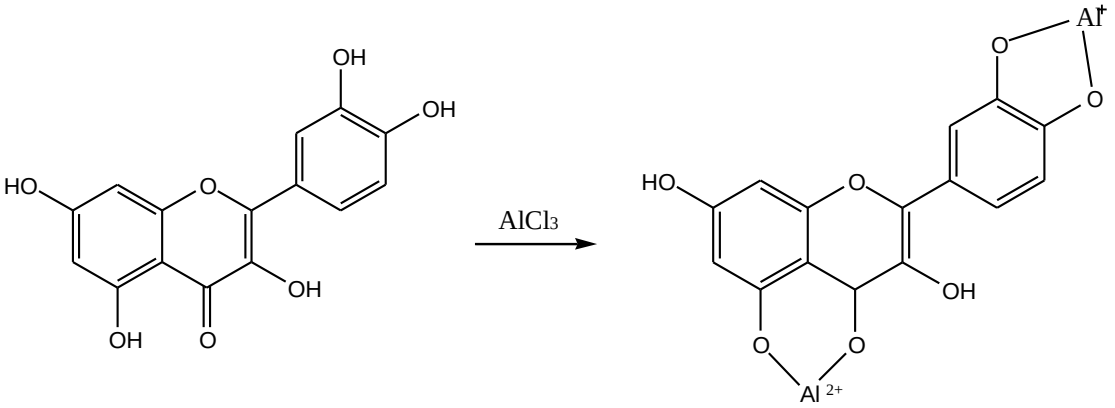

Figure 6. Reactions for the formation of complex compounds

Based on previous research, it is stated that secang samples have antioxidant activity due to their flavonoid compound content with an IC_{50} value for the DPPH test method of 101.8 ppm, the ABTS test method for 26.7 ppm and the FRAP test method for 11.04 ppm (Setiawan et al., 2018).

Tabel 2. The total flavonoid content of the total sample is secang

Repetition	Sample Absorbance (λ=439nm)	Total Flavonoid Content (%w/w)	Average Total Flavonoid Content (%w/w)
1	0,094	0,2356	0,2370
2	0,095	0,2385	

These data show that the greater the absorbance of the sample, the greater the flavonoid content, so that an average flavonoid content of 0.2370% w/w is obtained.

IV. CONCLUSION

The conclusion from the results of this research is that the secang plant samples contain flavonoid compounds which have the potential to act as antioxidants to increase the body's immunity with total flavonoid levels in a 0.05 gram serving size of 0.2370% w/w. Suggestions for further research are to carry out antioxidant activity tests to obtain IC_{50} values.

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