Analysis of Phenols and Antioxidants Infused Sappan Wood (*Caesalpiniasappan* L.)

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***Abstract*—*Caesalpinia sappan* L. is one of Caesalpiniaceae family found in Indonesia and a health drink that is often consumed by society at large in Indonesia. This study aims to theanalysis of phenol and flavonoid infuse a sappan wood *(Caesalpinia*sappan L.*)* and determine the activities of the wooden cup remedy by using DPPH (1,1-picrylhydrazyl-diphenyl-2). Sappanwood extracted using distilled solvent was conducted using infuse for 15 minutes at a temperature of 90^0C. Extracts infuse obtained analyzed qualitatively using the reactive dye and quantitative determination using spectrophotometry UV-Vis at a wavelength of 730 nm for analysis of phenol in total and 430 nm for analysis flavonoidtotal average level of phenols total of 554,86 mgGAE/L samples and flavonoid total of 1,0377 mgQAE/L samples. Samples wood sappan made with a concentration of 1%, 2%, 3%, 4%, 5% and 6% with the addition of DPPH by using a UV -Visible spectrophotometer. The results showed that the samples of the sappan wood 6% have the highest antioxidant activity at a wavelength of 513.1 nm.**

***IndexTerms*—Sappan Wood (*Caesalpiniasappan* L.), phenol, DPPH, antioxidant**

I. Introduction

On a variety of plant species have been known to contain many chemical compounds that can be used as medicine. At this time, many people are returning to the use of natural materials both in treatment and in practice to get used to living in avoiding chemicals synthesis.

One of the compounds responsible for addressing various degenerative diseases are antioxidant compounds, but the power activity of antioxidant compounds each material in different plants. Antioxidant compounds that have been shown to have potential and high antioxidant activity is of the fruits or vegetables rich in vitamin C, vitamin E, beta-carotene, and plants rich in polyphenols class of compounds such as flavonoids, phenolic acids, tannins, and lignin.Many types of plants are rich in polyphenol and used for the treatment, such as tea, mahkotadewa, sikaduduak, and mengkudu from Indonesia, clove leaves, etc.[1]–[3].

One of the plants that have high antioxidant activity is the sappan wood (*Caesalpiniasappan* L.). Plants containing flavonoids and phenols are known to have strong antioxidant activity[4].

Results of previous research turn the sappan wood, in vitro contains antioxidant compounds that indicate potential as an antioxidant that can prevent the formation of free radicals[5]. Sappan wood including traditional medicinal plants and some places in Indonesia utilising wooden cup as a dye and as a medicine[6].

Based on the above background, it is necessary to study this in the hopes of obtaining information about the content of phenols and flavonoids contained in infusesappan wood (*Caesalpiniasappan* L.). It also aims to determine the ability of the wooden cup infuse in counteracting free radicals. The method used was DPPH (1,1-diphenyl-2-picric-hydrazide). DPPH is a free radical molecule are unstable because ofdecolization electrons whole molecules so that the molecules were not DPPH dimerisation. Decolonization this electron will leave a deep purple colour ethanol solution measured at a wavelength of 520 nm[7].

II. Material and Methods

Method of Work

This research was carried out experimentally by the method[8], [9] using a UV-Vis spectrophotometer. And methods to be used in determining the antioxidant activity is DPPH (1,1-diphenyl-2-picric-hydrazide).

Tools and Materials

The tools used are micropipette (dragon lab), bath water, aultraviolet-visible spectrophotometer (PD 302UV Apel, analytical balance (Carat series).

The materials used are, liquid extract infusion sappan wood (*Caesalpinia sappan* L.), methanol (pa, Merck), reagent Folin-Ciocalteau (pa, Merck), aluminum chloride (pa, Merch), sodium carbonate (pa, Merck), potassium acetate (pa, Merck), gallic acid (pa, Merck), quercetin (pa, Merck), and DPPH (Sigma, Chem.Co).

Procedures

Retrieval and Processing Samples

Samples derived from thewood cup in Bone regency of South Sulawesi. Taken part is a rod with a diameter greater than 7 cm in red. Wood samples cut into small pieces and aerated in a place not exposed to direct sunlight to dry. Subsequently, the samples were extracted by the method of infusion.

Making infusion sappan wood

Infusion sappanwood making by introducing 20g simplicia of sappanwoodinto the pot infuse added 100 ml of distilled water into a measuring cup.

The solution was heated at 90^0C for 15 minutes, after which it is filtered using a flannel cloth. Here in after called the filtered water infuse wooden cup. Sappanwood infusion can be stored at a temperature of 4^oC

Qualitative Analysis Test phenol and flavonoid

Testing with FeCl3 Phenol[10],The positive results of a phenol compound shown by the formation of black in the mixture.

Test Quantitative Analysis Flavonoids

Make a stock solution of quercetin 1000 ppm. Quercetin 10 mg was weighed and dissolved in ethanol up to 10 mL[9].

Preliminary test antioxidant radical catchers

Test antioxidant radical catcher conducted according to the methods[11]. The chromatogram is dried and sprayed with a solution of 0.2% DPPH in methanol. Chromatogram checked 30 minutes after spraying. The active compound free radical catcher will show patches of yellowish white colour with a purple background.

Test antioxidant activity

The solution isolates in chloroform at several concentrations (1-32 pg / ml) by 1.2 ml plus 0.3 ml of 0.5 mM DPPH solution in chloroform so that the total volume of 1.5 ml mixture and the mixture shaken strongly. After allowed to stand at room temperature for 30 minutes, the remaining DPPH metrispektrofoto determined at a wavelength of 515.967 nm. This test is also carried out measurements of the blank (DPPH solution not containing the test material) as well as the positive control of quercetin. DPPH radical trapping activity (%) was calculated by the following formula:

$$\text{Activity} = \frac{A\ blank - A\ sample}{A\ blank} x\ 100\%$$

Data antioxidant activity DPPH radical catcher isolates and quercetin results were analyzed, and probit analysis calculates their respective IC50 values. IC50 is the concentration that can inhibit 50% of DPPH.

III. Result and Discussion

The results showed that infusion by using distilled water as much as 100 mL had a yield of 5,731% w/v (Table 1).

Table 1 Results rendemen infusion percentage sappan wood

The type of solvent	The amount of solvent (mL)	Ekstract (mL)	Yield (%b/v)
distilled water (infuse)	100	70	5.731

In Table 2 shows the results of qualitative analysis of phenol and flavonoid extracts infusesappan wood. The results of the analysis of the wooden cup on total phenol extract with two drops of reagent FeCl3 showed positive results and total flavonoids extract also showed positive results either using a reagent Mg powder and zinc powder.

Table 2 Results of the qualitative analysis of phenol and flavonoid extracts infuse sappan wood.

Qualitative Analysis	Reagent	OnservationResult
1. Total phenolic extract	2 drops $FeCl_3$	(+)
2. Total flavonoid extract	– Mg^+ Powder 10 drops HCl concentrated – seng$^+$HCL$^+$Powder	(+) (+)

Table 3 shows the sappanwoodinfuse the total phenol content replication first initial 54 153 mk/L and successive replica 2 and 3 are 54 613 and 57 692 mg/L.

Table 3. The results of the quantitative analysis of phenolic extracts infuse sappan wood.

Extract	Replicatio n	Absorbance (y)	The total content of phenolic early (mg/L)	The total content of phenolic (mgGAE/mL)	The average content of total phenolic (µgGAE/mL)
Infuse-sappan wood	I II III	0.524 0.530 0.570	54.153 54.613 57.692	41.530 546.130 572.920	554.860

Table 4, shows the sappanwoodinfuse the total flavonoid content replication first initial 10 016 mk/L and successive replica 2 and 3 is 10 416 and 10.7 mg/L.

Table 4. Results of quantitative analysis of flavonoids in extracts infuse sappan wood.

Extract	Replication	Absorbanc e (y)	The total content of flavonoids		The average content of total flavonoids (mgQAE/ mL)
			early(mg/L)	last (mgQAE/ mL)	
Infuse-sappan wood	I II III	0.535 0.559 0.576	10.016 10.416 10.7	1.0016 1.0416 1.07	1.1037

Table 5. Percentage of barriers quercetin and DPPH with a wavelength of 515 967 nm

Concentration	Absorbance	% Inhibition	IC 50 (µg/ml)	Information
4 ppm	0.865	4.419	5.054	Very Strong
6 ppm	0.756	16.464		
8 ppm	0.664	26.629		
10 ppm	0.569	37.127		
12 ppm	0.451	50.165		
Rata-rata	0.661	26.961		

Table 5, shows the percentage of quercetin and DPPH barriers at a wavelength of 515 967 nm. The results showed that the higher the concentration, the higher the percentage resistance. The IC 50 value is equal to 5,054 pg / ml.

Table 6.Percentage of obstacles sappan wood with a wavelength of 513.08 nm

Concentration	Absorbance	% Inhibition	IC 50 (µg/ml)	Information
1 %	0.831	-0.241	0.047	Very Strong
2 %	0.845	-1.930		
3 %	0.686	17.249		
4 %	0.481	41.974		
5 %	0.368	55.609		
6 %	0.250	69.843		
Rata – rata	0.576	30.518		

Table 6, shows the greater percentage of the sappanwoodbarriers at 513.08 nm wavelength, the higher the percentage of the wooden cup with IC 50 values of 0.047 ug / ml.

Sappan wood (*Caesalpiniasappan* L.) is a plant commonly grown in the open up to an altitude of 1000 m above sea level as in the rocky mountainous area, has a woody stem, globular and brownish green. Plants wooden cup containing gallic acid, brasilin, delta-a phellandrene, oscimene, resin, resorcin, essential oils and tannins. 0:16 to 0:20% while the leaves contain essential oils that smell bad and colourless[12].

The chemical content of the wooden cup comes from the stem that is; brazilin, phenols, flavonoids, dyes, tannins.Sappan wood used as a sample obtained from the Bone district of South Sulawesi province which then performed at the Laboratory of Pharmacognosy-determination Phytochemicals,Faculty of Pharmacy, Indonesian Muslim University. Determination of samples intended to ensure the correctness of the sample used is wood that comes from the plant cup.

Gallic acid standard solution at a concentration of 40, 50, 60, 70 and 80 ppm measured at a wavelength of 730 nm. Then the absorbance values obtained agallic acid standard solution at each concentration, then a linear equation that will be used to determine the total phenol content in the sample extracts infuse sappan wood.

To determine total phenols analysis, first performed measurements gallic acid standard solution of 20 ppm with a wavelength range of 650-800 nm, the maximum wavelength of 730 nm is obtained. Gallic acid is an acid with three phenolic hydroxyl group so it can be used as a comparison compound for setting total phenolic compound content. Additionally, gallic acid is available in high purity, stable and relatively cheaper price.

In the measurement of total phenolic compounds make three of replication for theaccuracy of data and obtaining anabsorbance of the sample, and the sample absorbance values were plottedas the line of linear equations generated in the measurement of a standard solution of gallic acid. From the results of this study showed total phenol content in the extract infuse sappan wood of 551.663 mgGAE/mL infusion.

Likewise, the absorbance measurement sample extracts infusedsappan wood made in three replications for theaccuracy of data and obtained anabsorbance of the sample, and the sample absorbance values were plottedas the line of linear equations generated in the measurement of a standard solution of gallic acid. From the results of this study showed total flavonoid content in the extract of the wooden cup infusion of 1.103 mgGAE/mL infusion.

The method used in testing the antioxidant activity DPPH radical absorbance method is a method that is simple, easy and using the samples in small quantities with a short time[13]. Measuring the antioxidant activity of samples carried out at a wavelength of 515.967 nm which is the wavelength of maximum DPPH, with a concentration of 50 mM DPPH. The presence of the antioxidant activity of the sample resulting in discolouration on DPPH solution in methanol was originally concentrated into a violet coloured pale yellow[14].

The antioxidant activity of the sappanwood expressed as a percentage inhibition against DPPH radical. UV-Vis spectrophotometer measured the percentage of inhibition obtained from the difference between the absorbance of DPPH absorbance with the absorbance of samples. The amount of antioxidant activity characterised by IC50, namely the concentration of the sample solution is needed to inhibit 50% of DPPH free radicals.

The test results showed the wooden cup active as an antioxidant with IC50 0.047 ug / ml. Isolates IC50 value smaller than quercetin which has a value of IC50 was 5.04 ug/ml. This shows that the sappanwood andquercetinhas strong antioxidant activity due to having IC50 of less than 200 pg/ml[15]. Testing the antioxidant activity in various concentrations of turns in the highest concentration showed higher antioxidant activity, but when compared with quercetin, then quercetin have lower antioxidant activity.

Sappan wood is a natural source of antioxidants. There have been many studies on the efficacy of crop cup, both as antimicrobials, antioxidants, and natural dyes. Components of bioactive compounds contained in the wooden cup, which brazilin, brazilein, 3'-O-metilbrazilin, sappanone, chalcone, sappancalchone and other standard components, such as amino acids, carbohydrates and palmitic acid which are relatively very small. Brazilian particularpart is the wooden cup that can give a brownish red colour when oxidised or under alkaline conditions. Also, this brazilin allegedly also can protect the body from being poisoned by chemical radicals [6].

IV. Conclusion

Based on the research results infuse sappan wood (*Caesalpiniasappan* L.) using distilled water solvent were analyzed by using a phenolic compound gallic acid standard solution of 551.663 mgGAE/mL infusion, while the analysis test flavonoid quercetin using a standard solution of 1.103 mgQAE/mL infusion.

Infuse sappan wood can reduce free radical DPPH. Capacity to reduce free radical DPPH sappan wood is greater than the ability of quercetin. IC50 value of the sappan wood is 0.047 mg/mL higher than quercetin is 5054 mg/mL.

References

[1] T. Anggraini and P. Lewandowsky, “The exotic plants of Indonesia: mahkota dewa (Phaleria macrocarpa), sikaduduak (Melastoma malabathricum Linn) and mengkudu (Morinda citrifolia) as potent antioxidant sources,” *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 5, no. 2, pp. 115–118, 2015.

[2] N. N. binti Ismail, U. Utra, and S. Abdullah, “Determination of Phenolic and Antioxidant Properties in Tea and Spent Tea Under Various Extraction Method and Determination of Catechins, Caffeine and Gallic Acid by HPLC,” *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 5, no. 3, pp. 158–164, 2015.

[3] A. Munisa, W. Manalu, T. Wresdiyati, and N. Kusumorini, “The effect of clove leaf methanol extract on the profiles of superoxide dismutase and malondialdehyde in the liver of rabbits under hypercholesterolemia condition,” *Transl. Biomed.*, 2015.

[4] S. Badami, S. Moorkoth, S. R. Rai, E. Kannan, and S. Bhojraj, “Antioxidant activity of Caesalpinia sappan heartwood,” *Biol. Pharm. Bull.*, vol. 26, no. 11, pp. 1534–1537, 2003.

[5] R. Safitri, “Karakterisasi Sifat Antioksidan In Vitro Beberapa Senyawa Yang Terkandung Dalam Tumbuhan Secang (Caesalpinia sappan L.),” *Disertasi. Progr. Pasca Sarj. Univ. Padjadjaran. Bandung*, 2002.

[6] O. Rina, “Efektifitas Ekstrak Kayu Secang (Caesalpinia Sappan L.) Sebagai Bahan Pengawet Daging Effectiveness of Extract Wood of Secang (Caesalpinia Sappan. L) as Meat Preservative,” *J. Pertan. Terap.*, 2012.

[7] P. Molyneux, "The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity," *Songklanakarin J. Sci. Technol*, vol. 26, no. 2, pp. 211–219, 2004.

[8] A. E. Nugroho, A. Malik, and S. Pramono, "Total phenolic and flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentale L.).," *Int. food Res. J.*, vol. 20, no. 1, 2013.

[9] C.-C. Chang, M.-H. Yang, H.-M. Wen, and J.-C. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *J. food drug Anal.*, vol. 10, no. 3, 2002.

[10] J. B. Harborne, "Metode Fitokimia, Penuntun Cara Modern Menganalisa Tumbuhan, terjemahan K," *Padmawinata dan I. Soediro, Penerbit ITB, Bandung*, 1987.

[11] L. Ö. Demirezer, A. Kuruüzüm-Uz, I. Bergere, H.-J. Schiewe, and A. Zeeck, "The structures of antioxidant and cytotoxic agents from natural source: anthraquinones and tannins from roots of Rumex patientia," *Phytochemistry*, vol. 58, no. 8, pp. 1213–1217, 2001.

[12] H. A. Hariana, *Tumbuhan Obat & Khasiatnya 3*. Niaga Swadaya, 2006.

[13] E. Hanani, A. Munim, and R. Sekarini, "Identifikasi senyawa antioksidan dalam spons Callyspongia sp dari Kepulauan Seribu," *Pharm. Sci. Res.*, vol. 2, no. 3, 2012.

[14] D. Permana *et al.*, "Antioxidative constituents of Hedyotis diffusa Willd," *Nat. Prod. Sci.*, vol. 9, no. 1, pp. 7–9, 2003.

[15] M. S. Blois, "Antioxidant determinations by the use of a stable free radical," *Nature*, vol. 181, no. 4617, pp. 1199–1200, 1958.