 ISBN: 978-623-92207-1-6

Antioxidant Activity of Ethanol Extract of Secang Wood (*Caesalpinia sappan L.*), Gotu Kola (*Centella asiatica L.*), and Their Combinations with DPPH Assay

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***Abstract*— Antioxidants are the properties of active compounds that can prevent the formation or binding of free radicals and other molecules and inhibit oxidation reactions. Secang wood (*Caesalpinia sappan L.*) and Gotu kola (*Centella asiatica L.*) are plants that contain lots of phytochemical compounds used as traditional drinks such as steeping drinks to date. Combining several natural ingredients is believed to provide more benefits and a more effective dosage if used properly. This study aims to determine the antioxidant activity of the ethanol extract of secang wood and gotu kola and their combinations (1:1, 2:1, 1:2) based on the IC50 value with various concentrations and replication 3 times for each sample from the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Ascorbic acid was also used as a positive control for this assay. Secang wood and gotu kola were extracted using maceration method with 96% ethanol as solvent. The results showed that the IC50 antioxidant activity of 34.173 ppm of secang wood ethanol extract was more active (very strong) compared to 160.236 ppm of gotu kola extract (weak). The best combination of ethanol extract mixture of secang wood and gotu kola which resulted in the strongest activity among the three combinations was a 1:1 ratio of 59.431 ppm (strong).**

Keywords—antioxidant, secang wood, gotu kola, combination, DPPH assay

I. INTRODUCTION

Several bioactive compounds in food ingredients have been indicated to have antioxidant activity, such as vitamin C, flavonoids, vitamin E, polyphenols, and phenolics. Antioxidants play a role in inhibiting oxidative stress which is influenced by the type and level of these active substances [1]. Food ingredients as a source of natural antioxidants commonly consumed as traditional drinks with various health benefits are secang wood (*Caesalpinia sappan L.*) and gotu kola (*Centella asiatica L.)* [1] [2]. Secang wood contains phytochemical compounds, including xanton, coumarin, chalcone, flavones, homoisoflavonoids, and brazilin. Brazilin is the main active compound that belongs to the flavonoid class as homoisoflavonoids [3]. Gotu kola contains active substances, namely flavonoids, phenolic compounds, phytosterols, essential oils, and pentacyclic triterpenoids (the main ingredients of this plant are *asiaticoside*, *centelloside*, *madekosside*, and *aciatic* acid) [4]. Gotu kola has anti-inflammatory and anti-microbial properties [5].

Oxidative stress plays an important role in the etiology of degenerative diseases [6]. Various scientific evidence shows that antioxidant compounds play a very positive role on health. Antioxidants are substances that can counteract or reduce free radicals by donating one electron so that atoms with unpaired electrons get an electron pair [7]. Now, there is in increasing use of antioxidant compounds for both food and for medicinal raw materials due to its proven therapeutic effect with strong antioxidant activity [8] [9]. Many people combine herbs or other components in traditional recipes that have synergistic effects or are more effective than single-ingredient recipes in reducing the dosage or side effects [10]. Previous research on DPPH IC50 free radical scavenging assay for ethanol extract of secang wood was 74.439 ppm while the ethanol extract of gotu kola was 56.60 ppm [11], [12]. No studies have reported the antioxidant activity of the combination of these two ingredients to date, so the researcher conducted a study combining the ethanol extract of secang wood and gotu kola to determine the antioxidant activity with DPPH assay.

II. METHOD

This in vitro laboratory experimental study tested the antioxidant activity of the ethanol extract of secang wood, gotu kola, and their various combinations.

A. *Materials and Tools*

The main materials used include secang wood and gotu kola obtained from Materia Medica Batu, Malang City. Other ingredients include 96% ethanol, ascorbic acid, and 1-diphenyl-2-picrylhydrazyl (DPPH). The tools used in this study include glass (Pyrex), stirring rod, analytical balance, rotary evaporator, spectrophotometry Uv-Vis, incubator, vortex, micropipette, test tube, and aluminum foil.

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B. *Sample preparation*

The samples were sorted and washed under running water. They were drained to dry. Then, they were aerated at room temperature (± 25 ºC) for 3 days or using a cabinet dryer at 40 ºC for 2 days. The clean and dry sprigs of secang wood or gotu kola leaves were mashed using stainless steel to produce smaller and smoother (100 mesh).

C. *Extraction*

Extraction was made using maceration method by adding 350 grams of secang wood and gotu kola powder with 96% ethanol solvent until completely submerged and macerating it 3x24 hours at a room temperature by stirring occasionally. The macerated filtrate was filtered and collected in a container. It was then concentrated using a rotary evaporator.

D. *Antioxidant Activity with DPPH Assay*

A main solution (stock) of 1000 ppm was made for each sample (ethanol extract of secang wood, gotu kola, and various combinations (1:1, 2:1, 1:2) and ascorbic acid (vitamin C) as a positive control. Then, the solution was diluted using ethanol with various concentrations. Ethanol extract of secang wood was carried out with a concentration of 5, 10, 20, 40, 60 ppm while gotu kola ethanol extract and their combinations was 5, 10, 20.40, 60, 100 ppm and vitamin C was 2, 3, 4, 5, 7.5, 10 ppm.

Then, a 0.15 mM DPPH solution was prepared by dissolving 0.0059 g of solid DPPH with ethanol as a solvent. For each test sample, 0.3 ml was prepared, and 0.3 ml of 0.15 mM DPPH stock solution was added to the test tube. It was incubated for 30 minutes at 27 ºC until the color changed. All samples were made in triplo.

The analysis of antioxidant testing with the DPPH method was carried out by observing the color change of each sample after incubation. If all the DPPH electrons are paired with the electrons in the extract sample, there will be a change in the color of the sample from dark purple to bright yellow [13]. Then, the absorbance of the sample was measured using a spectrophotometer Uv-Vis at a wavelength of 517 nm. The percentage of DPPH inhibitory activity (antioxidant activity) was calculated using the following formula.

% inhibition = ((Ac-As))/Ac x 100

Ac = Absorbance of control

As = Absorbance of sample

The percentage result is made a curve between the percent of free radical scavenger against the sample concentration. From the linear regression equation, the IC50 (Inhibitory Concentration 50%) value can be determined, namely the inhibition concentration of the test sample solution which can ward off 50% of free radicals. Sample concentration (ppm) is represented as the x-axis and percent inhibition as the y-axis (%). From the equation y = a + bx, the IC50 value is calculated using the following formula:

$y = ax + b$

$50 = ax + b$

$x\ (IC50) = (50-b)/a$

III. RESULT

Secang wood and gotu kola were extracted by maceration method. The extraction aimed to obtain specific active compounds. This method was used as it is one of the cold methods that can minimize the damage to active compounds which are not resistant to heating [14]. The solvent used in the extraction process is 96% ethanol because the active compounds found in wood (flavonoids) or gotu kola (triterpenoids) dissolve in polar solvents such as ethanol [15] [16]. The result of the extraction took the form of thick liquid obtained from the maceration results of 55 grams with a yield of 10.71% of the ethanol extract of secang wood. Meanwhile, the yield of ethanol extract of gotu kola 37.5 gr was 15.71%.

DPPH acts as a synthetic free radical model in organic solvents at room temperature by a compound having antioxidant activity. This free radical scavenging process is through taking hydrogen atoms from antioxidant compounds by free radicals so that free radicals capture an electron from the antioxidants [17]. The antioxidant activity of the samples was measured at a wavelength of 517 nm (maximum wavelength of DPPH) [13]. The following are the results of the percentage of DPPH inhibitory activity against various sample concentrations as shown in Figure 1.

Based on the Figure 1 shows the DPPH inhibitory activity of each sample. Ascorbic acid has the strongest activity due to its lower concentration (10 ppm) than that of the ethanol extract of secang wood (60 ppm). It has almost the same ability to ward off DPPH free radicals. These results are then regressed to obtain the IC50 value (Table I).

TABLE I. IC50 VALUE

Sample	Replication	Line equation	IC50 (ppm)	Mean IC50 (ppm)
S	1	y = 1.3288x + 4.7276	34.070	34.172
	2	y = 1.4329x + 0.4518	34.578	
	3	y = 1.4271x + 1.6658	33.868	
P	1	y = 0.2714x + 5.1708	165.177	160.236
	2	y = 0.3279x + 1.5858	147.649	
	3	y = 0.2883x + 1.5996	167.882	
S1:P1	1	y = 0.8021x - 1.4905	60.478	59.43
	2	y = 0.7991x - 1.1799	61.093	
	3	y = 0.8518x - 1.6856	56.720	
S2:P1	1	y = 0.6922x + 5.6414	64.083	61.085
	2	y = 0.7571 + 2.4429	62.814	
	3	y = 0.8477x - 2.2229	56.360	
S1:P2	1	y = 0.5197 + -0.8717	97.886	91.981
	2	y = 0.5806x + 1.9228	89.429	
	3	y = 0.584x - 1.7591	88.628	
VC	1	y = 6.3045x + 24.898	3.981	3.902
	2	y = 7.1588x + 22.581	3.830	
	3	y = 6.7738x + 23.603	3.896	

Remark;
S : Secang wood ethanol extract
P : Gotu kola ethanol extract
S1:P1: Secang wood ethanol extract: gotu kola ethanol extract (1:1)
S2:P1: Secang wood ethanol extract: gotu kola ethanol extract (2:1)
S1:P2: Secang wood ethanol extract: gotu kola ethanol

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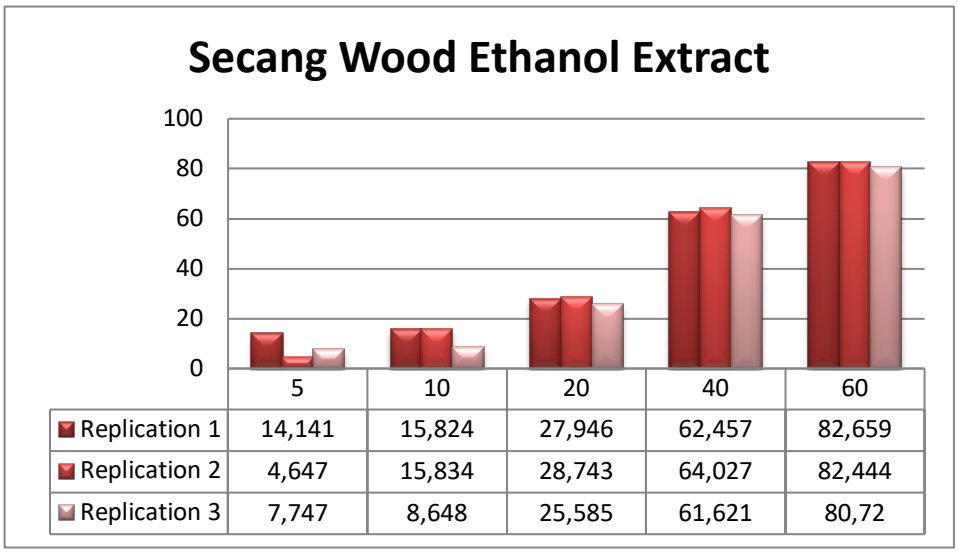
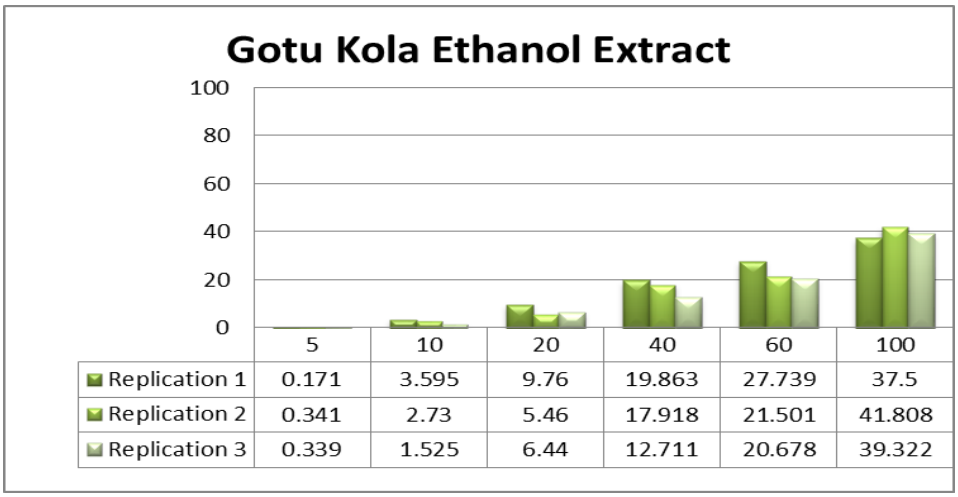


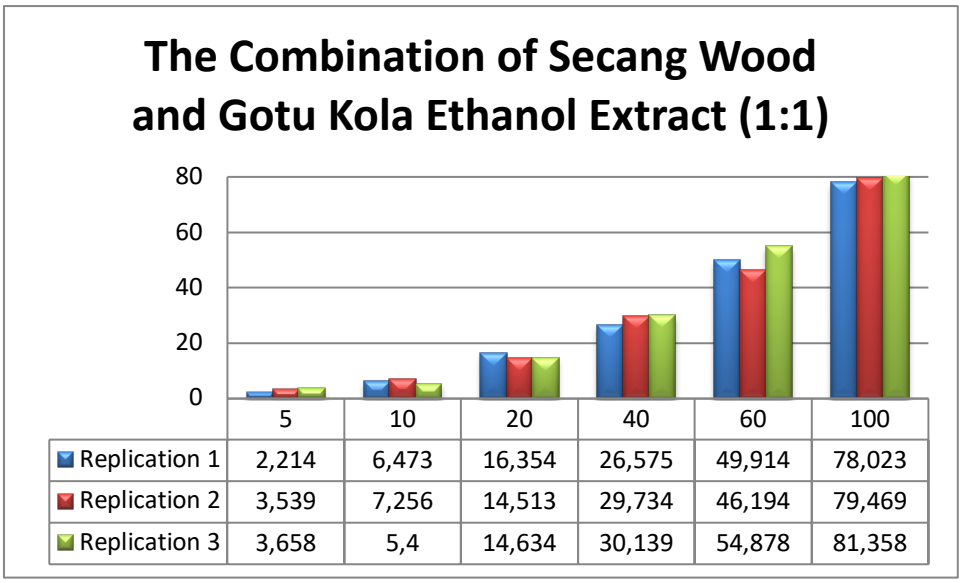
	5	10	20	40	60
Replication 1	14,141	15,824	27,946	62,457	82,659
Replication 2	4,647	15,834	28,743	64,027	82,444
Replication 3	7,747	8,648	25,585	61,621	80,72

(a) Secang wood ethanol extract



	5	10	20	40	60	100
Replication 1	0.171	3.595	9.76	19.863	27.739	37.5
Replication 2	0.341	2.73	5.46	17.918	21.501	41.808
Replication 3	0.339	1.525	6.44	12.711	20.678	39.322

(b) Gotu Kola Ethanol Extract



	5	10	20	40	60	100
Replication 1	2,214	6,473	16,354	26,575	49,914	78,023
Replication 2	3,539	7,256	14,513	29,734	46,194	79,469
Replication 3	3,658	5,4	14,634	30,139	54,878	81,358

(c) Secang wood and gotu kola ethanol extract (1:1)

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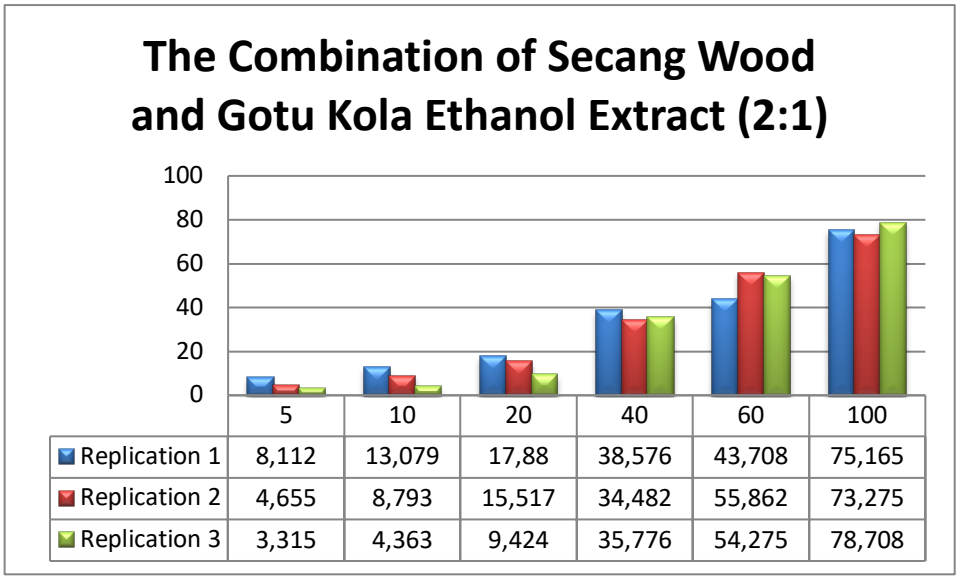
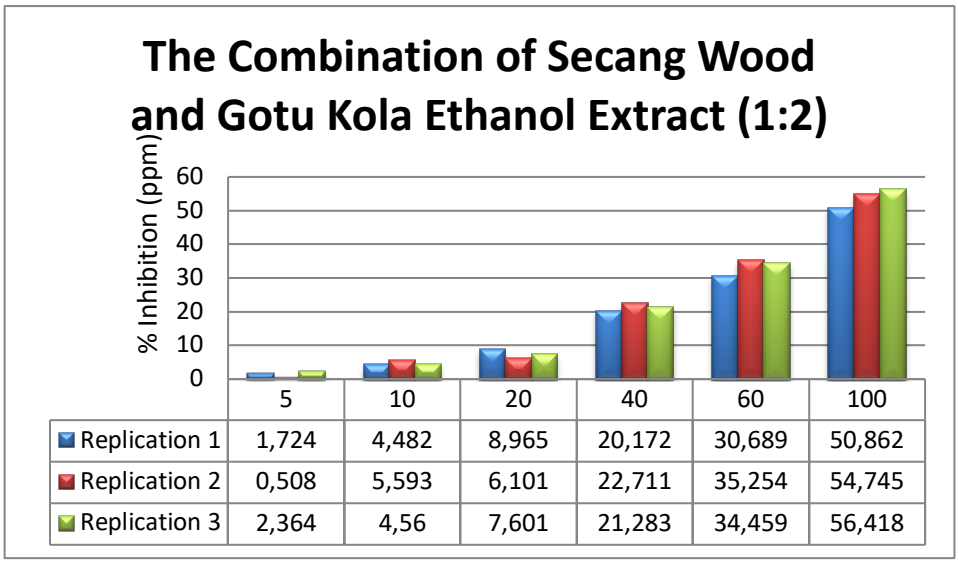


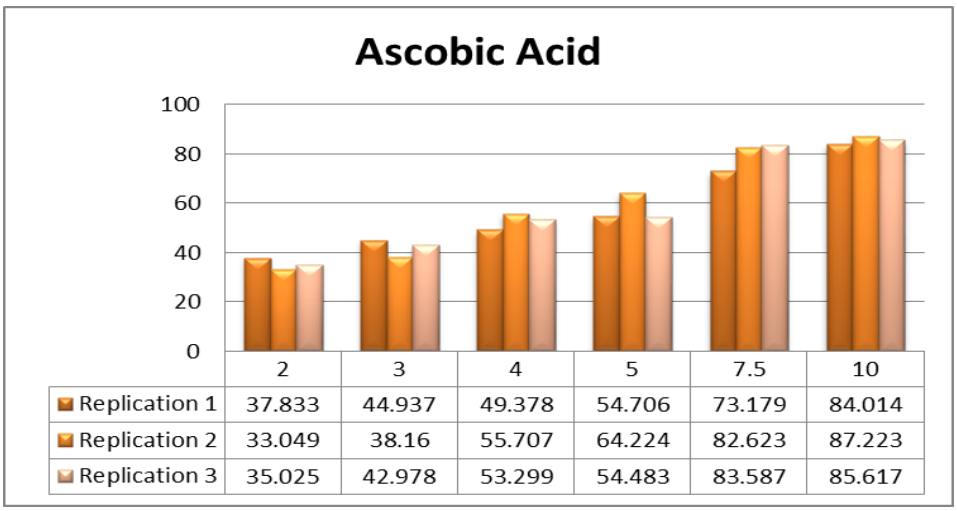
	5	10	20	40	60	100
Replication 1	8,112	13,079	17,88	38,576	43,708	75,165
Replication 2	4,655	8,793	15,517	34,482	55,862	73,275
Replication 3	3,315	4,363	9,424	35,776	54,275	78,708

(d) Secang wood and gotu kola ethanol extract (2:1)



	5	10	20	40	60	100
Replication 1	1,724	4,482	8,965	20,172	30,689	50,862
Replication 2	0,508	5,593	6,101	22,711	35,254	54,745
Replication 3	2,364	4,56	7,601	21,283	34,459	56,418

(e) Secang wood and gotu kola ethanol extract (1:2)



	2	3	4	5	7.5	10
Replication 1	37.833	44.937	49.378	54.706	73.179	84.014
Replication 2	33.049	38.16	55.707	64.224	82.623	87.223
Replication 3	35.025	42.978	53.299	54.483	83.587	85.617

(f) Ascobic acid

Figure 1. Percentage of DPPH inhibitory activity by antioxidant compounds of the ethanol extract of secang wood, gotu kola, their combination, and ascorbic acid

The smaller the IC50 value is, the more active the sample is as an antioxidant [18]. The following are the IC50 level categories (Table II) [13].

TABLE II. IC50 LEVEL

IC50	Category
< 50 ppm	Very strong
50-100 ppm	Strong
100-150 ppm	Medium
150-200 ppm	Weak
> 200	Very weak

Based on the Tables I and II indicate that the sample of secang wood ethanol extract (S) is a very strong antioxidant with an IC50 value of 34.172 ppm. The compounds with antioxidant activity from the ethanol extract of secang wood are flavonoids, saponins, alkaloids, tannins, phenolics, and brazilins. However, the antioxidant activity of secang wood ethanol extract is lower than ascorbic acid (VC) with an IC50 value of 3.902 ppm. This is because vitamin C is a very pure compound which is a reducing agent because the hydrogen atoms are easily released from the hydroxyl groups attached to C2 and C3 atoms (C atoms in the double bond), reducing free radicals [17].

Meanwhile, the antioxidant classified as weak from these data is the ethanol extract of gotu kola (P) with an IC50 value of 160.236 ppm. This is probably due to the low levels of active compounds that act as antioxidants such as triterpenoids, alkaloids, asiaticosides, asiatic acids, flavonoids, or tannins. The combination of ethanol extract of secang wood and gotu kola has potential as a strong antioxidant with IC50 values of 59.43 ppm (S1:P1), 61.085 ppm (S2:P1), and 91.981 ppm (S1:P2).

IV. CONCLUSION

The results of the antioxidant activity test using the DPPH method on the ethanol extract of secang wood were stronger than those of gotu kola and its various combinations (1:1, 2:1, and 1:2) indicating an IC50 value of 34.173 ppm (very strong). The weakest antioxidant activity was 160.236 ppm (weak). The result of the strongest combination includes a 1:1 ratio of 59.43 ppm (strong). It is necessary to conduct qualitative and quantitative tests of compounds that act as antioxidants.

ACKNOWLEDGMENT

The author would like to thank Supriono S.P and Yuliana Sabarina Lewar S.TP as a laboratory assistant at Politeknik Pertanian Negeri Samarinda, who have helped and guided the author during the study.

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