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Antioxidant Activity of Ethanol Extract of Turmeric Rhizome (*Curcuma domestica* Val), Trengguli Bark (*Cassia fistula* L), and Its Combination with DPPH Method

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Abstract

Turmeric rhizome (*Curcuma domestica* Val) and Trengguli bark (*Cassia fistula* L) contain antioxidant compounds which can be determined by 1,1-Diphenyl-2- Pycrylhydrazyl (DPPH) free radical inhibition method. This research was conducted to determine DPPH free radical inhibition by ethanol extract of turmeric rhizome, the ethanol extract of trengguli bark, and a combination of turmeric rhizome extract - trengguli bark extract (1:1.5) with ascorbic acid as a comparison. Identification of secondary metabolite classes is performed by phytochemical screening. Antioxidant activity was performed by inhibition of free radical color of DPPH using UV-Vis spectrophotometry. The study showed IC_{50} value of ascorbic acid, as a comparison, is 3.14 µg/mL. While ethanol extract of trengguli bark has the best antioxidant activity with IC_{50} value 10.98 µg/mL compare to combination ethanol extract of turmeric rhizome - trengguli bark (1 :1.5) and ethanol extract of turmeric rhizome with IC_{50} value is 13.70 µg/mL and 41.95 µg/mL, respectively.

Keywords: Antioxidant, *Cassia fistula*, *Curcuma domestica*, DPPH Method.

Aktivitas Antioksidan Ekstrak Etanol Rimpang Kunyit (*Curcuma domestica* Val), Ekstrak Etanol Kulit Batang Trengguli (*Cassia fistula* L) dan Kombinasinya dengan metode DPPH

Abstrak

Rimpang Kunyit (*Curcuma domestica* Val) dan Kulit batang trengguli (*Cassia fistula* L) mengandung senyawa yang bersifat antioksidan yang dapat ditentukan dengan metode peredaman radikal bebas 1,1-Difenil-2- pikrilhidrazil (DPPH). Penelitian ini dilakukan untuk mengetahui peredaman radikal bebas DPPH oleh ekstrak etanol rimpang kunyit, ekstrak etanol kulit batang trengguli, kombinasi ekstrak rimpang kunyit – ekstrak kulit batang trengguli (1 : 1.5) dengan asam askorbat sebagai pembanding. Metode skrining fitokimia dilakukan untuk mengidentifikasi metabolit sekunder pada ekstrak. Pengujian aktivitas antioksidan dilakukan dengan metode DPPH yang dianalisa menggunakan spektrofotometri UV-Vis. Penelitian ini menunjukkan nilai IC_{50} asam askorbat, sebagai perbandingan, adalah 3.14 µg/mL. Sedangkan ekstrak etanol kulit batang trengguli memiliki aktivitas antioksidan dengan nilai IC_{50} 10,98 µg / mL paling baik daripada kombinasi ekstrak etanol rimpang kunyit – ekstrak etanol kulit batang trengguli (1: 1.5) dan ekstrak etanol rimpang kunyit yang memiliki nilai IC_{50} sebesar 13.70 µg/mL dan 41.95 µg/mL.

Kata Kunci: Antioksidan, *Cassia fistula*, *Curcuma domestica*, metode DPPH

1. Introduction

There has been the attention toward the field of free radical chemistry in recent year. Free radicals such as reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different pathological states or physiochemical conditions[1]. Free radicals generally form naturally as part of our body's metabolic processes. However, free radicals can also be affected by environmental factors pesticide use in food including, smoking habits, radiation, and pollution[2]. Free radicals are molecules that have one or more unstable electron[3]. Research on antioxidant activity is conducted for the purpose of improvement of food quality and treatment.

Antioxidants have the ability to neutralize free radicals without becoming free radicals themselves[4]. When antioxidants neutralize free radicals by receiving or donating electrons, they will not turn into free radicals and remain stable. Antioxidants are widely found in vegetables, fruits, and medicinal plants[5].

The use of natural product as traditional medicine is not enough only based on experience passed down from generation to generation, but also need to be proven scientifically. As we known medicinal plants contain active substances that can be efficacious for the cure of disease. Various studies have been conducted to prove the pharmacological activity and determine the chemical content of the natural materials. One of these natural materials has been reported some pharmacological activity is *Cassia fistula* L.[6] and *Curcuma domestica* Val.[7].

Trengguli plant is proven to have some pharmacological activity. Trengguli bark ethanol extract has antioxidant activity by inhibiting DPPH[8,9]. Trengguli plant studies show strong relation between hepatoprotective activity and antioxidant activity that can be seen from liver biochemical and oxidative stress parameters[10]. Meanwhile, the study of turmeric rhizome shows that the ethanol extract of turmeric rhizome has hepatoprotective activity in paracetamol-induced rats[11]. Curcumin as one of the active compound in turmeric rhizome has activity as antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, anticoagulant, antihypertensive, and antidislipidemia[12]. Research on the effectiveness of *C. domestica* Val. as hepatoprotectors and immunomodulators show that turmeric can decrease Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) levels and may increase the activity of nonspecific immune systems in mice induced by CCl_4[8]. Other studies have shown that turmeric rhizome has hepatoprotective activity and prevent the increase of SGOT or SGPT due to toxic dosage of acetaminophen[13].

Various studies above have demonstrated various pharmacological activities from turmeric rhizome and different parts of the trengguli plant. The aim of this research is to know the potency of in vitro antioxidant activity on combination of ethanol extract from turmeric rhizome and trengguli bark compare to each extract and ascorbic acid as standard with DPPH method because of its accuracy and ease.

2. Materials and Methods

2.1. Material and Apparatus

Turmeric rhizome from species *C. domestica* Val. and trengguli bark from species *C. fistula* L. obtained from Manoko plantation, Lembang, Bandung. Determination of plants is evaluated at the Laboratory of Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The chemicals used in this study include ethanol 70% (Merck), ethanol 96% p.a (Merck), DPPH (2,2-diphenyl-1-pycryl-hydrazil) (Sigma), ascorbic acid (Merck). Absorbance of sample is analyzed using UV-Vis spectrophotometer (TECAN M200Pro).

2.2. Method

2.2.1. Extraction

The extraction simplicia of turmeric rhizomes and simplicia of trengguli bark plant were done on different maserator by maceration with 70% ethanol solvent for 3

times 24 hours. Liquid extract concentrated with a rotary evaporator at a temperature of 60 ºC then steamed above a water bath with a temperature of 40 ºC until constant weight of the extract. The yield of the extract can be calculated by the formula:

$$\text{Rendement (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Simplicia}} \times 100\%$$

Furthermore, the examination of organoleptic extract consisting of form, color, odor, and taste extract.

2.2.2. Thin Layer Chromatography Patterns

Thin layer chromatography was performed to determine the chemical content in the extract. In the thin layer chromatography analysis, silica gel phase GF_{254} was used in this experiment with mobile phase toluene : ethyl acetate : formic acid (5: 4: 1) for trengguli bark extract[8] and chloroform : methanol (95 : 5) for turmeric rhizome extract. The extract was first dissolved in ethanol and then applied to a silica gel plate of size 10 cm x 2.0 cm and inserted into a chromatographic vessel previously saturated mobile phase. The chromatographic process was stopped when the mobile phase reached the finish line. The chromatogram pattern observed with visible and UV light (λ254 and λ366 nm), then calculated the value of Rf.

2.2.3. Determination of IC_{50} with DPPH Method

The ethanol extract of turmeric rhizome, trengguli bark and a combination of ethanol extract of turmeric rhizome - trengguli bark (1:1.5) were added 1 mL DPPH for each concentration, then vortex and incubated for 30 minutes at room temperature. The absorbance was measured at 516 nm. The inhibition percentage was calculated using the equation :

$$\text{\% Inhibition} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100\%$$

Note :
Ab = DPPH absorbance
As = Sample Absorbance

The % inhibition obtained then used for determined IC_{50} that showed the concentration of sample to inhibit 50% free radical

2.2.4. *In vitro* Antioxidant Activity Test of Extract

a. Sample Preparation

0.01% w/v ethanol extracts were prepared with a stock solution of 100 ppm. 100 ppm stock solution is diluted to concentration 4, 6, 12, 16, 20 ppm.

b. Preparation of Comparative Solutions

0.01 % w/v Ascorbic acid was prepared with a solution of 100 ppm. Diluted a stock solution untill concentration 10 ppm. 10 ppm stock solution made standard solution 1, 2, 3, 4, 5 ppm.

c. Preparation of DPPH solution (2,2-diphenyl-1-pycryl-hydrazyl)

DPPH was weighed and dissolved in ethanol p.a at a concentration of 0.01% w/v (100 ppm) for immediate use and maintained in low temperatures and protected from light exposure.

d. Maximum Wavelength Determination

DPPH solution of 1 mL was dissolved with ethanol to 5 mL in 5-ml flask, measured at 500-530 nm wavelength to obtain an absorbance of ± 0.2-0.8.

3. Results

3.1. Determination

The results of plant determination show turmeric rhizome belongs to the Family: Zingiberaceae, Genus: Curcuma, Species: *Curcuma domestica* Valeton. Trengguli bark included in the Family: Fabaceae, Genus: Cassia, Species: *Cassia fistula* L.

3.2. Extraction

Turmeric rhizome extraction (1100 g) was macerated with 70% ethanol solvent resulting rhizome extract of 250.25 g (rendement = 22.75%). While the extraction of trengguli bark (850 g) in maceration with 70% ethanol solvent resulting trengguli bark extract of 276.25 g (rendement = 32.50 %).

Table 1. Phytochemical Screening Results of Ethanol Extracts of Turmeric Rhizome (EETR) and Ethanol Extract of Trengguli Bark (EETB)

Parameter	EETR	EETB
Alkaloid	-	-
Tanin	-	+
Poliphenolic	+	+
Saponin	-	+
Flavonoid	+	+
Monoterpenoid & Sesqiterpenoid	+/+	+/+
Steroid	-	-
Triterpenoid	+	+
Quinone	+	+

Table 2. Result of Organoleptic Test from Extract

Parameter	EETR	EETB
Form	Viscous Liquid	Viscous Liquid
Odor	Specific for Turmeric Rhizome	Specific for Trengguli Bark
Color	Brown	Dark Brown
Taste	Bitter	Bitter

Table 3. Result of TLC Patterns

Sample	Spot	Rf		
		Visible	λ254	λ366
EETR	1 (Yellow)	0.30	0.31	0.31
	2 (Yellow)	0.51	0.51	0.50
	3 (Yellow)	0.74	0.75	0.70
EETB	1 (Blue)	-	0.66	-

3.3. Phytochemical Extract Screening and Organoleptic Test Results

The results of phytochemical screening of extract can be seen in Table 1 and organoleptic test can be seen in Table 2.

3.4. TLC results

TLC results seen in visible light, UV λ254 nm and UV λ366 nm TLC results can be seen in Table 3.

3.5. Antioxidant Activity Test Results

The results of in vitro antioxidant activity test of ethanol extract of turmeric rhizome, ethanol extract of trengguli bark, combination both of them and ascorbic acid can be seen in Table 4 - 7. For comparasion, ascorbic acid used because known as a strong antioxidant.

4. Discussion

Turmeric rhizome ethanol extract from several studies with the DPPH method exhibited strong antioxidant activity. In that studies obtained data IC_{50} were 73.31 µg/mL14 and 24 µg/mL[15]. In this study value IC_{50} of ethanol extract turmeric rhizome is 41.95 µg/mL (Table 5). The value of IC_{50} ethanol

Table 4. Result of Ethanol Extract of Trengguli Bark Antioxidant Activity

Concentration (ppm)	Absorbance	% Inhibition	Regression	IC_{50}
0	0.8845	0.00	y=3.5641x+10.8886	10.98 µg/ml
4	0.6922	21.74		
8	0.4968	43.83		
12	0.4168	52.87		
16	0.2670	69.81		
20	0.1766	80.03		

*Result from triplo sample test ; *Absorbance Control is 0,8845

Table 5. Result of Ethanol Extract of Turmeric Rhizome Antioxidant Activity

Concentration (ppm)	Absorbance	% Inhibition	Regression	IC_{50}
0	0.8845	0.00	y=1.2193x-1.1419	41.95 µg/ml
4	0.8528	3.58		
8	0.8082	8.63		
12	0.7628	13.76		
16	0.7218	18.39		
20	0.6803	23.09		

*Result from triplo sample test ; *Absorbance Control is 0,8845

Table 6. Result of Combination of Turmeric Rhizome Ethanol Extract and Trengguli Bark Ethanol Extract (1:1,5) Antioxidant Activity

Concentration (ppm)	Absorbance	% Inhibition	Regression	IC_{50}
0	0.8845	0.00	y=3.5562x+1.2934	13.70 µg/ml
4	0.7665	13.34		
8	0.6091	31.14		
12	0.4806	45.66		
16	0.3597	59.33		
20	0.2621	70.37		

*Result from triplo sample test ; *Absorbance Control is 0,8845

Table 7. Result of Ascorbic Acid Antioxidant Activity

Concentration (ppm)	Absorbance	% Inhibition	Regression	IC_{50}
0	0.8845	0.00	y=16.2295x-0.8672	3.14 µg/ml
4	0.7654	13.46		
8	0.5873	33.60		
12	0.4470	49.47		
16	0.3331	62.34		
20	0.1748	80.24		

*Result from triplo sample test ; *Absorbance Control is 0,8845

extract of trengguli bark with DPPH method from several studies were 27.94 µg/mL[16] and 10.61 µg/mL[9]. In this research, IC_{50} extract of trengguli bark extract is 10.98 µg/mL (Table 4). The different results of studies above are probably due to differences in conditions and location test and the origin of medicinal plants that affect the content of antioxidant compound.

The combination of both extract (1 : 1.5), chosen to illustrate the combination of the minimum dose of the two extracts, shows IC_{50} values is 13.70 µg/mL (Table 6). The minimum dose that still has antioxidant activity for turmeric rhizome extract is 100 mg/kg bw and trengguli bark extract is 150 mg/kg bw[9]. From the results of research that IC_{50} values of ethanol extract of trengguli bark is better than ethanol extract of turmeric rhizome which causes antioxidant activity becomes stronger than single extract of turmeric rhizome, but the combination ethanol extract of turmeric rhizome - trengguli bark (1: 1.5) has not been able to produce IC_{50} value smaller than ethanol extract of trengguli bark. This is probably due to the comparison between the two extracts based on the minimum dose can not reach the IC_{50} value under a single extract of the trengguli, but the combination can still prove that with a combination ethanol extract of turmeric rhizome - trengguli bark (1: 1.5) including a very strong antioxidant category.

5. Conclusion

The results showed that ascorbic acid, ethanol extract of turmeric rhizome, ethanol extract of trengguli bark and combination of ethanol extract of turmeric rhizome – trengguli bark (1:1.5) gave antioxidant activity by DPPH method. Ascorbic acid, as a comparasion, shows IC_{50} value 3.14 µg/mL. While ethanol extract of trengguli bark has the best antioxidant activity with IC_{50} value 10.98 µg/mL compare to combination of ethanol extract of turmeric rhizome – trengguli bark (1:1.5) and ethanol extract of turmeric rhizome with IC_{50} value is 13.70 µg/mL and 41.95 µg/mL, respectively.

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