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Standardization of Purified Extract Mahoni Seed and Antioxidant Activity

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Abstract: Indonesia has many plants that have been used for treating many diseases such as diabetic. One of the Indonesian plants has been shown the antidiabetic activity such as mahoni. In this study, we have investigated the standardization of the purified extract of the mahoni seeds and its antioxidant activity. The standardization methods include organoleptic test, determination of drying loss, determination of, total ash level, determination of acid-insoluble ash level, determination of water-soluble essence level, determination of ethanol soluble essence level, phytochemical profile test: alkaloid test, flavonoid test, phenolic test, saponin test, terpenoid, and steroid test. The results show the purified extract mahoni seed qualify as a raw material for herbal medicine and also has potential as an antioxidant IC_{50} 33.86 ug/ml that compare with the standard.
Keyword: *antioxidant, standardization, Swietenia mahagoni (L.) Jacq, purified extract.*

Introduction

Indonesia has many plants that have been used for treating many diseases such as diabetic. One of the Indonesian plants has been shown the antidiabetic activity namely Mahon (*swietenia mahagoni* (L.) Jacq). Previous reports have been reported mahoni as antidiabetic, anti-inflammation, anti-mutagenesis, anti-hypertension, anti-malaria, and anti tumors. Another researcher also was reported mahoni as an anti-diabetic, nevertheless not clear with the mechanism[1,2]. The major compound of the mahoni is switenine, however in seeds also contain a lot of lipids[3]. Switenine has been investigated to decrease cholesterol levels, triglycerides, and liver glycogen. The dose of the swietenine 25 and 50 mg/kg weight for 5 days could decrease the fasting blood glucose approximately 47,34 mg/dL and 55,85 mg/dL, respectively[4]. In addition, mahoni seeds also have activity as an antioxidant. In this study, we have investigated the standardization of the purified extract of the mahoni seeds and its antioxidant activity.

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Material and Method

Material

Mahoni seeds. Dichloromethane (teknis), methanol (teknis), *n*-butanol (teknis), *n*-hexane (teknis), TLC plate (Merck cat. 1.05554, Jerman), TLC kit, the *rotary evaporator* (Buchi R-215, Jerman), spectrophotometer UV-Vis (Hitachi U 2000, Jepang). DPPH (Sigma, USA).

Method

Extraction and purification

The seeds of the *S. mahagoni* have been collected in Makassar. The specimens have identified in Laboratory Pharmacognosy and Phytochemistry UMI. The seeds have ground and ready to extract. To purify the extract from the lipid, we have used hexane solution. Furthermore, we extracted with ethanol solution.

Standardization

The purification extract has been standardized by following the protocol of the "Standarisasi Ekstrak Indonesia" (2000)[5] and Ahmad et al., 2014[6]. The measurement-includes organoleptic test, etermination of drying loss, the persistence of, total ash level, determination of acid-insoluble ash level, determination of water-soluble essence level, determination of ethanol soluble essence level, and phytochemical profile test (alkaloid test, flavonoid test, phenolic test, saponin test, terpenoid and steroid test)[6].

Determination of Flavonoids content

The purified extract 10 mg has been measured with various concentration of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. The each standard solution (1 mL) was added with 0.2 mL AlCl 10%, 0.2 mL potassium acetate 1M, 3.0 mL methanol and 5.6 mL aqua bidestillata, and then incubated for 30 minutes. Furthermore, absorbance was measured at a maximum wavelength of 415 nm[7].

Antioxidant activity

The purified extract has been tested by using DPPH method. The extract with the various concentration. As much as 0.5 ml sample added 3.5 ml DPPH, thus incubated for 30 minutes. The DDPH has been detected by using Spectrophotometer UV-Vis at wavelength 570 nm. The positive control used quercetin. The inhibition of the antioxidant has calculated with a linear curve[8].

Figure 1. TLC profile of switenine. Stationary phase: silica gel F254 (7x1 cm) plate, mobile phase :chloroform : methanol (1:1). (a) Before spraying, (b) Detection with Vanillin-sulphate acid reagent.

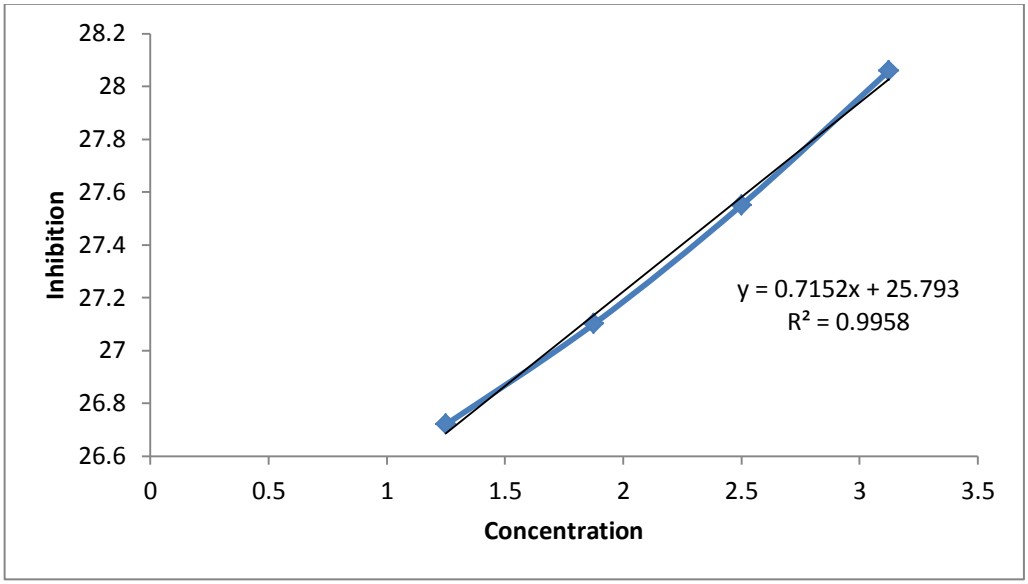

Figure 3. Antioxidant activity of the purified extract mahoni seed.

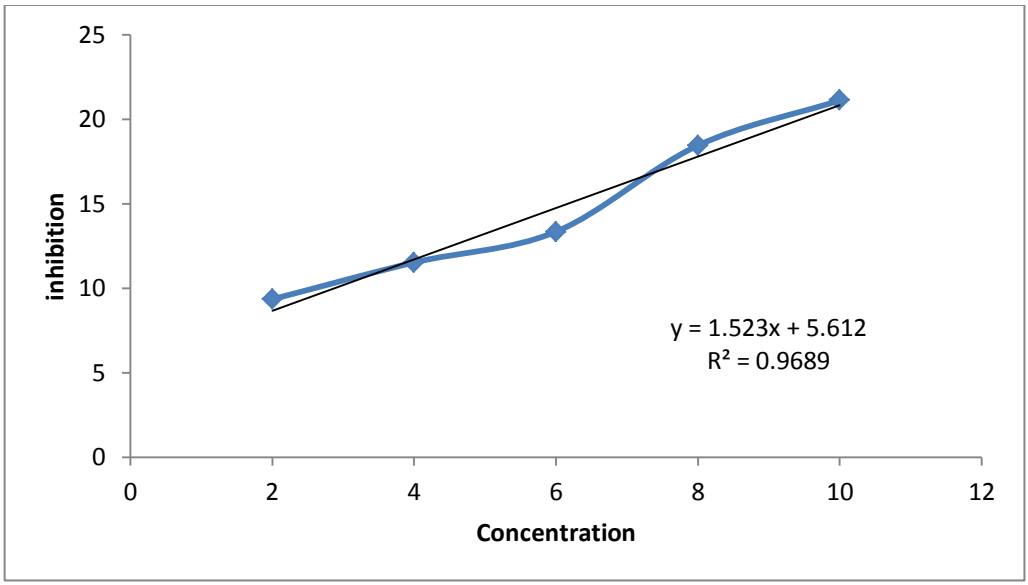

Figure 2. Antioxidant activity of the standard quercetin.

Results and Discussions

The standardization parametric according to Farmakope Herbal Indonesia (FHI) and Parameter Ekstrak Indonesia include organoleptic test, determination of drying loss, determination of, total ash level, determination of acid-insoluble ash level, determination of water-soluble essence level, determination of ethanol soluble essence level, phytochemical profile test: alkaloid test, flavonoid test, phenolic test, saponin test, terpenoid and steroid test.

In this experiment amount, 3750 g was extracted and got the purified extract about 73.86 g or 1.9% (Table 1).

The standardization was investigated include the parametric of standardization, metal contaminant, and microbiology test. In the organoleptic test, the extract has a red-brown color and bitter taste (Table 2). The determination of total ash level was not more than 1.71 % with acid insoluble not more than 0.38%, the result showed to consist of an inorganic compound, but lower than 2%. The water-soluble level was not more

than 14.84%, and ethanol soluble was not more than 15.38%. The results of phytochemical screening showed that the extract contains a few compounds such as a flavonoid, sapiens, terpenoid, and alkaloid (Table 2).

The metal contamination test aimed to observe the presence of a metal compound in the extract which intolerable in drug compounds such as Pb and Cd. From the result of the test, it was found that contains the Pb and Cd but in the low concentration (tolerable). It was caused by the contamination of air pollution where the plant was growing (Table 3).

The important material for herbal medicine does not contain the microorganism. From the result have been shown the extract can be tolerable the microorganism (Table 4).

A purified extract of mahoni seeds has been detected for total flavonoid by using spectrophotometer UV-Vis and Rutin as standard[9]. That result showed contain 2,3ug/g extract (Table 5). The swietenine is the major compound of the mahonee seed. The extract has been applied in the TLC plate, evaluated by using chloroform: methanol (1:1) and can separate the swietenine compounds with the rate of flow value approximately 0.21-0.9 (Suliman, 2017)[11]. In this result, the spot is shown in the Rf about 0.773 and also has been detection by Vanillin-sulphate acid. Furthermore, the compound identified by using Image J. Then the result in 10 ul extract solution contains about 2.49 ug/ul swieteni.

The *Swietenia mahagoni* extract (methanolic extract) has been shown the significant hypoglycemic and antioxidant activity in diabetic rats[10]. The purified extract has been tested with antioxidant assay by using scavenging DPPH method. The purified extract has IC_{50} 33.86 ug/ml (Table 6) and 29.14 ug/ml (Table 7) for quercetin as a standard. The results show that purified extract has activity as an antioxidant by the mechanism scavenging free radical.

Table 1. Extract rendamen

Dried sample (g)	Crude Extract (g)	Purified extract (%)	Rendamen (%)
3750	218.59	73.89	1.97

Table 2. Standardization parameters

Test	Result
Organoleptic Test	Taste: Bitter Colour: Red-brown
Determination of drying losses	Not more than 0.22%
Total ash level	Not more than 1.71 %
Acid-Insoluble ash level	Not more than 0.38 %
Water-soluble essence level	Not less than 14.84%
Ethanol-soluble essence level	Not less than 15.38%
Phytochemical screening (chemical compound)	Essential oil (-) Flavonoid (+) Saponin (+) Terpenoid (+) Alkaloid (+)

Table 3. Metal contamination test

Parameter	Unit	Result
Timbal (Pb)	ug/g	0,0607
Kadmium (Cd)	ug/g	<0,003

Table 4. Microbiology test

ALT Bacteria	**Concentration**				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Purified mahoni seed	10	3	3	3	1
	Concentration				
ALT Fungi	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Purified mahoni seed	4	3	3	1	0

Table 5. Flavanoid total

Abs (Y)	**Flavonoid total (mg/L)**	**Flavonoid content (gr RE/ gram)**	**Flavonoid content (gram RE/ gram)**	**% Flavonoid content**
0,163	46,615	2,33		
0,161	45,846	2,29	2,33	0,233
0,165	47,384	2,36		

Table 6. Antioxidant assay

Sample	Concentration (ppm)	Absorbance	Scavenging (%)	IC_{50}
Blank		0.784	-	-
Purified mahoni seed	20	0.365	53.44	33.86
	30	0.359	54.2	
	40	0.352	55.1	
	50	0.344	56.12	
	y = 0.7152x + 25.793		R^2 = 0.995	

Table 7. Antioxidant activity of the standard (quercetin)

	Concentration (ppm)	**Absorbance**	**Scavenging (%)**	**IC_{50}**
Blank	30	0.824	-	-
Quercetin	2	0.747	9.34	29.145
	4	0.729	11.52	
	6	0.714	13.34	
	8	0.672	18.44	
	10	0.650	21.11	
	y = 1.523x + 5.612		R^2 = 0.9689	

Table 8. Antioxidant assay

Sample	Concentration (ppm)	Absorbance	Inhibition (%)	IC_{50}
Purified mahoni seed	20	0.365	53.44	33.86
	30	0.359	54.2	
	40	0.352	55.1	
	50	0.344	56.12	
Quercetin	2	0.747	9.34	29.145
	4	0.729	11.52	
	6	0.714	13.34	
	8	0.672	18.44	
	10	0.650	21.11	

Conclusions

The purified extract mahoni seed qualify as a raw material for herbal medicine and also has potential as an antioxidant IC_{50} 33.86 ug/ml.

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