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## ***In Vivo* and *In Vitro* Antidiabetic Assay of Purified Mahoni Seeds Extract (*Swietenia mahagoni* (L.) Jacq)**

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**This study examined the future of Mahoni seed as an aspirant drug for treating diabetes. Mahogany seeds were washed using n-hexane solvent and followed by ethanol solvent. Extracts were measured using a standard spectrophotometric plate reader with acarbose (Glucobayâ). Glucose tolerance was tested in alloxan-challenged mice. The results indicated that the extract had potency against  $\alpha$ -glucosidases inhibited through a non-competitive mechanism. The  $IC_{50}$  value of the extract is 4.71g/ml, which was lower than 5.71g/ml for acarbose and has less activity on glucose tolerance at doses of 120 and 240 mg/kg. As a conclusion, purified mahogany seed extract got the ability to be developed as a new antidiabetic drug candidate.**

**Keywords:** Antidiabetic, purified extract, mahoni seeds (*Swietenia mahagoni* (L.) Jacq).

Diabetes can be a set of metabolic diseases characterized by hyperglycemia resulting from defects in hypoglycemic drug secretion, hypoglycemic drug action, or both. Diabetes mellitus (DM) can be a progressive chronic disease with aldohexose, protein, and lipid metabolism pathologies. DM is one of all aerobic stress conditions requiring additional exogenous antioxidants<sup>1</sup>.

Methanolic extracts from *Swietenia mahagoni* seeds were tested by various methods such as scavenging method ( $IC_{50}$  value 2.3 mg/ml), XOI assay (47.2%), HPSA method (49.5%) and FRAP method (0.728 mmol/Fe(II)). analyzed by the method. g). The total phenols and flavonoids are 70.83 mg gallic acid equivalents (GAE) and  $2.5 \pm 0.15$  mg catechin equivalents per gram of dry extract (Shagal ., 2009). A methanol extract

from the bark of *Swietenia mahagoni* protects against paracetamol-induced liver injury in Wistar rats (Haldar , 2011). An ethanol extract of mahogany seed (*Swietenia mahagoni* (L.) Jacq) contains flavonoids and saponins that inhibit the enzymatic activity of  $\alpha$ -glucosidase and may have hypoglycemic effects in mice. Extract at doses of 25 and 50 mg/kg body weight. It is significant in lowering blood glucose levels in diabetic rats ( $p < 0.001$ ) (Panda ., 2010). The 100 ppm ethanolic extract showed 18.147% inhibitory activity on  $\alpha$ -glucosidase ( $P < 0.05$ ). A hypoglycemic test (in mice) at different doses showed that a dose of 100 mg/kg BB extract had a hypoglycemic effect (Febriyany, 2014).

*Swietenia mahagoni* extract (L.) Jacq could modify anti-diabetic disease and associated complications such as aerobic stress

and hyperlipidemia (De D , 2011) . On the other hand, cats showed a good preference for Ethanol Mahogany Seed Extract and diets containing optimal concentrations of Ethanol Mahogany Seed Extract, with an average diet consumption of  $48.96 \pm 8.84$  (g/h). Cats consumed mahoni seed ethanol extract at mean consumption levels  $(Y) \pm 0.02$  (g/h) (Puspita, 2017). Extracts from *Swietenia mahagoni* (L.) Jacq may be a candidate for the development of promising dietary supplements for the treatment of anti-diabetic diseases.

In this study, we investigated the antidiabetic effects of purified mahoni seed extract (PMSE) by in vitro and in vivo assays in order to develop new candidate herbal medicines for diabetic diseases.

## MATERIALS AND METHODS

### Materials

Mahoni seeds (the specimen was determined number. 0925 and deposited at Laboratory of Pharmacognosy, UMI), dichloromethane (technical), methanol (technical), *n*-butanol (technical), *n*-hexane (technical), TLC plate (Merck cat. 1.05554, Germany), TLC kit, the *rotary evaporator* (Buchi R-215, Germany), spectrophotometer UV-Vis (Hitachi U 2000, Germany). DPPH (Sigma, USA), *p*-nitrophenyl a-D-glucopyranoside (Sigma Chemical), a-glucosidase (Sigma), acarbose (Glucobay®), glibenclamide.

### Method

#### Extraction and purification

The seeds were grounded and extracted by using hexane and ethanol. The purified extract was reported<sup>20</sup>.

#### Antidiabetic in vitro test

In vitro antidiabetic assay using a-glucosidase enzyme inhibitory. The extract was treated with a reagent containing 25 L of 2 mM *p*-nitrophenyl a-D-glucopyranoside (Sigma Chemical) and 49.5 L of pH 7.0 phosphate buffer. Furthermore, the solution was incubated at 37° C for 5 minutes. Then 25 L a-glucosidase (Sigma) was added, and the incubation was maintained for 30 minutes. 1 ml of 0.01 M Na<sub>2</sub>CO<sub>3</sub> was added to stop the reaction. The activity of a-glucosidase was evaluated at 400 nm using acarbose (Glucobay®) as a positive control<sup>2, 16, 21</sup>.

#### Kinetics of Inhibition Against a-glucosidase

By increasing the concentration of *p*-nitrophenyl a-D-glucopyranoside as a substrate in the absence or presence of samples at varied concentrations, the sample's capacity to inhibit glucosidase was examined. Lineweaver-Burk plot analysis of data derived from Michaelis-Menten mechanics was used to measure the mechanism<sup>3</sup>.

#### In vivo assay of oral glucose tolerance test (OGTT) in diabetic mice

The mice (100 mg/kg) (approved by Komisi Etik Penelitian Kesehatan, Universitas Muslim Indonesia No. UMI011811593) were fasted before receiving glucose intragastrically

**Table 1.** α-glucosidase inhibition activity of purified mahoni seeds extract

Concentration (ug/ml)	Inhibition (%)	IC <sub>50</sub> (ug/ml)
10	15.89	4.70
20	21.46	
30	25.46	
40	27.42	
50	34.80	
60	36.98	
70	41.31	
80	44.37	
90	50.78	
100	54.73	

**Table 2.** Control glucobay

Concentration (ug/ml)	Inhibition (%)	IC <sub>50</sub> (ug/ml)
100	4.20	5.79
200	13.67	
300	16.07	
400	29.98	
500	39.21	
600	43.17	
1000	59.71	
2000	61.75	
3000	63.19	
4000	66.91	
5000	70.38	
6000	72.54	

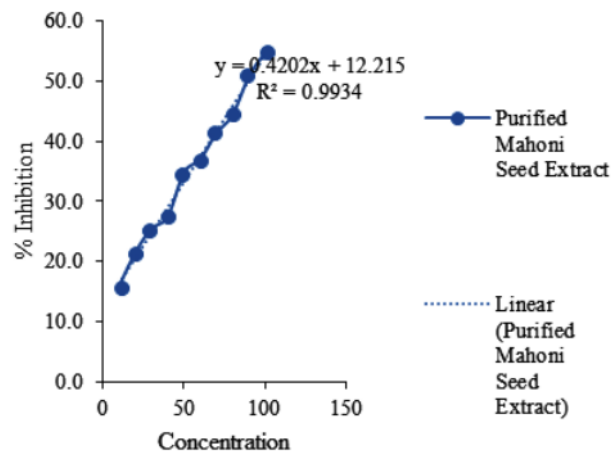
at 2 g/kg weight dose. Blood samples were taken from the orbital sinus before and after being treated with glucose for 0, 30, 60, 90, and 120 minutes. The experiment used glibenclamide as a control. Glucose tolerance was calculated and performed by analyzing SPSS<sup>5</sup>.

**RESULTS AND DISCUSSION**

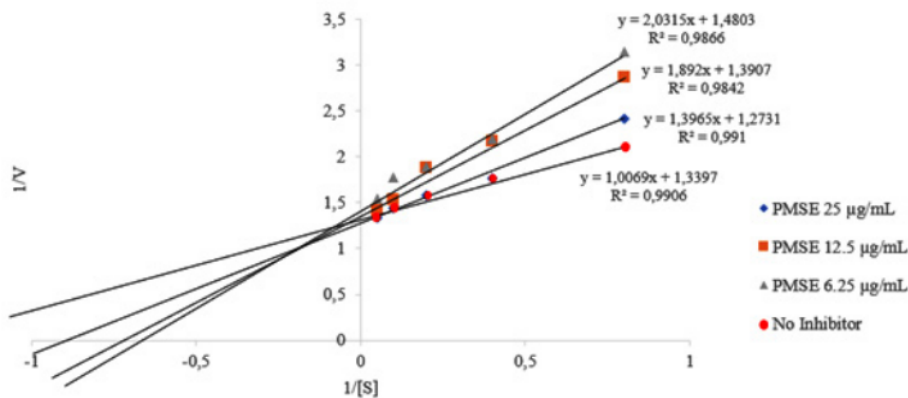
The secondary metabolites contained in the extract determine the potential of biological

activity. A few researchers have reported the biological activities based on the compounds such as phenolic, alkaloid, and terpene<sup>2</sup>. In addition, several compounds can inhibit alpha-glycosidase, for example, alkaloids and terpene. *Swietenia mahagoni* Jacq contains the swietenine (terpene) constituent with antidiabetic activity. Several researchers have reported *Swietenia mahagoni* Jacq as antidiabetic<sup>17</sup>.

The purified extract mahoni seeds (PMSE) was purified following the method of Virsa . (2018).



**Fig. 1.** %  $\alpha$ -glucosidase inhibition of purified mahoni seed extract



**Fig. 2.** Lineweaver-Burk plot of  $\alpha$ -glucosidase inhibition of the PMSE

Description:  
 PMSE (Purified Mahoni Seed Extract) 25  $\mu\text{g/mL}$   
 PMSE (Purified Mahoni Seed Extract) 12.5  $\mu\text{g/mL}$   
 PMSE (Purified Mahoni Seed Extract) 6.25  $\mu\text{g/mL}$

The antidiabetic effect has been tested using in vitro and in vivo methods. In vitro method by inhibiting  $\alpha$ -glucosidase enzyme and in vivo by using mice induced by glucose.

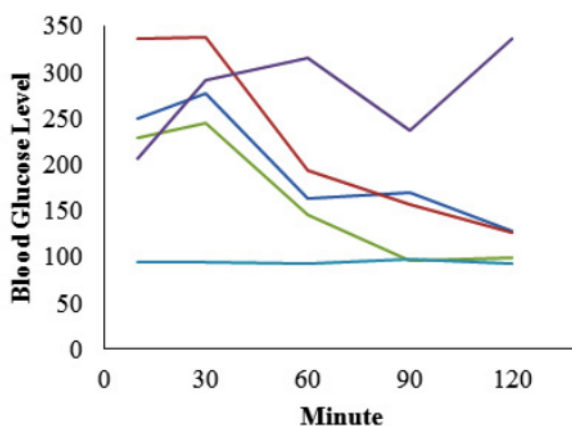
The in vitro test consisted of four test articles: sample with its control and blank with its control. Test substances were prepared at different concentrations (10; 20; 30; 40; 50; 60; 70; 80; 90, and 100  $\mu$ g/ml) (Figure 1).  $\alpha$ -Glucosidases can bind to  $\alpha$ -1,4 of various substrates and hydrolyze terminal non-reducing glucose residues that generate  $\alpha$ -D-glucose.  $\alpha$ -Glucosidase hydrolyzes  $\alpha$ -glycosidic bonds of oligosaccharides and  $\alpha$ -D-glycosides. The breakdown of carbohydrates is key in the method used, and inhibiting this enzyme activity may lead to lower postprandial blood glucose levels (Najib

., 2013 & 2016 and Hamidu ., 2018). The result showed that the purified mahoni seeds could inhibit the activity of  $\alpha$ -glucosidase with 4.7  $\mu$ g/ml (table 1), which is the result compared with the positive control (Glucobay) with 5.7  $\mu$ g/ml (table 2).

Reaction kinetic mechanisms based on the Lineweaver-Burk plot analysis of the samples were calculated and shown in FIG. These results indicated the presence of non-competitive inhibition. Data analysis of Lineweaver-Burk and Michaelis-Menten constants indicated that the extract exhibited a non-competitive inhibitory mechanism. Compounds in the extract bind to different sites on the enzyme, resulting in decreased enzyme activity<sup>3,10</sup>.

**Table 3.** Results of measurements the average glucose level in each treatment group

Treat	Initial levels	The average reduction in glucose levels at the minute					An average of % decrease
		0	30	60	90	120	
PMSE 120 mg	78 $\pm$ 7.1	250.2 $\pm$ 20.1	277 $\pm$ 54.3	162.6 $\pm$ 19.3	169.8 $\pm$ 45.8	128.2 $\pm$ 28.9	48.7
PMSE 240 mg	80 $\pm$ 81.4	336.8 $\pm$ 68.5	338 $\pm$ 37.9	193.6 $\pm$ 46.3	157.2 $\pm$ 47.3	125.8 $\pm$ 15.4	61.9
Glibenklamid	82 $\pm$ 4.1	228.6 $\pm$ 15.0	244.8 $\pm$ 78.6	146.2 $\pm$ 46.7	95.6 $\pm$ 19.9	99.6 $\pm$ 32.6	56.5
Induction	87.8 $\pm$ 6.3	206.4 $\pm$ 6.1	291.4 $\pm$ 36.5	316.2 $\pm$ 50.9	236.6 $\pm$ 63.8	336.4 $\pm$ 61.3	-63.7
Control							
No treatment	79.8 $\pm$ 6.2	94.6 $\pm$ 4.3	94.4 $\pm$ 6.8	93.4 $\pm$ 11.7	98 $\pm$ 13.5	92 $\pm$ 10.4	2.4



**Fig. 3.** Average results of decreased blood glucose levels

Description:

PMSE (Purified Mahoni Seed Extract) 120 mg

PMSE (Purified Mahoni Seed Extract) 240  $\mu$ g/mL

Glibenklamid

Initial Control/No Treatment

The *in vivo* glucose tolerance test (GTT) evaluates a human's capacity to employ glucose, the body's primary energy intake. This test can tell whether you have prediabetes or diabetes<sup>4,11</sup>. When collated with the control group of rats, purified mahoni seed extract significantly decreases blood glucose (Figure 3). The mean standard deviation (n=5) is used to express all values. \*P 0.05 against positive control values. A one-way ANOVA test was used to examine the comparisons, followed by LSD. The glucose tolerance test (GTT) results at 120, and 240 mg/kg doses of body weight were compared to glibenclamide as a control (Table 3). According to this experiment, the Glucose Tolerance Test (GTT) assesses the body's capability to use glucose, the body's primary energy intake. The oral treatment of pure mahoni seed extract in mice resulted in a glucose-lowering effect<sup>8,9</sup>.

### CONCLUSION

In summary, purified mahoni seed extract (PMSE) can inhibit  $\alpha$ -glycosidases with an  $IC_{50}$  value of 4.7  $\mu$ g/ml, affecting glucose tolerance at 120 and 240 mg/kg doses. A purified mahogany seed extract positive can potentially develop new antidiabetic drug candidates.

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