

Research Article

## **$\alpha$ -Amilase Inhibitory Activity of Fraction of Ethanolic Extract of Lebug Seed (*Cajanus cajan* (L.) Millsp.) Grown in West Nusa Tenggara**

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**Abstract:** Diabetes is a metabolic syndrome characterized by high blood sugar levels or hyperglycemia. Alpha-amylase inhibitors can be used as an antidiabetic that controls postprandial hyperglycemia. Alpha-amylase inhibitors are found in plants as protein and non-protein bioactive content such as in lebug seed. The purpose of this study was to determine the activity of alpha-amylase inhibition of the fraction of ethanol extract of lebug seed. This research was carried out by extraction of lebug seed and then fractionated lebug extract to obtain n-hexane fraction (HF), ethyl acetate fraction (EAF), and water fraction (WF). The fractions then tested to alpha-amylase inhibition activity in vitro by measuring the reducing sugar using 3,5-dinitrosalicylic (DNS) reagent. Furthermore, the identification of the bioactive content of each fraction was carried out using thin layer chromatography. The results of alpha-amylase inhibition activity test showed that all fractions had the ability to inhibit alpha-amylase. The IC<sub>50</sub> value of the n-hexane fraction, ethyl acetate fraction, and water fraction were 173.05 mg/ml, 9.98 mg/ml, and 137.19 mg/ml, respectively. Ethyl acetate fraction has the smallest IC<sub>50</sub> value compared to other fractions. Based on the identification of the bioactive content, bioactive content that is only found in the ethyl acetate fraction but not in other fractions are tannins. Therefore, the chemical content in lebug seed which acts as an alpha-amylase inhibitor might be tannins.

**Keywords :** lebug seeds (*Cajanus cajan* (L.) Millsp.);  $\alpha$ -amilase; fraction of ethanol extract

### 1. INTRODUCTION

Diabetes mellitus is a metabolic syndrome caused by the inability of the pancreas to produce insulin or the loss of the body's ability to effectively use insulin which causes hyperglycemia that can lead to various macrovascular and microvascular complications [1,2]. Based on data of IDF, in 2015 there were 415 million adults with diabetes. By 2040, this number is predicted to increase to 642 million people.

Treatment of diabetes mellitus aims to control the blood sugar levels of patients to minimize the risk of macrovascular and microvascular complications.  $\alpha$ -amylase inhibitors can inhibit the breakdown of carbohydrates to glucose so that glucose absorption decreases.  $\alpha$ -Amylase works to hydrolyze carbohydrates into oligosaccharides and dextrans which will be further hydrolyzed to glucose. Glucose will be absorbed by the intestinal epithelium and enter the

bloodstream [3]. Therefore, inhibition of the  $\alpha$ -amylase enzyme can delay and prolong the digestion of carbohydrates so that the rate of absorption of glucose decreases and prevents the postprandial hyperglycemia [4].

Lebui seed (*Cajanus cajan* (L.) Millsp) is one of the 5 most important nuts in the world along with soybeans, peanuts, green beans and red beans. Protein extract of lebui seed has an  $\alpha$ -amylase inhibitor isoform which inhibits amylase in human saliva and bovine pancreas [5]. Besides protein, non-protein bioactive compounds from plants such as tannins, flavonoids, alkaloids, and terpenoids also have  $\alpha$ -amylase inhibitory activity [6-9]. Lebui seeds have known to contain flavonoids, tannins, alkaloids, saponins, cyanogenic glycosides, glycosides, and anthocyanins [10]. However, research on the inhibition activity of  $\alpha$ -amylase from the fraction of the lebui seed grown in West Nusa Tenggara and identification of the active content has not been done yet. Therefore this research is needed to obtain information about the  $\alpha$ -amylase inhibitory activity of bioactive compounds non-protein of the lebui seed.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Porcine pancreas  $\alpha$ -amylase type VI-B (Sigma A-3176), 3,5-dinitrosalisilat acid (Sigma D-0550), soluble starch (Merck), glucobay tablet (Bayer), ethanol, aquadest, n-heksane, ethyl acetate, dragendorff reagent, amonia, FeCl<sub>3</sub>, Liebermann Burchard reagen, silica gel G60 F254 (Merck).

### 2.2. Plant material

Lebui (*Cajanus cajan* L. Millsp) seed was collected from Kebun Roek Traditional Market, North Ampenan, Mataram, West Nusa Tenggara. The lebui seed has been determined in Biology Pharmacy Departement of Faculty of Pharmacy, Universitas Gadjah Mada.

### 2.3. Preparation of plant extract

Lebui seed was immediately washed by distilled water and dried in the oven at 50°C. The dried sample was ground to a fine powder. The crude ethanolic extract was obtained by extracting 200 g of dried plant powder in 1.500 ml ethanol and soaked for 48 hours. The extract was further concentrated using vacuum rotary evaporator at 50°C and stored in a refrigerator at 4-8°C until required.

### 2.4. Preparation of Fraction of Plant Extract

The fractionation of lebui seed extract was done using the liquid-liquid partition method. Fifteen grams of lebui seed extract was dispersed with 150 ml warm aquadest. The extract then partitioned with 150 ml of n-hexane and the n-hexane phase was taken. Repeat the step until a clear n-hexane phase is obtained. Furthermore, the extract was partitioned with 150 ml of ethyl acetate and the ethyl acetate phase was taken. Repeat the step until a clear ethyl acetate phase is obtained. Each fraction includes n-hexane fraction, ethyl acetate fraction, and extract residue as water fraction were evaporated using vacuum rotary evaporator at 50°C and stored in a refrigerator at 4-8°C until required.

### 2.5. $\alpha$ -Amilase inhibitory activity of fraction of plant extract

The method used for  $\alpha$ -amylase inhibition assay was a modification of McCue and Shetty (2004). The assay mixture containing 200  $\mu$ L  $\alpha$ -amylase 2U/ml in 0.02 M sodium phosphate buffer, and 200  $\mu$ L fraction of lebui seed extract in concentration range of 10-200 mg/ml, were incubated at 25°C for 10 minutes. Then, added 200  $\mu$ L of 1% starch in 0.02 M sodium phosphate buffer and

incubate for 10 minutes at 25°C. The reaction was terminated with the addition of 800 µL DNS reagent and placed in boiled water for 5 minutes. The reaction mixture was diluted with 4 ml aquadest and the absorbance was measured at 540 nm using spectrophotometer. The control samples were prepared with the same procedure using 0.02 M sodium phosphate buffer instead of fraction of lebuli seed extract.

$$\% \text{ Inhibition} = \frac{(\text{Abs.control} - \text{Abs.sample})}{\text{Abs.control}} \times 100\%$$

The IC<sub>50</sub> value was determined from the regression equation of % Inhibition mean value vs concentration of the sample. Acarbose was used as the α-amylase inhibitor reference. All tests were performed in triplicate.

### 2.6. Analysis of bioactive compounds

The identification of bioactive compounds was carried out using thin layer chromatography with silica gel as a stationary phase.

#### 2.6.1. Flavonoids

The mobile phase used butanol: acetic acid: water (BAW) (4:1:5), with ammonia vapor as the detection reagent. Positive reactions are indicated by the formation of brown-yellow stains on visible and blue, and yellow observations on 366 nm UV light observations after ammonia was exposed [11,12].

#### 2.6.2. Alkaloids

The mobile phase that used was methanol: NH<sub>4</sub>OH (100:3), with dragendorff as the detection reagent. A positive reaction is indicated by the formation of orange spots on visible-light observations after spraying dragendorff [12].

#### 2.6.3. Tanin

The mobile phase that used was butanol: acetic acid: water (BAW) (4:1:5), with FeCl<sub>3</sub> as the detection reagent. A positive reaction is indicated by the formation of blackish-blue spots after being sprayed with FeCl<sub>3</sub> [13].

#### 2.6.4. Triterpenoids and steroids

The mobile phase used was chloroform: methanol (9:1), with Liebermann-Burchard as the detection reagent. The positive reaction of terpenoid is shown by the formation of pink spots, magenta to purple after being sprayed by Liebermann-Burchard on visible-light [14]. Where as the positive results of triterpenoid are indicated by the presence of brown spots, and positive results of steroid are indicated by the presence of green or blue spots on visible-light observations [15].

## 3. RESULTS AND DISCUSSION

### 3.1. Extraction and Fractionation

The result of extraction using ethanol found that rendemen of lebuli seed ethanolic extract was 7.29%. While the rendemen of n-heksane fraction, ethyl acetate fraction, and water fraction were 16.64%; 2.20%; and 44.49%, respectively.

### 3.2. α-Amylase inhibitory of plant extract fraction

The α-amylase inhibitory activity of lebuli seed ethanolic extract, fraction of lebuli seed ethanolic extract, and acarbose were investigated in this study and the results are shown in Figure

1, Figure 2 and Table 1. Figure 1 shows % of the inhibition and then the value were changed to IC<sub>50</sub> value such as summarized in table 1. The results indicate that the lebui seed ethanolic extract and fraction of lebui seed ethanolic extract have the  $\alpha$ -amylase inhibitory activities, but no better than acarbose. Ethyl acetate fraction has the smallest IC<sub>50</sub> value of 9.9878 mg/ml which indicates it has the highest potential to inhibit  $\alpha$ -amylase activity than ethanolic extract and other fractions.

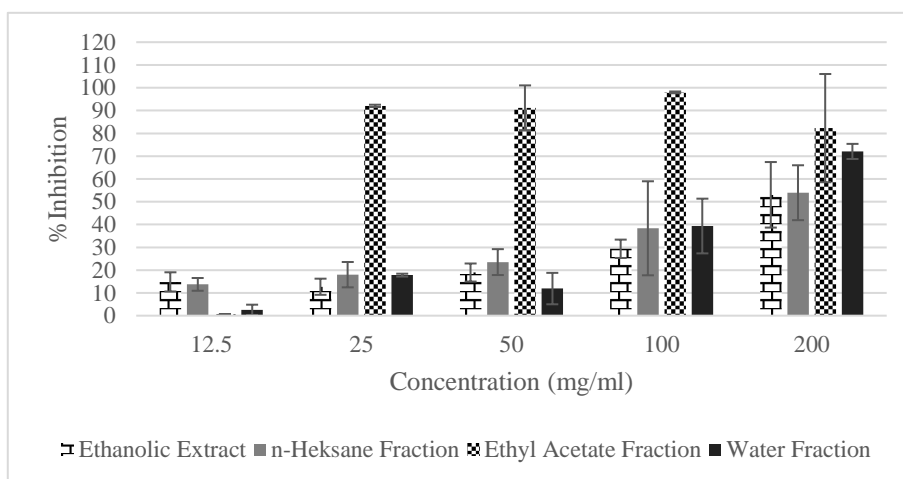


Figure 1. % Inhibition value of fraction of lebui seed ethanolic extract (triplicate)

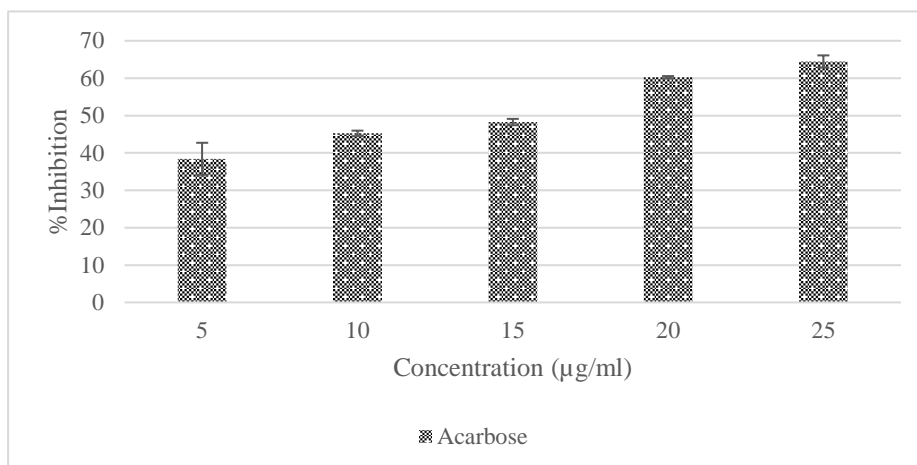


Figure 2. % Inhibition value of acarbose (triplicate)

Table 1. IC<sub>50</sub> value

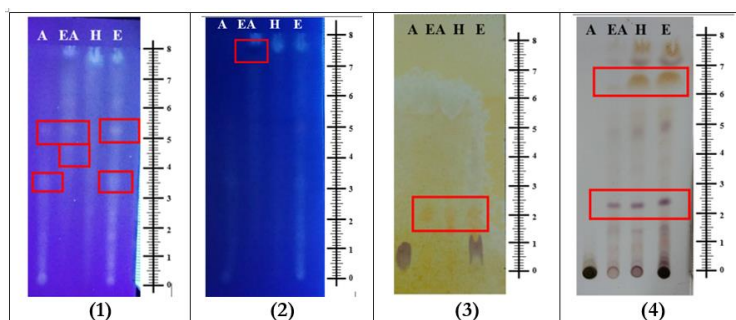
Sample	IC <sub>50</sub> (mg/ml)
Ethanolic extract	190.0543
n-Heksane fraction	173.0489
Ethyl acetate fraction	9.9878
Water fraction	137.1920
Acarbose	0.0140

### 3.3. Analysis of bioactive compounds

Analysis of bioactive compounds in this research aims to determine flavonoid, alkaloid, terpenoid, steroid and tannin compounds in the fraction and ethanol extract. In the identification of flavonoid compounds using thin layer chromatography, positive results were obtained on ethanol

extract, ethyl acetate fraction and water fraction (Figure 3, no,1). It can be shown from appearance of spots after evaporated with ammonia in UV 366 nm with Rf 0.40 at E, Rf 0.56 in at EA, and 0.40 at A. Based on Markham (1988), analysis of the spots found that the spots are flavonoid. The possibility of type of the flavonoid is isoflavones which does not have 5-OH free. In addition, analysis using the UV 366 nm also found red-orange spots on Rf 0.64 in E, EA, and A which were suspected to be anthocyanin compounds.

The results of the identification of tannin compounds can be seen in Figure 3 no.2. Identification of tannin group compounds obtained positive results on EA as shown by the presence of black spots with Rf 0.93 in observations of visible light after spraying FeCl<sub>3</sub> reagents.



**Figure 3.** The presence of black spots with Rf 0.93 in visible light observations after spraying FeCl<sub>3</sub> reagents. A = water fraction, EA = ethyl acetate fraction, H = n-heksane fraction, E = ethanolic extract

On the identification of alkaloid group compounds using thin layer chromatography, positive results were obtained on E, H and EA as shown in Figure 3 no. 3. This can be shown by the presence of orange-colored spots on visible light observations with Rf 0.25 in E, H, and EA after being sprayed with dragendorff reagent.

The identification results of the terpenoid group compounds content can be seen in Figure 3 no. 4. Based on these results, it is known that E, H, and EA gave positive results. This can be proved by the presence of spots on Rf 0.31 on E and Rf 0.28 in H and EA where these spots are purple at visible light appearance after spraying Liebermann-Burchard reagent. According to Sharifa et al (2012), terpenoid compounds will form pink color, magenta to purple after spraying Liebermann-Burchard reagent [14]. In visible light appearance of Rf 0.83 E, 0.81 H, and 0.80 EA, there are brown spots. This also confirms the positive results of triterpenoid compounds in E, H, and EA.

**Table 2.** Bioactive compounds

	Flavonoid	Tannin	Alkaloid	Terpenoid
Ethanolic extract	✓	×	✓	✓
n-Heksane fraction	×	×	✓	✓
Ethyl acetate fraction	✓	✓	✓	✓
Water fraction	✓	×	×	×

The  $\alpha$ -amylase inhibitor of the plant can be either protein or non-protein bioactive compound. Non-protein type  $\alpha$ -amylase inhibitors can be plant compounds such as flavonoids [2,6,7], alkaloids [8], terpenoids [9], and tannins [6]. The all results of bioactive compounds identification in this study shows that ethanolic extract contains flavonoids, alkaloids and

terpenoids; n-heksane fraction contains alkaloids and terpenoids; ethyl acetate fraction contains flavonoids, tannins, alkaloids and terpenoids; and water fraction contains flavonoids.

The ethyl acetate fraction was the fraction which had the highest  $\alpha$ -amylase activity compared to the activity of ethanolic extract, n-heksane fraction, and water fraction but it was still less active when compared with acarbose. Based on these results, bioactive compounds that play a dominant role in the inhibitory activity of  $\alpha$ -amylase from lebei seed are more likely to be tannins. As described in Table 2, these are due to the ethyl acetate fraction contains flavonoids, tannins, alkaloids, and terpenoids, whereas in ethanolic extract, n-heksane fraction, and water fraction there are no tannin compounds.

#### 4. CONCLUSION

Based on the results of the research and discussion, it can be concluded that lebei seed has an  $\alpha$ -amylase inhibitory activity. The  $IC_{50}$  value of n-hexane fraction, ethyl acetate fraction and water fraction are 173.0489 mg/ml, 9.9878 mg/ml and 137.1920 mg/ml, respectively. The bioactive compounds in n-hexane fraction are alkaloids and terpenoids. While in ethyl acetate fraction are flavonoids, alkaloids, terpenoids, and tannins and in water fraction is flavonoids. Ethyl acetate fraction is supposed to be a fraction that has the highest  $\alpha$ -amylase inhibition activity in which the possible bioactive compound is tannin.

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