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The Effectiveness of Flavonoids in Pigeon Pea (*Cajanus cajan*) as Inhibitors of α-Glucosidase Enzyme in Anti-diabetes

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ABSTRACT

Diabetes mellitus is a metabolic disease characterized by hyperglycemia, due to relative and absolute insulin deficiency of the body. Acarbose is a drug that can be used to treat diabetes mellitus, especially type II diabetes mellitus. Type II diabetes mellitus is an endocrine disease that causes 95 % of deaths worldwide. Gude bean extract (*Cajanus cajan*) is one alternative to the use of natural anti-diabetic medicine, containing saponins, flavonoids, phenolics, and tannins. Flavonoids are antioxidant compounds that have a hypoglycemia effect in people with diabetes mellitus. This study aimed to test the inhibitory activity of alpha-glucosidase enzyme from gude bean extract. The enzyme α-glucosidase activity was quantified by measuring the quantity of para-nitrophenol generated at a wavelength of 405 nm. The inhibitory power of the α-glucosidase enzyme was seen from the IC_{50} value. Gude Bean Extract has the ability to inhibit the enzyme α-glucosidase resulting in an IC_{50} value of 69.67 ppm strong level activity. Gude Bean Extract has the ability to inhibit the enzyme α-glucosidase resulting in an IC_{50} value of 69.67 ppm with a strong strength level.

Keywords: *α-Glucosidase inhibition; pigeon pea; flavonoids*; *antidiabetic*

INTRODUCTION

Inadequate insulin function is caused by impaired or insufficient insulin production by pancreatic β cells or a lack of cells in the body to respond to insulin. Type II diabetes mellitus is the most common type of DM and accounts for nearly 95 % of all cases. The high prevalence of diabetes is accompanied by the increasing use of synthetic oral preparations and insulin injections as pharmacological treatment in patients. But in general, synthetic oral drugs are not immune to unwanted side effects, so it is necessary to develop other therapies that are safer and easier to use, including drugs using natural ingredients as alternative therapies are more considered, because of the potential and minimal side effects given. One approach, the search for compounds capable of inhibiting the enzyme α-glucosidase is to develop new antidiabetic drugs (Dwi Apriliani & Amelia Saputri, 2018).

Nuts are one of the foods that are reported to have the effect of lowering blood sugar, therefore diabetics should consume them. One of them is Gude Bean (*Cajanus cajan*). Gude beans are local beans that are widely grown by farmers. Gude beans are nuts that are rich in flavonoids, including kajanol, quercetin, and luteolin. Flavonoids are low molecular weight phenolic compounds composed of 2-phenyl-chromone from acetic acid derivatives. Flavonoids are antioxidant compounds that have a hypoglycemia effect in people with diabetes mellitus (Azzahra et al., 2022). According to previous research, giving gude beans was able to lower blood sugar by 33.40 % in diabetic rats and help regenerate hypoglycemic pancreatic β cells. However, so far there has been no publication on the

antidiabetic activity of gude beans with the mechanism of inhibition of carbohydrate-breaking enzymes, such as α-glucosidase. The results of previous research, that gude bean leaf extract (400 and 600 mg/kg) suppressed the peak postprandial increase in blood glucose of normal rats by 101.8 and 57.40 %, respectively, showed the potential of gude bean leaf as an antidiabetic therapy, which may be caused by hypoglycemic activity and increased postprandial hyperglycemia in diabetes mellitus. Regarding to these conditions, the aim of this study was to test the antidiabetic activity of α-glucosidase enzyme inhibitors from gude bean extract; to find out the content of flavonoid compounds; This is expected to be a reference for the development of more effective diabetes therapy.

METHODS

Sample preparation

The determination of Gude Bean Plant was determined at the STIKES Tujuh Belas Pharmaceutical Biology Laboratory. Gude beans are thoroughly washed, wet sorted to select good quality materials, then thinly sliced and dried in the sun for 6-12 days or until the ingredients are dry. Furthermore, the powder is sprayed with the dried ingredients that are ground separately with a blender until sample powder is obtained that is not too fine, then sift the powder using a mesh 40 sieve. The unsifted powder is ground again until the entire powder is sifted (Ginaris et al., 2022). Gude Bean Powder can then be stored in an airtight container for further extraction.

Maceration Extraction

Extraction by maceration method using gude bean powder as much as 250 g using 70 % ethanol solvent, put 75 parts of the powder mixture into the macerator, soak for 3 days and stir occasionally, filtered and the remaining pulp allowed to stand for 2 days until the filtrate was obtained, then it is added the remaining ethanol until 100 parts of the juice (Ginaris, 2020). The filtrate was evaporated on a rotary evaporator at 40 °C, thick gude bean extract was obtained. The resulting viscous extract is poured into the beaker of the evaporator and placed on a waterbath to remove any residual ethanol solvent.

Identification of Compound Groups by Thin Layer Chromatography (TLC)

Identification of Gude Bean extract compound class using Thin Layer Chromatography (TLC) (Jawa La et al., 2020). Phytochemical tests were carried out to determine the chemical content contained in gude bean extract including saponins, flavonoids, phenolics, tannins. The Phytochemical tests using the TLC method, to determine the presence of active compounds in the extract preparation sample, by comparing the color of the spots and RF (range factor) to the Gude Bean extract. This TLC method uses silica gel GF254 as a stationary phase and the eluent developer as the mobile phase.

Mobile phase identification of saponins with chloroform: methanol: water (64: 50: 1) action Burchardar's Liebermen gives a blue-violet color. Mobile phase identification of flavonoids with n-butanol: acetic acid: water (4: 1: 5) ammonia vapor spray reagent gives yellow color. Take 0.1 g of sample, add 2-3 drops of 5 % FeCl3. Phenolic identification changes its color, if the sample is positive then the color will change to blue-black. Each sample is reduced to 0.1 g, 2 mL of water is added, and then boil for a few minutes. Determination of tannins by filtering the solution and adding 1 % $FeCl_3$ to the filtrate by 2-3 drops is shown dark green or greenish-black color (Vinholes & Vizzotto, 2017).

Inhibitory Activity of α-glucosidase enzyme

In this study, the enzyme α-glucosidase was derived from Saccharomyces cerevisae and the substrate p-nitrophenyl-α-D-glucopyranoside (pNPG). These inhibitors act by blocking the α-glucosidase enzyme in the small intestine where breakdown of complex carbohydrates occur. This enzyme reaction reduces carbohydrate hydrolysis and glucose absorption into the bloodstream and thus lowering postprandial blood glucose levels. p-nitrophenyl-α-D-glucopyranoside is a good substrate for α-glucosidase because it is specific for this enzyme and is not

hydrolyzed by other glycosidases (Zaharudin et al., 2018).

The enzyme α-glucosidase 100 µL (1.0 units/mL) using micropipettes was incubated with 50 µL of varying extract concentrations for 10 min. The concentration of extract variations is 20, 40, 60, 80 and 100 µg/mL. Then, dissolve 50 µL pNPG (3.0 mM) in 20 mM phosphate buffer pH 6.9 and add to the mixture, pH measured using a pH meter. The reaction is incubated at 37 °C for 20 minutes, adding 2 mL of Na_2CO_3 (0.1 M). The activity of the enzyme α-glucosidase can be measured using a spectrophotometer with a wavelength of 405 nm. The spectrophotometer measures the yellow color of para-nitrophenol released from pNPG (Swargiary & Daimari, 2020).

Acarbose as a positive control. The mechanism of action of acarbose is to inhibit the upper gastrointestinal enzymes (alpha-glucosidases) that convert complex polysaccharide carbohydrates into monosaccharides in a dose-dependent fashion. This slows the absorption of dietary carbohydrate, which is potentially beneficial in diabetes (He et al., 2014). The inhibitory activity of α-glucosidase is expressed as percent inhibitory control. Inhibition percentage was calculated using Equation 1.

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100\ \% \quad (1)$$

Where, Abs control means absorbance of assay mixture without extract and acarbose. Abs sample means absorbance of assay mixture with extract or acarbose. The value of IC50 is obtained from the linear equation of concentration and percentage of inhibition (Kifle et al., 2021).

Data Analysis

Statistical analysis was performed using SPSS one-way analysis of variance (ANOVA) software, taking into account significant differences in p-values <0.05 (Venditti et al., 2015).

RESULTS AND DISCUSSION

Sample preparation

The material used in this study was Gude Bean (*Cajanus cajan*) from the family Fabaceae obtained in the Tawangmangu area. Gude Bean Plant determined at STIKES Tujuh Belas Pharmaceutical Biology Laboratory. The obtained gude bean raw materials are then washed, wet sorted to classify good quality raw materials, weighed, sliced and then dried in indirect sunlight, for 6 to 12 days or until the raw materials are dried. The weight of wet gude beans was 500 grams. Drying was carried out to reduce the moisture content in sample, so as to prevent the destruction of sample from decay (Senduk et al., 2020). The weight of dried gude beans was 325 grams. The dried plant parts were ground into powder form using a mechanical grinder, then sift the powder with a mesh sieve 40. The unsifted powder was ground again until the entire powder was sifted. Gude Bean Powder could then be stored in a closed container for further extraction. Sample powder was stored in place in an airtight container protected from sunlight. The yield is 65 %. The ideal yield is 100 %, if the yield is above 50 % it is called fair (Wibowo et al., 2018).

Yield Maceration Extraction

Through maceration, 250 grams of gude bean powder were extracted. The advantage of using the maceration method is the simple way of working and equipment. The solvent uses 70 % ethanol, because it has the ability to extract with wide polarity ranging from non-polar to polar compounds. The maceration method was suitable for attracting active substances that were not heat-resistant. The yield is 34 %, the result is in accordance with the requirements of not less than 22.7 % (Surbakti et al., 2022).

Identification of Gude Bean Extract Compound Groups by Thin Layer Chromatography (TLC)

Identification of Gude Bean extract compound class using Thin Layer Chromatography (TLC). The compound groups identification groups using TLC is shown in Table 1. Identification of saponins gives a blue-violet color to 366 nm UV light, Rf results in samples of 0.82 indicating the presence of saponins. Identification of flavonoids gives yellow color to 366 nm uv rays, Rf results in samples of 0.71 indicate the

presence of flavonoids. Phenolic identification of a color change to blue-black in 366 nm uv light produced Rf 0.41 indicates the presence of phenolics. Determination of tannins added 1 % $FeCl_3$ to the filtrate by 2-3 drops. Dark green color indicates the presence of tannins at Rf 0.40. The results of the observation of flavonoids, phenolic, tannins, and saponins compounds by using TLC plate (Figure 1). These compounds are biologically active compounds with antioxidant, anti-inflammatory, antibacterial and antidiabetic roles (Vinholes & Vizzotto, 2017).

Table 1. The Compound Groups Identification Of Gude Bean Extract using TLC

Compounds	Result	Rf
Saponins	+	0.82
Flavonoids	+	0.71
Phenolic	+	0.41
Tanins	+	0.40

Information: (+) Detected, (-) Undetectable

Inhibitory activity of gude bean extract against α-glucosidase enzyme

Test of inhibitory activity of gude bean extract against α-glucosidase enzyme, shown in Table 2. Inhibition of the activity of the enzyme α-glucosidase causes a delay in the breakdown of carbohydrates into glucose, thereby, it causes a decrease in postprandial hyperglycemia. Acarbose is one of the drugs that can be used to treat diabetes. Acarbose is a α-glucosidase inhibitor commonly used to control postprandial blood glucose (Churia, 2018). Exhibited alpha-glucosidase inhibitory activity with IC50 values 945.5 µM, and 163.6 µM for acarbose the most potent inhibitor (Dirir et al., 2022).

Acarbose is a competitive and reversible inhibitor of glucosidase brush border of the small intestine, which inhibits the breakdown of starch and sucrose and slows the absorption of glucose and fructose in the upper small intestine (He et al., 2014). One of the searches for compounds that can inhibit α-glucosidase enzymes is using Gude Bean (*Cajanus cajan*), contains saponins, flavonoids, phenolics, and tannins. The ability or potential of gude bean extract in the treatment of diabetes has been tested for its ability to inhibit the enzyme α-glucosidase in vitro.

The results have showed an IC50 value of gude bean extract of 69.67 ppm, it is able to inhibit the enzyme α-glucosidase with a strong level of strength (IC50 = 50-100). Acarbose as a positive control has a smaller IC50 value of 48.07 ppm which is very strong (IC50 <50). Based on the results of the research conducted, acarbose produces an IC50 value of 45.72 (µg/ mL. The smaller the IC50 value, the greater the inhibitory activity of the enzyme α-glucosidase (AK et al., 2019).

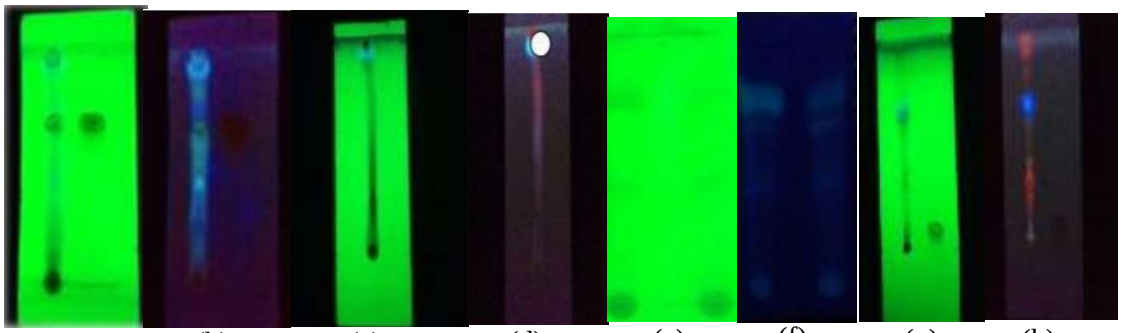

Figure 1. Thin Layer Chromatography Plate of the observation (a) flavonoids UV 254 nm, (b) flavonoids UV 366 nm, (c) saponins UV 254 nm, (d) saponins UV 366 nm, (e) phenolic UV 254 nm, (f) phenolic UV 366 nm, (g) tanins UV 254 nm, and (h) tanins UV 366 nm

Table 2. Inhibitory activity of gude bean extract

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (ppm)
Extract	20	22.180	69.67
	40	29.091	
	60	43.636	
	100	66.777	
Acarbose	20	43.464	48.07
	40	46.732	
	60	54.902	
	100	62.092	

The results of previous studies have indicated that gude bean leaf extract (400 and 600 mg / kg) suppressed the peak postprandial increase in blood glucose of normal rats by 101.8 and 57.40 %, respectively, showed the potential of gude bean leaf as an antidiabetic therapy, which may be caused by hypoglycemic activity and increased postprandial hyperglycemia in diabetes mellitus (Yusasrini & Jambe, 2018). Researchers attribute the antidiabetic properties of some medicinal plants to bioactive compounds such as phenolics, flavonoids, and tannins. Polyphenols, especially flavonoids, can be considered a better treatment for diabetes as well as chronic complications associated with this disorder. Flavonoid compounds have been believed to have an anti-diabetic effect because they can inhibit α-glucosidase enzymes. This interaction between the enzyme and flavonoids leads to reduced digestion of starch and postprandial glycemia. Another role of flavonoids is to interact with starch and form a complex which is difficult to digest (resistant). Flavonoids can also inhibit the process of glucose absorption by inhibiting glucose transporters (Cahyana & Adiyanti, 2021). Based on previous research, flavonoid compounds can lower blood sugar levels by stimulating pancreatic cells to produce more insulin.

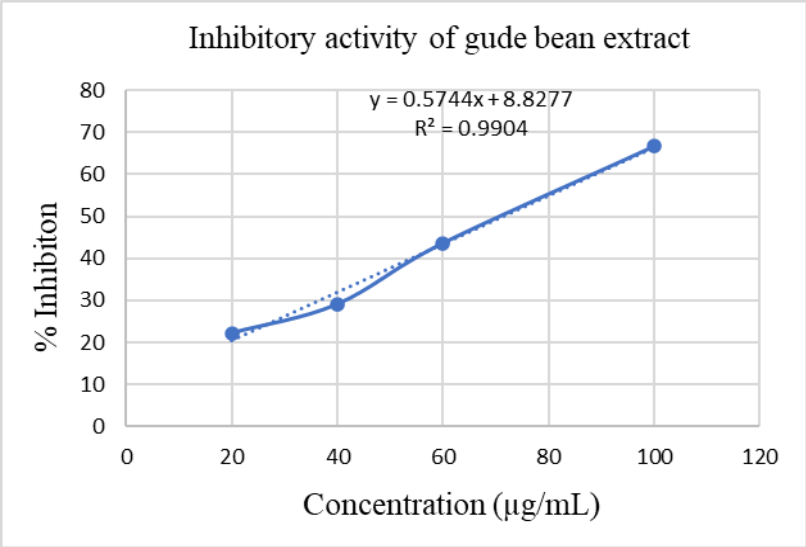

Figure 2. Value absorbance and linear equation curve gudean bean extract

CONCLUSIONS

Gude Bean Extract contains Saponins, flavonoids, phenolics, and tannins. Gude Bean Extract has the ability to inhibit the enzyme α-glucosidase resulting in an IC_{50} value of 69.67 ppm with a strong strength level. Gude Bean Extract has potential as an antidiabetic drug

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