α –Glucosidase inhibitory activity of breadfruit leaf extract (Artocarpus altillis (parkinson) fosberg)

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Abstract. Breadfruit plant has been empirically used by the society as antidiabetic. The aim of this study is to understand the α-glucosidase enzyme inhibitory activity of breadfruit leaves (Artocarpus altillis) and their phytochemical profile. The varieties of leaf used were the yellow and green breadfruit leaves. Each was extracted with multiple maceration using n-hexane and 70% ethanol as solvents. From the phytochemistry identification, it was found that the ethanol extract of green breadfruit leaf contains alkaloid, flavonoid, triterpene/steroid, polyphenol, while the yellow breadfruit leaf contains flavonoid and triterpene/steroid. The result of α-glucosidase inhibitory activity test shows that ethanol extracts of both yellow and green breadfruit leaf (Artocarpus altillis (Parkinson) Forberg) have the greatest enzyme inhibitory activity with IC50 of 9.07 and 11.01 ppm, respectively, compared to the n-hexane extracts of both yellow and green breadfruit leaf with IC50 of 16.16 and 23.24 ppm, respectively. Meanwhile, the positive control, acarbose has the strongest inhibitory activity with IC50 of 6.79 ppm.

1. INTRODUCTION

The prevalence of degenerative diseases, particularly in Indonesia, keeps increasing over time, especially diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by increased blood glucose level caused by the inability of pancreas to secrete insulin [1]. Another factor that may induce diabetes mellitus is that the body fails to produce insulin because of damaged beta cells in pancreas [2]. In 2015, around 415 million were diagnosed with diabetes mellitus in the whole world and this number is expected to increase up to 642 million in 2040 [3]. The cases of diabetes mellitus in the world keep on increasing with the complication of disability and hospitalization, which may lead to significant financial burden [4].

The increase of diabetes mellitus prevalence each year suggests the necessity of serious attention for the therapy of this disease. Therapies using synthetic pharmaceuticals often fail because of adverse effects, insulin resistance, and high cost for prolonged therapy. This encourages research to find alternative medicines with better efficacy and allows the patients to have many treatment options, so as to increase the chances of being cured, at least with controlled blood glucose levels and minimal side effects and relatively cheaper costs [5].

Indonesia is one of the megadiversity countries that has abundant biodiversity which has the potential as a medicinal plant. One medicinal plant that has been used empirically by the community as an antidiabetic drug is breadfruit leaf (Artocarpus altillis (Parkinson) Fosberg) [6].
Artocarpus altilis, which belongs to the family Moraceae, is usually called breadfruit. Breadfruit is also known as a carbohydrate-rich plant. The Artocarpus genus consists of around 50 species and is widespread in the tropics and subtropics [7]. Artocarpus extract and metabolites from leaves, stems, fruits and peels contain many beneficial biological active compounds and these compounds are used in various biological activities including antibacterial, antitubercular, antiviral antifungal, antiplatelet, anti-arthritis, tyrosinase inhibition and cytotoxicity [8], antidiabetic mellitus [9].

According to Lotulung, et al., 2014 [10], in the study of the antidiabetic activity of breadfruit leaf flavonoids (Artocarpus altilis) through the mechanism of inhibition of the α-glucosidase enzyme was found in ethanol, n-hexane, ethyl acetate and butanol fractions. Ethyl acetate fraction has the strongest antidiabetic activity compared to ethanol, n-hexane and butanol fractions. Visto Tjahjadi (2010) conducted a study on the effect of blood glucose reduction from the infusion of breadfruit leaves (Artocarpus altilis (Parkinson) Fosberg) in male white rats showing that at doses of 27 and 54 g/kg bw, the reduction of blood sugar level was found to be statistically significant at half and one hour after administration of glucose [11].

The aim of this study is to understand the α-glucosidase enzyme inhibitory activity of breadfruit leaves (Artocarpus altilis) and their phytochemical profile.

2. MATERIALS AND METHODS

2.1 Equipment and Materials

The equipment used in this study were maceration vessels, blenders, spraying bottles, porcelain evaporating dish, chambers, ELISA reader, micro pipette, Erlemeyer flasks, -200°C freezer, measuring cylinders, Beaker glass, filter paper, volumetric flasks, UV lights (254 nm and 366 nm), drying cabinets, TLC plates, Buchner filters, pH meters, rotary evaporator, incubator shaker, test tubes, analytical scales, vacuum pumps and 96-well plates.

The materials used were extracts of green and yellow breadfruit leaves. The chemicals used were ethanol, methanol, n-hexane, ethyl acetate, chloroform, distilled water, several reagents such as 1% FeCl₃ reagent, sulfuric acid, vanillin, anisaldehyde and nitroborate, TLC plate aluminum coated silica gel G 60 F254, aluminum foil and filter paper. The materials used to test enzyme activity were the α-glucosidase enzyme derived from Saccharomyces cerevisiae recombinant (Sigma Aldrich, USA), p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate (Sigma Aldrich, USA), dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), acarbose, sodium carbonate, buffer solution.

2.2 Sample Collection and Preparation

Breadfruit leaf samples (Artocarpus altilis (Parkinson) Fosberg were collected in Gowa Regency, South Sulawesi. Breadfruit leaves taken are yellow and green in the same size and were then collected, cleaned, and dried to reduce the water content of the leaves. After drying, the leaves were made powder to increase the contact area with the solvent so that all secondary metabolites can be extracted.

2.3 Extraction

Breadfruit leaf simplicia (Artocarpus altilis (Parkinson) Fosberg) each leaf is green and yellow as many as 600 grams and 300 grams for the green and yellow leaves, respectively, were inserted into the vessel then extracted by maceration using n-hexane. Maceration was carried out 3 x 24 hours, where every 24 hours the extract was filtered then macerated again with fresh n-hexane for 3 days. The results of maceration were filtered using a Buchner vacuum filter, all the filter results were mixed so that the n-hexane filtrate was obtained. The remaining n-hexane was evaporated using a rotary evaporator, thus, n-hexane extract was obtained. The simplicia residue of the n-hexane extract was soaked again using 70% ethanol, and the same was performed on n-hexane extract to obtain a 70% ethanol thick extract. The extracts obtained was then tested for the TLC profiles and α-glucosidase inhibitory activity test.
2.4 $\alpha$-Glucosidase Inhibitory Activity Test

The activity of the $\alpha$-glucosidase enzyme was analyzed using the Sancheti [12] method with modifications. The enzyme stock solution was dissolved in a phosphate buffer solution (pH 7). Enzymatic reaction was performed by mixing 15 μL of 25 mM p-NPG as substrate, 25 μL phosphate buffer solution and breadfruit leaf samples from each type of n-hexane and ethanol extract of both yellow and green leaves as much as 20 μL into 96-well plates which was then incubated for 5 minutes at 37°C, after that, 15 μL of $\alpha$-glucosidase enzyme was added.

The reaction mixture was then incubated at 37°C for 30 minutes. The reaction was stopped by adding 100 μL of 0.2 M Na$_2$CO$_3$ solution. The resulting P-nitrophenol was measured at $\lambda = 405$ nm using an Elisa reader. The experiment was conducted with 2 replications and % inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{C - S}{S} \times 100\%$$

$C$ = absorbance without sample $S$ = absorbance with sample

3. RESULTS AND DISCUSSION

Maceration was carried out using n- hexane and ethanol. The samples were macerated for 3 x 24 hours then the maceration results were concentrated with a rotary evaporator until thick extracts were obtained. From the extraction, the extracts of green and yellow breadfruit leaves were obtained (Table 1).

### Table 1. Extract Yield

| Test Sample      | Extraction Solvent | Simplicia Weight (g) | Thick Extract Weight (g) | Extract Yield (%) |
|------------------|--------------------|----------------------|--------------------------|-------------------|
| Green Breadfruit | n-hexane           | 350                  | 13.8                     | 3.94              |
|                  | ethanol            |                      | 37.2                     | 10.62             |
| Yellow Breadfruit| n-hexane           | 350                  | 8.4                      | 2.4               |
|                  | ethanol            |                      | 18.3                     | 5.22              |

**Figure 1.** Results of TLC with eluent n-hexane: ethyl acetate (3: 1) (A) n-hexane extract of yellow breadfruit leaves, (B) ethanol extract of yellow breadfruit leaves, (C) n-hexane extract of green breadfruit leaves, (D) ethanol extract green breadfruit leaves, silica gel 60 GF254 as stationary phase, (I) TLC results under 366 nm UV light without sulfuric acid, (II) after spraying sulfuric acid and heating at 105°C for 10 minutes, (III) After spraying sulfuric acid and heating then observed under 254 nm UV light, (IV) After being sprayed with sulfuric acid and heated then observed under 366 nm UV light.
Table 2. Rf value of each spots after sprayed with sulfuric acid and heated at 105°C for 10 minutes with the eluent n-hexane: ethyl acetate 3 : 1.

| Sample                        | Number of Spots | Rf                        |
|-------------------------------|-----------------|---------------------------|
| n-hexane yellow breadfruit leaves | 6               | 1, 0.97, 0.92, 0.87, 0.82, 0.75 |
| ethanol yellow breadfruit leaves | 4               | 0.75, 0.23, 0.17, 0.09    |
| n-hexane green breadfruit leaves | 11              | 1, 0.97, 0.89, 0.84, 0.77, 0.71, 0.66, 0.54, 0.48, 0.4, 0.35 |
| ethanol green breadfruit leaves | 13              | 0.95, 0.8, 0.71, 0.55, 0.51, 0.45, 0.37, 0.32, 0.29, 0.21, 0.12, 0.07, 0.05 |

α-glukosidase Inhibition

In this study the potential hypoglycemic activity of breadfruit leaves (Artocarpus altitís (Parkinson) Forberg) was tested in vitro by inhibiting the activity of α-glucosidase enzyme. Acarbose is used as a comparison which is an antidiabetic agent that works by inhibiting the action of the α-glucosidase enzyme. The activity test results showed that the ethanol extract of the yellow breadfruit leaves and the green breadfruit leaves showed the best activity compared to the green and yellow breadfruit leaf n-hexane extract with IC50 values of 9.07 ppm and 11.01 ppm, respectively. The high activity of ethanol extract compared with n-hexane extract is thought to be due to the many compounds that have the effect of inhibiting the activity of α-glucosidase enzymes. The results of testing the inhibitory activity against α-glucosidase can be seen in Table 3 below.

Table 3. Test results of α-glucosidase inhibitory activity of breadfruit leaf (Artocarpus altitís (Parkinson) Forberg) extracts

| Sample                        | Concentration (ppm) | Absorbance | % inhibition | Linear regression | IC50 |
|-------------------------------|---------------------|------------|--------------|------------------|------|
| n-hexane yellow breadfruit leaves | 5                  | 0.491      | 30.056        | y = 1.7094x + 22.365 | 16.16 |
|                               | 10                  | 0.413      | 41.168        |                  |      |
|                               | 15                  | 0.371      | 47.15         | $R^2 = 0.9709$   |      |
| ethanol yellow breadfruit leaves | 5                  | 0.393      | 44.017        | y = 1.3818x + 37.464 | 9.07  |
|                               | 10                  | 0.337      | 51.994        |                  |      |
|                               | 15                  | 0.296      | 57.834        | $R^2 = 0.9921$   |      |
| n-hexane green breadfruit leaves | 5                  | 0.561      | 20.085        | y = 1.567x + 13.58 | 23.24 |
|                               | 10                  | 0.478      | 31.908        |                  |      |
|                               | 15                  | 0.451      | 35.754        | $R^2 = 0.9205$   |      |
| ethanol green breadfruit leaves | 5                  | 0.495      | 29.487        | y = 3.1197x + 15.622 | 11.01 |
|                               | 10                  | 0.349      | 50.284        |                  |      |
|                               | 15                  | 0.276      | 60.683        | $R^2 = 0.9643$   |      |
| Acarbose                      | 5                   | 0.369      | 47.435        | y = 1.1439x + 6.79 |      |
|                               | 10                  | 0.318      | 54.672        | 42.222            |      |
|                               | 15                  | 0.288      | 58.874        | $R^2 = 0.9771$   |      |
Figure 2. Diagram of IC50 values from the result of α-glucosidase inhibitory activity of breadfruit leaves (*Artocarpus altilis* (Parkinson) Forberg)

Acarbose is an oligosaccharide compound from microorganism fermentation of *Actinoplanes utahensis*. Acarbose has a white powder form with a molecular weight of 645.6 and pKa 5.1 with an empirical formula C$_{25}$H$_{43}$NO$_{18}$. The chemical structure is as follows [14]. This complex oligosaccharide compound is a potential competitive inhibitor of the α-glucosidase enzyme that works in brush borders to break down starch, dextrin, maltose and sucrose to produce digestible monosaccharides. Through this characteristic, acarbose is one of the antidiabetic agents for patients with diabetes mellitus type II [15].

α-glucosidase is an enzyme that catalyzes the cutting of glycosidic bonds in oligosaccharides. Some glucosidases that work specifically in cutting glycosidic bonds depend on the position, number or configuration of hydroxyl bonds in the sugar molecule [16]. Therefore, in the condition of hyperglycemia, the inhibition of the action of the α-glucosidase enzyme can help overcome the condition of hyperglycemia because the amount of monosaccharide absorbed by the intestine becomes less and less.

Many studies have shown that the compounds contained in a plant have the ability to inhibit the action of α-glucosidase enzymes, such as compounds from the flavonoid [17], triterpenoid 18, alkaloid [19].

groups and inhibition of the activity of α-glucosidase enzymes by various phenolic compounds also found in various studies, including luteolin, myricetin, quercetin [20], and flavonol [21]. Thus,
the ability of breadfruit leaves (*Artocarpus altilis* (Parkinson) Forberg) in inhibiting the activity of the α-glucosidase enzyme is inseparable from the chemical components contained in it.

**Table 4. Result of phytochemical screening of green breadfruit leaf extract**

| Chemical compound | Rf  | Visible light            | UV 366 nm         | UV 254 nm         | Results |
|-------------------|-----|--------------------------|-------------------|-------------------|---------|
| **Flavonoid**     | 0.38| (Without reagent) Green | Yellowish green   | (Without reagent) Sитроборат reagent Yellow | Green | Yellowish Green | + |
|                   | 0.24| (Without reagent) Brownish Green | Purple Blue | (Without reagent) Vanillin reagent Blackish brown | Purplish blue purple | Vanillin reagent | + |
|                   | 0.63| (Without reagent) ~ | Anisaldehyde reagent Reddish orange | (Without reagent) Anisaldehyde reagent Grey | Light purple | Anisaldehyde reagent | + |
| **Terpenoid**     | 0.52| (Without reagent) Grey | FeCl$_3$ reagent Blackish green | (Without reagent) FeCl$_3$ reagent Greyish blue | Purple | FeCl$_3$ reagent Greyish black | + |
| **Polyphenol**    |     |             |                   |                   |         |

**Table 5. Result of phytochemical screening of yellow breadfruit leaf extract**

| Chemical compound | Rf  | Visible light            | UV 366 nm         | UV 254 nm         | Results |
|-------------------|-----|--------------------------|-------------------|-------------------|---------|
| **Flavonoid**     | 0.5 | (Without reagent) Green | Yellowish green   | (Without reagent) Sитроборат reagent Yellow | Green | Yellowish Green | + |
|                   | 0.11| (Without reagent) Brownish Green | Pурплиш Blue | (Without reagent) Vanillin reagent Blackish brown | Pурплиш blue purple | Vanillin reagent | + |
|                   | 0.07| (Without reagent) ~ | Anisaldehyde reagent Reddish orange | (Without reagent) Anisaldehyde reagent Grey | Light purple | Anisaldehyde reagent | + |
| **Terpenoid**     |     |             |                   |                   |         |
| **Polyphenol**    |     |             |                   |                   |         |
Figure 3. TLC profile of ethanol extract of green breadfruit leaves with eluent n-hexane: ethyl acetate (3: 1), silica gel 60 GF254 as stationary phase, (A) sprayed using sitroborate, (B) sprayed using anisaldehyde, (C) sprayed using FeCl$_3$, (D) sprayed using vanillin, (I) the result of TLC after spraying sulfuric acid and heating at 105°C for 10 minutes, (II) After spraying sulfuric acid and heating then observed under 366 nm UV light, (III) After spraying sulfuric acid and heating then observed under 254 nm UV light.

Figure 4. TLC profile of ethanol extract of yellow breadfruit leaves with eluent n-hexane: ethyl acetate (3: 1), silica gel 60 GF254 as stationary phase, (A) sprayed using sitroborate, (B) sprayed using FeCl$_3$, (C) sprayed using anisaldehyde, (D) sprayed using vanillin, (I) the results of TLC after spraying sulfuric acid and heating at 105°C for 10 minutes, (II) after spraying sulfuric acid and heating then observed under 366 nm UV light, (III) after spraying sulfuric acid and heating then observed under 254 nm UV light.

4. CONCLUSION

The test results of the inhibition of $\alpha$-glucosidase enzyme activity showed that yellow and green breadfruit leaf (*Artocarpus altilis* (Parkinson) Forberg) ethanol extract have the greatest $\alpha$-glucosidase enzyme inhibition activity with IC50 values of 9.07 and 11.01, respectively, compared to the IC50 of n-hexane extract of yellow and green leaves of 16.16 and 23.24, respectively.

5. ACKNOWLEDGEMENT

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