Inhibition of the α-glucosidase enzyme from Pelawan stem extract (*Tristaniopsis merguensis* Griff)

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**Abstract.** Diabetes mellitus continues to increase along with the increasing pattern of consuming ready-to-eat foods. The consequences of this habit will have a negative impact on the health of the body so that it can cause death. Treatment of diabetes mellitus is carried out in various ways, including administration of insulin and synthetic drug therapy. However, this medication has dangerous side effects. Therefore, research was carried out on Pelawan stems (*Tristaniopsis merguensis* Griff) which have the potential to be associated with secondary metabolites and bioactivity as antidiabetic so that they can be considered as raw material for herbal medicines in the future.

The total phenolic content of the methanol fraction of *T. merguensis* stems is 176.37 mg GAE/g DW. The total flavonoid content of the methanol fraction of *T. merguensis* stems is 9.85 mg QE/g DW. The results of the antidiabetic test for the methanol fraction of *T. merguensis* stems obtained an IC₅₀ of 5.31 µg/mL. When viewed from the qualitative results of phenolics and flavonoids that have been carried out, it is likely that the activity of the α-glucosidase enzyme in this study is more influenced by polyphenolic compounds.

1. **Introduction**

   Based on data from the World Health Organization revealed that people with diabetes mellitus in Indonesia in 2000 numbered 8.4 million people and will increase significantly to 21.3 million people in 2030. Several studies have shown that the mortality of COVID-19 patients with comorbid diabetes is very high compared to those without diabetes [1]. Based on the data obtained, it was found that 33.6% of patients had diabetes mellitus [2]. This problem is the focus of attention to immediately reduce the number of people with diabetes mellitus, so that the mortality of COVID-19 patients with co-morbidities with diabetes mellitus can be suppressed [3]. Due to current conditions, we must live side by side with the corona virus, especially those with comorbid diabetes.

   Some therapies to reduce blood sugar levels such as life style changes, oral agents, injectable agents. Life style changes are therapy by adjusting the diet including control of carbohydrates, sucrose and fructose [3]. Oral agents for the treatment of diabetes such as metformin, insulin secretagogues, Sulfonylureas and meglitinides. While injectable agents such as RA-GLP1 use exenatide, lixisenatide, liraglutide, albigrutide, dulaglutide. Another commonly used injectable is insulin. Insulin is a therapy that has the smallest side effect compared to other oral agents or injectable agents. For example, metformin has a high fatality rate which can cause sepsis, heart failure, dehydration, acute or progressive renal impairment [4]. There are several drugs that cause heart disease to heart failure such
as thiazolidinediones, sitagliptin, sexagliptin, alogliptin and many others. Unfortunately all antidiabetic agents have side effects and are expensive. Therefore, research on new antidiabetic agents with low side effects and cheaper is a challenge for current researchers.

Polyphenols from natural products can be the future treatment of diabetes. Polyphenols can regulate glucose metabolism through different pathways, such as restoring beta cell integrity, increasing insulin-releasing activity, and increasing cellular glucose uptake, which can increase insulin resistance [5]. Natural products that have high polyphenols for antidiabetic therapy such as blackberries, green tea, apricots, and others [3]. This study will use extracts of natural products that are high in polyphenols as antidiabetic therapy, which is a local plant of Bangka Belitung and has been used by the people of Bangka Belitung as a traditional medicine for antidiabetic therapy like Tristaniopsis merguensis Griff stems.

2. Method

2.1 Sample Preparation and Extraction

T. merguensis Griff stems used were collected from Bangka. The sample will be dried in the sun and ground into a dry powder that is ready for extraction. T. merguensis stem dry powder as much as 1 kg was macerated gradually with the solvent polarity gradient by maceration. Starting from a non-polar solvent using 10 L of n-hexane for 3x24 hours. The filtrate was taken and the powder was extracted with a semi-polar solvent using 10 L of ethyl acetate for 3x 24 hours. Then followed by polar solvent using methanol for 3x24 hours. The filtrate of each solvent was concentrated with a rotary evaporator vacuum to obtain a dry extract.

2.2 Determination of Total Phenolic Content

Analysis of total phenolic content was performed spectrophotometrically with gallic acid standard. The standard solution of gallic acid was made in various concentrations, then 0.5 mL of each concentration was taken and added with 0.5 mL of Folín Ciocalteu then vortexed for 30 seconds. After that, 2.5 mL of 7.5% sodium carbonate (Na2CO3) solution was added. The solution was vortexed again for 30 seconds. After that the absorbance was measured with a wavelength of 760 nm [5]. Sample solution was also taken as much as 0.5 mL added with 0.5 mL Folin-Ciocalteu and vortexed for 30 seconds. After that, 2.5 mL of 7.5% sodium carbonate (Na2CO3) solution was added. The solution was vortexed again for 30 seconds. After that the absorbance was measured with a wavelength of 760 nm [5].

2.3 Determination of Total Flavonoid Content

Analysis of total flavonoid content was carried out spectrophotometrically using aluminum chloride (AlCl3) and quercetin as standard solutions. [5]. Standard solution of quercetin with 0.5 mL of each concentration was pipetted, added with 0.1 mL of aluminum chloride (AlCl3), then 1.5 mL of methanol p.a and 0.1 mL of 1M sodium acetate (NaCH3COO) were added. Then 2.8 mL of distilled water was added. The solution was vortexed for 30 seconds. Then let stand at room temperature for 30 minutes. After that the concentration was measured absorbance at a wavelength of 420 nm. Samples of the methanol fraction of T. merguensis extract were pipetted with 0.5 mL added with 0.1 mL of aluminum chloride (AlCl3) then 1.5 mL of methanol p.a and 0.1 mL of sodium acetate (NaCH3COO) 1M were added, then 2.8 mL of distilled water were added. The solution was vortexed for 30 seconds. After that, the absorbance was measured at a wavelength of 420 nm [5].

2.4 Inhibition α-glucosidase enzymes

Antidiabetic testing was carried out in vitro at LIPI Kimia with -glucosidase enzyme inhibition method. Measurement of α-glucosidase activity inhibition was carried out by mixing 250 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution and 495/490 µL of phosphate buffer pH 7 0.1 M and added to a test tube containing 5 µL (standard solution). ) / 10 µL (sample solution) in DMSO with various concentrations. After the homogeneous solution was incubated for 5 minutes at 37°C, the
reaction was started by adding 250 µL of α-glucosidase solution. Incubation was continued for 15 minutes. The reaction was stopped by the addition of 1 ml of 0.2 M Na₂CO₃. Enzyme activity was measured based on the absorption of p-nitrophenol at a wavelength of 400 nm [6]. Calculation of IC50 from the regression equation from the concentration plot on the percentage of inhibition.

3. Result and Discussion

*T. merguensis* stem dry powder as much as 250 g was macerated gradually with the solvent polarity gradient by maceration. Starting from n-hexane, ethyl-acetate, and methanol. The purpose of this stepwise extraction is to fractionate the compounds contained in the stem of *T merguensis* into a non-polar fraction (n-hexane), a semi-polar fraction (ethyl acetate), and a polar fraction (methanol). The results of this stepwise extraction are presented in Table 1.

| Fractions  | Extract Mass (g) | Yields (%) |
|------------|------------------|------------|
| n-hexane   | 4.2320           | 1.69       |
| Ethyl Acetate | 3.0917         | 1.24       |
| Methanol   | 11.8010          | 4.72       |

The highest mass in the stem extract of *T. merguensis* is methanol fraction, which is 11.8010 grams. Followed by the n-hexane fraction at 4.2320 grams, and the ethyl acetate fraction at 3.0917 grams. These results indicate that the stem extract of *T. merguensis* contains compounds that are polar. There are fewer semipolar compounds because the mass of the ethyl acetate fraction is the smallest. In addition, this stepwise extraction to maximize test results. Generally, the methanol fraction contains polar compounds such as polyphenols (phenolic or flavonoid). So that this polyphenol compound is expected to be active as an antidiabetic. To ensure the presence of polyphenols, a quantitative test was carried out on the methanol fraction to determine the total phenolic and flavonoid content.

3.1 Total Phenolic Content (TPC)

The total phenolic content was carried out to determine of phenolic compounds present in the sample, this test as the basis for testing antidiabetic activity. The standard solution in this test is gallic acid. Gallic acid is one of the natural phenolic compounds so it is used as a standard. In this test also using Folin-Ciocalteu reagent. Gallic acid is reacted with Folin-Ciocalteu to form a yellow color and then the color changes to blue after the addition of sodium carbonate (Na₂CO₃). The high intensity of the blue color produced is equivalent to the amount of phenolic compound. So that the higher the content of phenolic compounds in the sample, the darker the blue color produced. The total phenolic content of the methanol fraction obtained an absorbance of 1.478. Based on the gallic acid standard, the total phenolic content was 176.37 mg GAE/g DW.

Total phenolic content of *T. merguensis* stems higher than the other *Myrtaceae* species such as *Pimenta dioica*, *M. vexator*, *S. cumini*, *M. alba* and *M. rubra* with several different solvents (Table 2). However, TPC of methanol fraction of stems was lower than leaves extract of *T. merguensis*.

3.2 Total Flavonoid Content

Analysis of the total flavonoid content in the sample was based on the quercetin standard. Quercetin was chosen as the standard because its structure is a flavonoid framework. The maximum wavelength of quercetin when interacting with AlCl₃ is 420 nm. The higher the concentration, the darker the yellow color produced. The total flavonoid in the sample was obtained from the absorbance of the sample on the quercetin standard. The total flavonoid content of the methanol fraction of *T. merguensis* stems is 9.85 mg QE/ g DW.
Table 2. Total Phenolic Content from *Myrtaceae* species

| Plants               | Extracts                         | Total Phenolic Content (mg GAE/g DW) | References |
|----------------------|----------------------------------|-------------------------------------|------------|
| *T. merguensis*      | Metanol fraction of stems        | 176.37                              | This Research |
|                      | Ethyl acetate fraction of leaves | 86.724                              |            |
|                      | Acetone extract of leaves        | 215.22                              | [7]        |
|                      | Ethanol extract of leaves        | 291.33                              | [7]        |
| *Pimenta dioica* (L.)| Leaf essential oil               | 99.09                               | [8]        |
| *Myrciaria vexator*  | Meoh-formic acid extract of fruit| 44.1                                | [9]        |
| *Syzygium cumini* (L)| Meoh-formic acid extract of fruit| 39.6                                | [9]        |
| *Morus alba* L       | Ethanolic extract of stems       | 10.58                               | [10]       |
| *Morus rubra*        | Methanolic extract of stem bark  | 2.54                                | [11]       |

Table 3. Total Flavonoid Content from *Myrtaceae* species

| Plants               | Extracts                         | Total Flavonoid Content (mg QE/g DW) | References |
|----------------------|----------------------------------|-------------------------------------|------------|
| *T. merguensis*      | Methanol fraction of stem        | 9.85                                | This Research |
| *Eugenia florida* DC.| Methanol extract of leaves       | 45.2                                | [12]       |
|                     | Ethyl acetate fraction of leaves | 93.3                                | [12]       |
|                     | Buthanol fraction of leaves      | 39.6                                | [12]       |
| *S. Polychepalum*    | Ethanolic extract of leaves      | 8.89                                | [13]       |
| *Psidium guajava* L  | Chloroform extract of leaves     | 112.71                              | [14]       |
|                     | Ethyl acetate extract of leaves  | 269.57                              | [10]       |
|                     | Buthanolic extract of leaves     | 68.37                               | [10]       |
| *M. glazioviana*     | Dichloromethanic extract of stem | 9.78                                | [15]       |
|                      | Methanolic extract of stem       | 7.62                                | [15]       |

The total flavonoid content in the methanol fraction of *T. merguensis* stems was not higher other species in the *Myrtaceae* family, such as leaf extract of *E. florida* DC, and *P. guajava*. However, it is still higher than the ethanolic extract of leaves of *S. Polychepalum*, dichloromethanic and methanolic extracts of *M. glazioviana* stems. Methanolic fraction or extracts of steam is indeed not too high in total flavonoid content when compared to leaf extract [15].

3.3 Inhibition α-glucosidase enzymes

The antidiabetic activity test was carried out in vitro by inhibiting the -glucosidase enzyme. The test sample and the p-nitrophenol-α-D-glucopyranoside (p-NPG) substrate were incubated and reacted with the α-glucosidase enzyme. The results of the analysis of antidiabetic activity in the methanol fraction of *T. merguensis* stems were able to inhibit the activity of the α-glucosidase enzyme. The value of this activity is expressed in IC\textsubscript{50}. The results of the antidiabetic test for the methanol fraction of *T. merguensis* stems obtained an IC\textsubscript{50} of 5.31 µg/mL. The IC\textsubscript{50} value obtained from plots of
concentration versus percentage inhibition (Figure 1). From Figure 1, we get the equation 
\[ y = 15.805 \ln(x) + 23.598 \]. Based on this equation, where \( y = 50 \) so the value of \( x \) or IC\textsubscript{50} obtained is 5.31 µg/mL.

![Figure 1](image)

**Figure 1.** The relationship between concentration and inhibition (%) of α-glucosidase enzyme

The IC\textsubscript{50} value better than the acetone extracts of leaves of *T. merguensis* ie 8.83 µg/mL [16]. Many natural compounds have been reported as potential antidiabetic agents such as terpenoids, flavonoids, and phenolics. Polyphenol compounds, flavonoids, and sugar derivatives are active as inhibitors of the α-glucosidase enzyme. When viewed from the qualitative results of phenolics and flavonoids that have been carried out, it is likely that the activity of the α-glucosidase enzyme in this study is more influenced by polyphenolic compounds contained in the methanolic fraction of *T. merguensis* stems.

### 4. Conclusion

Based on the results of this study, total phenolic content of the methanol fraction of *T. merguensis* stems is 176.37 mg GAE/g DW. The total flavonoid content of the methanol fraction of *T. merguensis* stems is 9.85 mg QE/g DW. The results of the antidiabetic test for the methanol fraction of *T. merguensis* stems obtained an IC\textsubscript{50} of 5.31 µg/mL. When viewed from the qualitative results of phenolics and flavonoids that have been carried out, it is likely that the activity of the α-glucosidase enzyme in this study is more influenced by polyphenolic compounds.

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