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Evaluasi Potensi Antidiabetes (Uji Inhibisi Enzim α-Glukosidase dan α-Amilase) dari Ekstrak Daun Mundu [Garcinia dulcis (Roxb.) Kurz]

Evaluation of Antidiabetic Potential (α-Glucosidase and α-Amylase Enzyme Inhibition Assay) of Mundu Leaf Extract [Garcinia dulcis (Roxb.) Kurz]

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Abstract

Diabetes Mellitus (DM) is a chronic disease that requires appropriate solutions for its management. To date, synthetic antidiabetic drugs frequently cause side effects in the body. The search for natural antidiabetic agents has therefore been carried out by exploring the potential of mundu leaves, which are considered safer as they originate from natural sources. Mundu leaves contain flavonoids, phenols, and tannins, which may act as natural antidiabetic agents due to their richness in hydroxyl and aromatic compounds. Antioxidant compounds play a role in protecting body cells from oxidative stress, which is strongly associated with diabetes. Several species from the Garcinia genus have been studied, but to date, no research has focused on the antidiabetic potential of mundu leaves. Through assays of mundu leaf extract in inhibiting α-amylase and α-glucosidase enzymes, as well as antioxidant tests, DM could potentially be better controlled, providing a reference for its use as a natural antidiabetic and antioxidant agent. Mundu leaves were prepared and macerated using ethanol as a solvent, followed by evaporation with a rotary evaporator. Phytochemical screening was conducted to detect the presence of active compounds. The inhibitory activity of mundu leaf extract against α-glucosidase and α-amylase enzymes was measured using a UV-Visible spectrophotometer to obtain absorbance values. The absorbance data were then used to determine the percentage of inhibition, allowing the calculation of IC50 values as a benchmark for antidiabetic activity. The results demonstrated that the mundu leaf extract exhibited antidiabetic properties, with IC50 values of 51.058 ppm and 33.004 ppm, categorized as strong and very strong, respectively.

Keywords**:** *α-Glucosidase; α-Amylase Enzyme; Antidiabetic; Mundu Leaf Extract*

1. INTRODUCTION

Diabetes mellitus (DM) is a type of chronic metabolic disease characterized by elevated blood glucose levels due to impaired insulin production [1]. DM poses a burden on patients' lives, affecting the healthcare system and leading to complications such as cardiovascular disorders, kidney damage, blindness, and chronic wounds that may result in amputation [2]. Type II DM, in particular, is closely associated with the increased production of free radicals, which can be reduced through the use of antioxidants [3].

According to the International Diabetes Federation, the number of diabetes patients continues to increase

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worldwide, with a projected prevalence of more than 700 million cases by 2045 [4]. Diabetes treatment can be performed by consuming drugs that inhibit the activity of α-glucosidase and α-amylase enzymes, thereby minimizing the postprandial increase in blood glucose levels [5].

The use of synthetic drugs such as acarbose often causes digestive side effects, including bloating and diarrhea. Therefore, it is necessary to explore bioactive compounds from natural sources as alternative antidiabetic therapies, such as plants from the Garcinia genus, including Garcinia dulcis. Mundu leaves are believed to exert antidiabetic and antioxidant effects due to their flavonoid, phenolic, and tannin content. Through inhibition of α-glucosidase and α-amylase enzymes, blood glucose levels can be better controlled, while oxidative stress can be minimized by antioxidant compounds [6].

Research conducted by Sunan Jaisamut and Boonyadist Vongsak (2021) demonstrated that leaf, fruit, and bark extracts of Garcinia schomburgkiana exhibited strong activity, with EC_{50} values of 20.40 ± 1.33 μg/mL against α-amylase and 2.81 ± 0.43 μg/mL against α-glucosidase [7]. Similarly, reported the antioxidant activity of Garcinia dulcis root extract with an IC_{50} value of 71.07 ± 0.02 μg/mL [8]. Furthermore, Nethengwe *et al*. (2024) confirmed that Garcinia livingstonei leaf extract exhibited α-glucosidase inhibitory, anti-inflammatory, and hypoglycemic effects [9]. Based on these findings, this study aims to evaluate the ability of mundu (Garcinia dulcis) leaf extract to inhibit α-glucosidase and α-amylase activities, as well as its antioxidant potential, as a preliminary step in developing safer and more effective herbal medicines for DM patients.

2. METHODS

Research Type and Design

This study is a laboratory based experimental research conducted in vitro with a descriptive quantitative approach to evaluate the antidiabetic and antioxidant activities of mundu leaf extract. The research involved a series of biological activity assays on the extract at various concentrations to determine its potential activity progressively and to measure the IC_{50} value as an indicator of the extract's effectiveness in inhibiting enzyme activity (Antidiabetic Assay).

Apparatus and Materials

Apparatus: Macerator, rotary evaporator, water bath, freezer, incubator, 96-well microtiter plate, micropipette, analytical balance, blender, UV-Visible spectrophotometer, volumetric flask, filter paper, aluminum foil, measuring flask, and beaker glass**.**

Materials: Mundu leaf powder, ethanol, methanol, ferric chloride ($FeCl_3$), magnesium powder, concentrated hydrochloric acid, α-glucosidase enzyme, p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate, α-amylase enzyme, starch solution substrate, DNS solution (3,5-dinitrosalicylic acid), sodium carbonate, acarbose, and potassium phosphate buffer.

Procedures:

Preparation and Maceration

Mundu leaves (5 kg) were cleaned and cut into smaller pieces. The leaves were dried and then blended into a fine powder. The mundu leaf powder was macerated for 24 hours using ethanol as a solvent. The residue was remacerated until all extract was obtained. The liquid extract was concentrated using a rotary evaporator at 78 °C with a rotation speed of 80 rpm until a dry extract was produced [10].

Qualitative Phytochemical Tests

Phenolic and Tannin Test: Mundu leaf extract was treated with 2–3 drops of ferric chloride ($FeCl_3$), resulting in a dark purple, blue, or black solution. Flavonoid Test: The extract was dissolved in methanol and mixed with magnesium powder, followed by the addition of concentrated hydrochloric acid until a red or orange solution appeared [11].

Antidiabetic Test

α-Glucosidase Inhibition Assay: A mixture of 100 µL potassium phosphate buffer (10 mM, pH 6.8), 20 µL α-glucosidase enzyme solution (1 U/mL), and 40 µL mundu leaf extract was added into wells of a 96-well microtiter plate. The mixture was incubated at 37 °C for 15 minutes. Then, 40 µL pNPG substrate solution (2 mM) was added and incubated for another 15 minutes. The reaction was terminated by adding 100 µL sodium carbonate solution (0.1 M), and absorbance was measured at 405 nm [12].

α-Amylase Inhibition Assay: A mixture of 40 µL extract and 40 µL starch solution (1%) was placed in a 1.5 mL microtube and incubated at 25 °C for 10 minutes. After incubation, 40 µL α-amylase enzyme solution (0.5 mg/mL) was added, followed by a second incubation at 25 °C for 10 minutes. Next, 80 µL DNS solution (3,5-dinitrosalicylic acid) was added, and the mixture was heated at 100 °C for 5 minutes. The reaction was then cooled at 0 °C for 5 minutes. Subsequently, 50 µL of the reaction mixture and 200 µL distilled water were transferred into a 96-well microtiter plate, and absorbance was measured at 540 nm. A mixture without extract was used as a blank, a mixture without enzyme and extract as a negative control, and acarbose as a positive control .

3. RESULTS

3.1 Extract

Mundu leaves extract was obtained in gel form, weighing 250 g, after extraction using ethanol as the solvent. The extract yield was 5.5%, calculated by comparing the weight of the extract to the initial sample powder of 4.5 kg. Qualitative tests using chemical reagents confirmed the presence of phenolic compounds, tannins, and flavonoids in the mundu leaf extract, as presented in Table 1.

Table 1. Results of Qualitative Tests

Metabolites	Reagents	Results	Indications
Phenolics	$FeCl_3$ 5%	Black	+
Tannins		Black	+
Flavonoids	Mg Trace + concentrated HCl	Reddish	+

The phytochemical screening results indicated that the sample tested positive for phenolic compounds, tannins, and flavonoids. The reaction between 5% $FeCl_3$ and the sample produced a black color, confirming the presence of phenolic compounds. A similar black coloration was observed in the tannin test, indicating the existence of tannins as part of the phenolic group. Meanwhile, the addition of magnesium powder and concentrated hydrochloric acid produced a reddish color, signifying a positive result for flavonoids. These three compounds are secondary metabolites known for their antioxidant activity.

3.2 α-Glucosidase Inhibition Test

The inhibitory activity of mundu leaf extract against the α-glucosidase enzyme was measured using a UV-Visible spectrophotometer at a maximum wavelength of 405 nm. The results showed that as the concentration of mundu leaves extract increased, the absorbance decreased, while the percentage of inhibition increased. The percentage of inhibition was calculated by subtracting the absorbance of the sample from the control, dividing by the control absorbance, and multiplying by 100%. The relationship between the extract concentration and inhibition percentage produced a linear regression equation, which was used to determine the IC_{50} value. Overall, the results of the α-glucosidase inhibition assay are presented in Table 2.

Table 2. α-Glucosidase Enzyme Inhibition

Concentration	Average Absorbance	Percent of Inhibition
Control	0.7030	-
10 ppm	0.6067	13.6564
20 ppm	0.5409	23.0499
30 ppm	0.4864	30.8075
40 ppm	0.4181	40.5282

Acarbose	0.2752	60.8535
Linear Regression Equation	y = 0.8837x + 4.9173	
Correlation	0.9991	
IC_{50} Value	51.058 ppm	

3.3 α-Amylase Inhibition Test

The inhibitory activity of mundu leaf extract was analyzed using a UV-Visible spectrophotometer at a maximum wavelength of 515 nm. The results showed that the extract exhibited decreasing absorbance values accompanied by increasing inhibition percentages, as presented in Table 3.

Table 3. α-Amylase Enzyme Inhibition

Concentration	Average Absorbance	Percent of Inhibition
Control	0.7094	-
10 ppm	0.5061	28.6547
20 ppm	0.4450	37.2633
30 ppm	0.3776	46.7694
40 ppm	0.3049	57.0227
Acarbose	0.2213	70.3000
Linear Regression Equation	y = 0.9461x + 18.775	
Correlation	0.9992	
IC_{50} Value	33.004 ppm	

4. DISCUSSIONS

Table 2 shows that the absorbance value of the control (without extract) was 0.7030, which was higher than that of the extracts, indicating that mundu leaf extract was able to inhibit the enzyme in accordance with the increasing concentrations. The inhibition percentage of mundu leaf extract ranged from 13.6564 to 40.5282, compared to acarbose with an inhibition percentage of 60.385. The correlation coefficient of 0.9991 indicated a very strong relationship between concentration and inhibition percentage, confirming that the measurement was valid and highly reliable [13].

The linear regression equation y = 0.8837x+4.9173 was used to determine the IC_{50} value, where the IC_{50} of mundu leaf extract against α-glucosidase enzyme inhibition was found to be 51.058 ppm, categorized as strong. This strong IC_{50} value can be attributed to the presence of secondary metabolites such as flavonoids, phenolics, and tannins in the mundu leaf extract. An IC_{50} value of 51.058 ppm against α-glucosidase indicates that mundu leaf extract is capable of lowering blood glucose levels by slowing down carbohydrate hydrolysis. These results position mundu leaf extract as a promising natural antidiabetic candidate, although further comparative studies with standard drugs and additional assays are still required

Table 3 shows that the control solution without extract had an absorbance value of 0.7094. In contrast, the absorbance values of the extract series varied from 0.5061 to 0.2213, with corresponding α-amylase inhibition percentages ranging from 28.6547 to 57.0227. The relationship between extract concentration and inhibition percentage produced a linear regression equation of $y = 0.9461\ x + 18.755$ y=0.9461x+18.755 with a correlation coefficient of 0.9992. The analysis revealed an IC_{50} value of 33.004 ppm, indicating that the extract can act as an antidiabetic agent with a very strong category.

The results of research related to Dej-adisai *et al.* (2022) identified several flavonoids from the inflorescence of Streptocaulon stramonifolium and demonstrated strong α-glucosidase inhibition. The crude extract exhibited 97.98 ± 0.64% inhibition at 2 mg/mL, with an IC_{50} value of 81.27 µg/mL. This potent activity was attributed to

its high phenolic and flavonoid contents, particularly compounds such as kaempferol and astragalin, which showed mixed-type inhibition based on kinetic and molecular docking analyses [14]. Similarly, Aguila-Muñoz *et al.* (2023) reported that methanolic extracts of Rumex crispus L. possessed notable α-glucosidase inhibitory activity, where the flower extract had an IC_{50} of 7.3 ± 0.17 µg/mL, the leaf extract 112.0 ± 1.23 µg/mL, and its purified fraction (F89s) 3.8 ± 0.11 µg/mL. The extract also exhibited significant antihyperglycemic effects in diabetic rats and showed a strong correlation between total phenolic content and enzyme inhibition ($R \approx 0.946$ – 0.997) [15]. In the research of Rijai *et al*. (2023), five Litsea species from East Borneo were screened for α-amylase and α-glucosidase inhibition. Among them, Litsea firma demonstrated the highest activity with IC_{50} values of 52.1 ± 1.01 µg/mL for α-amylase and 55.21 ± 1.46 µg/mL for α-glucosidase. The authors concluded that the strong inhibitory effects were linked to the abundance of phenolic and flavonoid compounds, highlighting Litsea species as promising natural antidiabetic agents [16].

5. CONCLUSION

Mundu leaf extract was proven to act as an antidiabetic agent, with an IC_{50} value of 51.058 ppm in inhibiting the α-glucosidase enzyme, categorized as a strong antidiabetic agent. Meanwhile, the IC_{50} value from the α-amylase inhibition assay indicated that mundu leaf extract could act as a very strong antidiabetic agent, with an IC_{50} value of 33.004 ppm.

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