e-ISSN: 2746-5152

Eureka Herba Indonesia

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Potential of Ethanol Extract of Red Gedi Leaves (*Abelmoschus manihot* L. Medik) Against Endogenous Antioxidant Activity in Rat Model of Diabetes Mellitus

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ARTICLE INFO

Keywords:
Abelmoschus manihot
Antioxidant
Diabetes mellitus
Ethanol extract
Oxidative stress

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/ehi.v5i3.123

A B S T R A C T

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and increased oxidative stress, contributing to disease complications. Red gedi leaves (*Abelmoschus manihot* L. Medik) are rich in bioactive compounds, including tannins, saponins, alkaloids and flavonoids, with antioxidant potential. This study aims to evaluate the effect of ethanol extract of red gedi leaves on endogenous antioxidant activity in rat model of DM induced by STZ-NA. Male rats were induced with DM with streptozotocin (STZ)-NA. Rats were divided into five groups: normal control (KN), DM control (KDM), glibenclamide positive control (KPG), and group treated with red gedi leaf extract at a dose of 100 mg/kgBW (GEDI 100), 200 mg/kgBW (GEDI 200), and 400 mg/kgBW (GEDI 400). The parameters observed included blood glucose levels, malondialdehyde (MDA) as a marker of oxidative stress, as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in liver tissue. Data analysis used one-way ANOVA and Tukey's post hoc test ($p<0.05$). The results showed that the yield of ethanol extract from red gedi leaves was 6.984%. Administration of red gedi leaf extract at a dose of 400 mg/kgBW (GEDI 400) significantly reduced blood glucose levels ($p<0.05$) and MDA ($p<0.05$), and increased the activity of SOD ($p<0.05$), CAT ($p<0.05$), and GPx ($p<0.05$) in DM rats compared to the DM control group (KDM). The effect of GEDI 400 was comparable to the effect of glibenclamide as a positive control. The ethanol extract of red gedi leaves at a dose of 400 mg/kgBW was effective in increasing endogenous antioxidant activity and reducing oxidative stress in STZ-NA-induced DM rats, showing potential as an antioxidant and antidiabetic agent.

1. Introduction

Diabetes mellitus (DM) has become an alarming global epidemic, with prevalence continuing to increase significantly in recent decades. The World Health Organization (WHO) estimates that in 2045, the number of DM sufferers worldwide will reach 700 million people. In Indonesia itself, DM is one of the main causes of death and imposes a large economic burden on individuals, families, and the national health system. DM is characterized by chronic hyperglycemia, namely an increase in blood glucose levels that exceed normal limits. This condition is caused by impaired secretion or action of insulin, a hormone that plays a role in regulating glucose metabolism. There are two main types of DM, namely type 1 DM which is caused by autoimmune damage to insulin-producing pancreatic β cells, and type 2 DM which is caused by insulin resistance and a progressive decrease in insulin secretion. Chronic hyperglycemia in DM triggers various micro and macrovascular complications that can reduce quality of life and increase the risk of death. Microvascular complications include diabetic retinopathy (retinal damage), diabetic nephropathy (kidney damage), and diabetic neuropathy (nerve damage). Meanwhile, macrovascular complications include coronary heart

disease, stroke, and peripheral vascular disease.[1,2]

One of the main pathophysiological mechanisms underlying DM complications is oxidative stress. Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to neutralize them through the endogenous antioxidant system. Free radicals are highly reactive and unstable molecules because they have unpaired electrons. These molecules can damage various cellular components, including lipids, proteins, and DNA, which can ultimately lead to cell dysfunction and cell death. Excess glucose can bind to proteins and lipids to form advanced glycation end products (AGEs). AGEs can trigger the production of free radicals and activate inflammatory signaling pathways that contribute to tissue damage. Hyperglycemia triggers activation of the polyol pathway, in which glucose is converted to sorbitol by the enzyme aldose reductase. Activation of this polyol pathway increases the production of free radicals and reduces levels of glutathione, an important antioxidant in cells. Hyperglycemia also triggers increased fatty acid oxidation in mitochondria, which is the main source of free radical production in cells. Oxidative stress can damage mitochondria, cell organelles that play a role in energy production. Mitochondrial damage can lead to mitochondrial dysfunction and increased production of free radicals. Increased production of free radicals and decreased endogenous antioxidant capacity in DM sufferers cause oxidative stress which plays an important role in the pathogenesis and development of DM complications. Oxidative stress can damage pancreatic β cells, impair insulin secretion, and worsen insulin resistance. In addition, oxidative stress can also damage vascular endothelial cells, accelerate atherosclerosis, and increase the risk of macrovascular complications. Therefore, antioxidant therapy is a promising approach to treating DM. Antioxidant therapy aims to increase the body's endogenous antioxidant capacity to fight oxidative stress, thereby preventing or slowing the development of DM complications.[3,4]

The use of medicinal plants as a complementary therapy for DM is increasingly in demand because of minimal side effects and multi-target potential. Medicinal plants contain various bioactive compounds that can work synergistically to overcome various aspects of DM pathophysiology, including hyperglycemia, insulin resistance, and oxidative stress. One of the medicinal plants that has potential as a complementary therapy for DM is red gedi leaves (*Abelmoschus manihot* L. Medik). Red gedi leaves are a plant native to Southeast Asia that has long been used in traditional medicine to treat various diseases, including DM. Red gedi leaves are rich in bioactive compounds, such as flavonoids, tannins, saponins, and alkaloids, which have antioxidant, anti-inflammatory, and anti-diabetic activities. Red gedi leaves contain a combination of bioactive compounds that have potential as antioxidants and antidiabetics. The flavonoids, tannins, saponins, and alkaloids in red gedi leaves can work synergistically to overcome various aspects of DM pathophysiology. The antioxidant activity of red gedi leaves can help protect pancreatic β cells from oxidative damage, increase insulin sensitivity, and improve pancreatic β cell function. This can contribute to lowering blood glucose levels and preventing DM complications. Apart from that, the content of bioactive compounds in red gedi leaves can also provide anti-inflammatory, antihypertensive, and antihyperlipidemic effects, all of which are important risk factors in the development of DM and its complications. Several in vivo studies have shown the potential of red gedi leaf extract in controlling DM in animal models. Red gedi leaf extract is reported to reduce blood glucose levels, increase insulin sensitivity, and protect organs from damage due to DM. However, research on the effect of red gedi leaf extract on endogenous antioxidant activity in STZ-NA-induced DM animal models is still limited.[5-7] This study aims to fill this gap by evaluating the effect of ethanol extract of red gedi leaves on endogenous antioxidant activity in the rat model of DM induced by STZ-NA. This study aims to evaluate the effect of ethanol extract from red gedi leaves on endogenous

antioxidant activity in the rat model of DM induced by STZ-NA. The parameters observed included blood glucose levels, malondialdehyde (MDA) as a marker of oxidative stress, as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in liver tissue.

2. Methods

Research materials

Red gedi leaves: Fresh red gedi leaves are obtained from medicinal plant gardens in the Bogor area, West Java, Indonesia. The leaves selected are healthy leaves and are not attacked by pests or diseases. The leaves are then washed clean, dried in the sun for 7 days, and ground using a blender until they become powder. Streptozotocin (STZ): STZ was obtained from Sigma-Aldrich (St. Louis, MO, USA) and used to induce diabetes in rats. Nicotinamide (NA): NA was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was used to partially protect pancreatic β cells from STZ-induced damage. Glibenclamide: Glibenclamide was obtained from PT. Kimia Farma (Jakarta, Indonesia) and used as a positive control. Ethanol 96%: Ethanol 96% is used as a solvent in the extraction process of red gedi leaves. Other Chemicals: Other chemicals used in this research, such as thiobarbituric acid (TBA), glacial acetic acid, pyrogallol, potassium phosphate buffer, Tris-HCl buffer, H_2O_2, reduced glutathione (GSH), NADPH, and other reagents, were obtained from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA).

Research equipment

Blender: Used to grind dried red gedi leaves into powder. Rotary Evaporator: Used to evaporate ethanol solvent from red gedi leaf extract. UV-Vis Spectrophotometer: Used to measure absorbance in measuring MDA levels, SOD, CAT, and GPx activity. Glucometer: Used to measure blood glucose levels. Analytical Scales: Used to weigh chemicals and tissue samples. Homogenizer: Used to homogenize liver tissue. Cooling Centrifuge: Used to separate supernatant from liver tissue homogenate. Micropipette: Used to accurately transfer small volumes of liquid. Reaction Tubes: Used to carry out chemical reactions. Filter Paper: Used to filter red gedi leaf extract.

Preparation of ethanol extract from red gedi leaves

The red gedi leaf powder was macerated with 96% ethanol in a ratio of 1:10 (w/v) for 3 x 24 hours at room temperature. Every 24 hours, stir and replace with new solvent. The macerate obtained was collected and filtered using Whatman filter paper no. 1. The filtrate obtained was then evaporated using a rotary evaporator (Buchi, Switzerland) at a temperature of 40°C and reduced pressure until a thick extract was obtained. The thick extract is weighed to determine the extract yield. Extract yield is calculated using the formula:

Extract yield (%) = (Weight of condensed extract / Weight of simplicia powder) x 100%

The thick extract obtained was then stored in a tightly closed container at 4°C until used. The water content of red gedi leaf simplicia powder was determined using the gravimetric method by drying in an oven at a temperature of 105°C until constant weight. Water content is calculated using the formula:

Water content (%) = (Initial weight - Final weight) / Initial weight x 100%

Phytochemical tests are used to identify secondary metabolite compounds contained in extracts, such as alkaloids, flavonoids, saponins, and tannins.

Diabetes mellitus induction and trial design

Diabetes mellitus was induced in rats using streptozotocin (STZ) followed by administration of nicotinamide (NA). Rats were fasted for 12 hours before DM induction. Rats were injected with STZ at a dose of 65 mg/kg body weight (BW) intraperitoneally (i.p.). STZ will cause selective damage to pancreatic β cells, thereby reducing insulin production. After 14 days of STZ injection, rats were given NA at a dose of 230 mg/kgBW i.p. NA will protect some of the remaining pancreatic β cells, thereby preventing

severe type 1 diabetes. This STZ-NA combination will induce a condition of type 2 diabetes similar to humans, characterized by insulin resistance and a progressive decrease in insulin secretion. After 72 hours of STZ injection, fasting blood glucose levels were measured. Rats with fasting blood glucose levels >200 mg/dL are considered to have DM. After confirmation of DM, the rats were randomly divided into five treatment groups: Normal Control Group (KN): Normal rats without treatment, DM Control Group (KDM): DM rats without treatment, Glibenclamide Positive Control Group (KPG): DM rats were given a dose of glibenclamide 10 mg/kgBB orally every day for 28 days. Glibenclamide is a standard antidiabetic drug used as a comparison of the effectiveness of red gedi leaf extract. GEDI 100 group: DM rats were given red gedi leaf extract at a dose of 100 mg/kgBW orally every day for 28 days. GEDI 200 group: DM rats were given red gedi leaf extract. dose of 200 mg/kgBW orally every day for 28 days, GEDI 400 Group: DM rats were given red gedi leaf extract at a dose of 400 mg/kgBW orally every day for 28 days. Red gedi leaf extract and glibenclamide were administered every day at the same time for 28 days.

Blood glucose levels

Blood glucose levels were measured using the glucose oxidase method. The blood samples that have been taken are centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was then analyzed using a commercial glucose oxidase kit (Randox, UK) according to the manufacturer's instructions. Absorbance was measured at a wavelength of 505 nm using a spectrophotometer. Blood glucose levels are calculated based on a standard curve made from standard glucose solutions.

Malondialdehyde (MDA)

MDA levels in liver tissue were measured using the thiobarbituric acid reactive substances (TBARS) method with several modifications. Liver tissue was homogenized in potassium phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was taken and reacted with 0.67% thiobarbituric acid (TBA) in glacial acetic acid. The mixture was heated at 95°C for 60 minutes, then cooled and centrifuged again. The absorbance of the supernatant was measured at a wavelength of 532 nm using a spectrophotometer. MDA levels were calculated based on a standard curve prepared from 1,1,3,3-tetramethoxipropane (TMP).

Superoxide dismutase (SOD)

SOD activity in liver tissue was measured using the pyrogallol autooxidation inhibition method. Liver tissue was homogenized in potassium phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was taken and reacted with 0.2 mM pyrogallol in Tris-HCl buffer (pH 8.2). The increase in absorbance was measured at a wavelength of 420 nm for 3 minutes using a spectrophotometer. One unit of SOD activity is defined as the amount of enzyme required to inhibit the autoxidation rate of pyrogallol by 50%.

Catalase (CAT)

CAT activity in liver tissue was measured using the H_2O_2 decomposition method. Liver tissue was homogenized in potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was taken and reacted with 30 mM H_2O_2. The decrease in absorbance was measured at a wavelength of 240 nm for 1 minute using a spectrophotometer. One unit of CAT activity is defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per minute.

Glutathione peroxidase (GPx)

GPx activity in liver tissue was measured using the NADPH oxidation method. Liver tissue was homogenized in potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was taken and reacted with 1 mM H_2O_2, 1 mM reduced glutathione (GSH), and 0.2 mM NADPH in potassium phosphate buffer (pH 7.0). NADPH oxidation was measured at a wavelength of 340 nm for

3 minutes using a spectrophotometer. One unit of GPx activity is defined as the amount of enzyme that oxidizes 1 μmol NADPH per minute.

Statistical analysis

The data obtained were analyzed using SPSS version 25.0 statistical software (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). The data normality test was carried out using the Shapiro-Wilk test. The homogeneity of variance test was carried out using Levene's test. Differences between treatment groups were analyzed using a one-way ANOVA test, followed by the Tukey HSD (Honestly Significant Difference) post hoc test to see significant differences between each treatment group. Differences were considered significant if $p < 0.05$.

3. Results and Discussion

The results showed that giving ethanol extract of red gedi leaves (GEDI) to STZ-NA-induced diabetes mellitus (DM) rats for 28 days had an effect on blood glucose levels. On the 3rd day after DM induction, there was a significant increase in blood glucose levels in the KDM group compared with the KN group ($p<0.05$). Administration of GEDI at a dose of 400 mg/kgBW (GEDI 400) and glibenclamide (KPG) can significantly reduce blood glucose levels on day 28 compared to the KDM group ($p<0.05$), as shown in Table 1.

Table 1. Blood glucose levels (mg/dL).

Group	Day 0	Day 3	Day 28
KN	80 ± 5	85 ± 6	83 ± 4
KDM	82 ± 6	290 ± 20	275 ± 18
KPG	84 ± 5	285 ± 15	140 ± 10
GEDI 100	81 ± 4	280 ± 12	240 ± 15
GEDI 200	83 ± 7	288 ± 16	200 ± 12
GEDI 400	82 ± 6	292 ±18	160 ± 9

Table 2 shows the levels of MDA (malondialdehyde), a marker of oxidative stress, in the liver tissue of rats in various treatment groups. Rats in the normal control group (KN) group had the lowest MDA levels (2.0 ± 0.2 nmol/mg protein), indicating normal basal oxidative stress levels. Untreated diabetic rats (KDM) showed a significant increase in MDA levels (4.5 ± 0.3 nmol/mg protein) compared with the KN group ($p < 0.05$). This indicates an increase in oxidative stress in DM rats. DM rats treated with glibenclamide (KPG) showed a significant reduction in MDA levels (3.0 ± 0.2 nmol/mg protein) compared with the KDM group ($p < 0.03$). This shows that glibenclamide has an antioxidant effect in reducing oxidative stress. Administration of red gedi leaf extract at a dose of 100 mg/kgBW did not show a significant difference in MDA levels (4.0 ± 0.2 nmol/mg protein) compared to the KDM group ($p > 0.05$). Administration of red gedi leaf extract at a dose of 200 mg/kgBW showed a decrease in MDA levels (3.5 ± 0.2 nmol/mg protein) compared to the KDM group, but this difference was not statistically significant ($p > 0.05$). Administration of red gedi leaf extract at a dose of 400 mg/kgBW showed a significant reduction in MDA levels (3.0 ± 0.2 nmol/mg protein) compared to the KDM group ($p < 0.02$). This effect is comparable to that of glibenclamide.

Table 2. MDA level (nmol/mg protein).

Group	MDA level (nmol/mg protein)	p-value
KN	2.0 ± 0.2	-
KDM	4.5 ± 0.3	0.01
KPG	3.0 ± 0.2	0.03
GEDI 100	4.0 ± 0.2	0.08
GEDI 200	3.5 ± 0.2	0.2
GEDI 400	3.0 ± 0.2	0.02

Table 3 presents data on the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in rat liver tissue in various treatment groups. These enzymes are part of the endogenous antioxidant defense system which plays an important role in neutralizing free radicals and protecting cells from oxidative damage. Rats in the Normal Control Group (KN) had the highest SOD, CAT, and GPx activities, indicating optimal antioxidant function under normal conditions. Untreated diabetic rats (KDM) showed a significant decrease in SOD, CAT, and GPx activities ($p < 0.05$) compared with the KN group. This indicates a disruption in the antioxidant defense system due to diabetes, which can cause increased oxidative stress and cell damage. DM rats treated with glibenclamide (KPG) showed a significant increase in SOD, CAT, and GPx activities ($p < 0.05$) compared with the KDM group. This indicates that glibenclamide, as a standard antidiabetic drug, has a positive effect in increasing endogenous antioxidant activity and reducing oxidative stress in DM rats. Administration of red gedi leaf extract at a dose of 100 mg/kgBW showed an increase in SOD, CAT, and GPx activity compared to the KDM group, but this difference was not statistically significant ($p > 0.05$). This indicates that a dose of 100 mg/kgBW may not be effective enough in increasing antioxidant activity significantly. Administration of red gedi leaf extract at a dose of 200 mg/kgBW showed a higher increase in SOD, CAT, and GPx activity compared to GEDI 100, but this difference was also not statistically significant ($p > 0.05$). Administration of red gedi leaf extract at a dose of 400 mg/kgBW showed a significant increase in SOD, CAT, and GPx activity ($p < 0.05$) compared to the KDM group. This effect is comparable to the effect of glibenclamide, indicating that a dose of 400 mg/kgBW is an effective dose in increasing endogenous antioxidant activity and reducing oxidative stress in DM rats. The data in Table 3 shows that the ethanol extract of red gedi leaves (GEDI) has a positive effect in increasing endogenous antioxidant activity (SOD, CAT, and GPx) in the STZ-NA-induced DM rats model. This effect is dose-dependent, with a dose of 400 mg/kgBW (GEDI 400) showing the most significant effect and comparable to glibenclamide.

Table 3. Endogenous antioxidant activity.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
KN	20.0 ± 1.0	15.0 ± 0.5	71.92 ± 2.00
KDM	12.0 ± 1.0*	8.0 ± 0.5*	20.06 ± 1.22*
KPG	18.0 ± 1.0*	12.0 ± 0.5*	64.67 ± 3.36*
GEDI 100	14.0 ± 1.0*	9.0 ± 0.5*	28.40 ± 1.84 *
GEDI 200	16.0 ± 1.0*	10.0 ± 0.5*	39.82 ± 2.09 *
GEDI 400	18.0 ± 1.0*#	12.0 ± 0.5*#	62.05 ± 2.00 *#

*p value<0.05 VS KN; #p value>0.05 VS KPG.

Table 4 presents the results of characterization of the ethanol extract of red gedi leaves, including extract yield, the water content of simplicia powder, and phytochemical tests. The extract yield value shows the weight percentage of the thick extract obtained from red gedi leaf simplicia powder. The yield of 6.984% indicates that red gedi leaves contain a large number of bioactive compounds that can be extracted with ethanol solvent. This value is in line with other research that reports the ethanol extract yield of red gedi leaves ranging from 6-10%. The high extract yield shows the efficiency of the extraction process and the potential for utilizing red gedi leaves as a source of bioactive compounds for various applications, including as a raw material for herbal medicine. The water content of simplicia powder is an important parameter in determining the quality of simplicia. High water content can cause microbial growth and degradation of bioactive compounds in simplicia. The water content of red gedi leaf powder of 7.16% meets the requirements for good simplicia, namely no more than 10%. This shows that red gedi leaf simplicia powder has good quality, is stable, and is not easily damaged during storage. Phytochemical tests were carried out to identify the groups of secondary metabolite compounds contained in the ethanol extract of red gedi leaves. The results of the phytochemical test showed that the extract positively contained tannins, saponins, alkaloids, and flavonoids. Tannins are polyphenolic compounds that have antioxidant, anti-inflammatory, and antimicrobial activity. Tannins can inhibit the formation of free radicals, inhibit pro-oxidant enzymes, and protect cells from oxidative damage. Saponins are glycoside compounds that have antidiabetic, anti-inflammatory, and immunomodulatory activities. Saponins can increase insulin sensitivity, inhibit glucose absorption in the intestine, and increase insulin secretion. Alkaloids are organic compounds containing nitrogen with various biological activities, including antidiabetic and antioxidant activities. Alkaloids can increase insulin secretion, increase insulin sensitivity, and inhibit glucose absorption in the intestine. Flavonoids are polyphenolic compounds that have antioxidant, anti-inflammatory, and anti-diabetic activities. Flavonoids can scavenge free radicals, inhibit pro-oxidant enzymes, and protect cells from oxidative damage. The presence of these bioactive compounds in the ethanol extract of red gedi leaves supports its potential use as an antioxidant and antidiabetic agent.

Table 4. Extract yield, water content, and phytochemical tests.

Parameter	**Results**
Extract yield (%)	6.984
Powder water content (%)	7.16
Phytochemical test	
Tannin	+ (Positive)
Saponin	+ (Positive)
Alkaloid	+ (Positive)
Flavonoid	+ (Positive)

The significant reduction in blood glucose levels in the group of rats given 400 mg/kgBB GEDI showed a promising antidiabetic effect. This effect can be explained through several potential mechanisms involving bioactive compounds in red gedi leaf extract, namely flavonoids, tannins, saponins, and alkaloids. Flavonoids are a group of polyphenolic compounds that are abundant in red gedi leaves. This compound has been shown to have antidiabetic activity through various mechanisms, including increasing insulin sensitivity and improving pancreatic β-cell function. One of the main mechanisms by which flavonoids

increase insulin sensitivity is through modulation of the insulin signaling pathway. Flavonoids can activate protein kinase B (Akt), which is a key component in the insulin signaling pathway. Activated Akt will phosphorylate the insulin receptor substrate (IRS), thereby increasing the translocation of glucose transporter type 4 (GLUT4) to the cell plasma membrane. GLUT4 plays a role in facilitating the entry of glucose into cells, thereby increasing glucose utilization and reducing blood glucose levels. In addition, flavonoids can also increase GLUT4 gene expression through activation of the transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ). PPAR-γ is a nuclear receptor that plays an important role in the regulation of glucose and lipid metabolism. Activation of PPAR-γ by flavonoids can increase GLUT4 gene transcription, thereby increasing the amount of GLUT4 in the plasma membrane and increasing glucose uptake by cells. Tannins are a group of polyphenolic compounds which are also abundant in red gedi leaves. This compound has the ability to inhibit the activity of the enzymes α-glucosidase and α-amylase, which play a role in the digestion of carbohydrates in the small intestine. α-glucosidase is the enzyme responsible for hydrolyzing oligosaccharides into glucose, while α-amylase is the enzyme responsible for hydrolyzing polysaccharides into oligosaccharides. By inhibiting the activity of these two enzymes, tannins can slow down carbohydrate digestion and reduce glucose absorption in the small intestine, thereby reducing blood glucose levels after eating.[8-10]

Saponins are a group of glycoside compounds that have a triterpenoid or steroid structure. This compound has been shown to have antidiabetic activity through various mechanisms, including increased insulin secretion and increased insulin sensitivity. One of the main mechanisms by which saponins increase insulin secretion is through increasing intracellular calcium levels in pancreatic β cells. Saponins can open calcium channels in the plasma membrane of pancreatic β cells, thereby increasing calcium entry into the cells. An increase in intracellular calcium will trigger the release of insulin from the secretory vesicles of pancreatic β cells. In addition, saponins can also increase insulin gene expression through activation of the transcription factor PPAR-γ. Activation of PPAR-γ by saponins can increase insulin gene transcription, thereby increasing insulin production by pancreatic β cells. Alkaloids are a group of organic compounds that contain nitrogen in their heterocyclic structure. Several alkaloids have been reported to have antidiabetic activity by inhibiting gluconeogenesis, namely the process of forming glucose from non-carbohydrate sources in the liver. Gluconeogenesis is one of the main mechanisms that contributes to increasing blood glucose levels in DM sufferers. By inhibiting gluconeogenesis, alkaloids can help lower blood glucose levels and control hyperglycemia.[11-13]

The decrease in MDA levels and increase in the activity of the antioxidant enzymes SOD, CAT, and GPx in the group of rats given 400 mg/kgBB GEDI shows the strong antioxidant effect of red gedi leaf extract. This effect can be explained through several potential mechanisms involving bioactive compounds in red gedi leaf extract. Flavonoids are one of the most powerful antioxidant compounds found in nature. This compound has the ability to donate hydrogen atoms or electrons to free radicals, thereby neutralizing free radicals and preventing oxidative damage to cells. Apart from that, flavonoids can also inhibit the activity of pro-oxidant enzymes, such as xanthine oxidase and lipoxygenase, which are involved in the production of free radicals. By inhibiting these enzymes, flavonoids can reduce the production of free radicals and protect cells from oxidative damage. Tannin is a polyphenolic compound which has the ability to bind iron and other heavy metals. Iron and other heavy metals can act as catalysts in the Fenton reaction, which produces highly reactive hydroxyl radicals and can cause lipid peroxidation. By binding iron and other heavy metals, tannins can inhibit the Fenton reaction and reduce the production of hydroxyl radicals. In addition, tannins can also inhibit the activity of the lipoxygenase enzyme, which is involved

in lipid peroxidation. By inhibiting lipid peroxidation, tannins can protect cell membranes from oxidative damage.[14-16]

Alkaloids are organic compounds that contain nitrogen in their heterocyclic structure. Some alkaloids have been reported to have antioxidant activity by inhibiting protein oxidation. Protein oxidation can cause damage to protein structure and function, which can disrupt various cellular processes. By inhibiting protein oxidation, alkaloids can protect cells from oxidative damage and maintain normal cell function. Apart from acting as a free radical scavenger and inhibitor of oxidative reactions, red gedi leaf extract can also increase the activity of endogenous antioxidant enzymes, such as SOD, CAT, and GPx. SOD is the enzyme responsible for converting superoxide radicals into hydrogen peroxide (H_2O_2). CAT and GPx then play a role in decomposing H_2O_2 into water (H_2O) and oxygen (O_2), thereby preventing the formation of more reactive hydroxyl radicals. The increase in SOD, CAT, and GPx activity in the group of rats given GEDI shows that red gedi leaf extract can increase the body's endogenous antioxidant capacity in fighting oxidative stress. Bioactive compounds in red gedi leaf extract, such as flavonoids, can activate the Nrf2 transcription factor which plays a role in increasing the expression of endogenous antioxidant genes, including SOD, CAT, and GPx. The bioactive compounds in red gedi leaf extract can also protect antioxidant enzymes from oxidative damage, thereby increasing the stability and activity of these enzymes. Several bioactive compounds in red gedi leaf extract, such as vitamin C and vitamin E, can act as cofactors in antioxidant enzymatic reactions, thereby increasing the efficiency of the enzyme's work.[17-19]

The antioxidant and antidiabetic effects of red gedi leaf extract are not only caused by one bioactive compound, but by a synergistic combination of various bioactive compounds contained therein. The flavonoids, tannins, saponins and alkaloids in red gedi leaf extract can work together through various mechanisms to provide protective effects against oxidative stress and hyperglycemia. For example, flavonoids and tannins can work together as free radical scavengers and lipid peroxidation inhibitors. Flavonoids can also increase insulin sensitivity, while tannins can inhibit glucose absorption in the intestine. Saponins can increase insulin secretion and protect pancreatic β cells from oxidative damage, while alkaloids can inhibit gluconeogenesis. The combination of synergistic effects of various bioactive compounds makes red gedi leaf extract a potential antioxidant and antidiabetic agent. This also explains why red gedi leaf extract has a stronger effect compared to single bioactive compounds isolated from red gedi leaves.[19,20]

4. Conclusion

Red gedi leaf ethanol extract (GEDI) shows promising potential as an antioxidant and antidiabetic agent in the STZ-NA-induced DM rat model. GEDI is effective in lowering blood glucose levels, reducing oxidative stress, and increasing the activity of endogenous antioxidant enzymes. This effect is thought to be related to the content of bioactive compounds in GEDI, such as flavonoids, tannins, saponins, and alkaloids.

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