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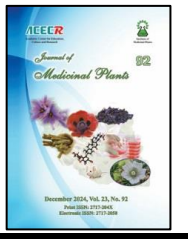

Research Article

Antioxidant activity of dandang gendis leaf extracts *Clinacanthus nutans* L. ethyl acetate solvent on free radical inhibition and increasing number of pancreas beta cells

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ABSTRACT

Background: Excessive free radicals can cause oxidative stress, which is one of the main factors of pancreatic beta cell damage, thus contributing to the development of diabetes. Dandang Gendis leaves (*Clinacanthus nutans* L.) are known to contain active compounds that have potential as antioxidants. **Objective:** This study aims to reveal the antioxidant effect, expression of pancreatic β cells and to screen the phytochemical obtained of dandang gendis. **Methods:** The dandang gendis leaf extract were screen the phytochemical, test the antioxidant activity, analyze flavonoid compounds contained in dandang gendis leaves using FT-IR, and determine the increase in the number and expression of pancreatic β cells due to alloxan induction by histopathological examination of pancreatic β cells. The sample was divided into 5 groups: healthy control, alloxan only, alloxan with Dandang Gendis at 50 mg/kg BW (M50), 100 mg/kg BW (M100), 150 mg/kg BW (M150), and alloxan with glibenclamide (MG). **Results:** The results of the phytochemical screening of dandang gendis leaf extract with ethyl acetate solvent contained flavonoids, alkaloids, steroids, saponins, terpenoids, tannins, and phenolics. Antioxidant activity is moderately active, with an IC_{50} value of 160 mg/L. FT-IR analysis containing the functional groups OH, CH aliphatic, C=C aromatic and C-O indicated that this isolate is a flavonoid compound. At a concentration of 100 mg/kg BW of mice, dandang gendis leaf extract worked optimally in increasing the expression of pancreatic β cells. **Conclusion:** It can be concluded that the dandang gendis leaf extract showed antioxidant activity and increased the expression of pancreatic β cells.

1. Introduction

Degenerative diseases have become a problem for every country worldwide and are the most significant cause of death [1]. One of the degenerative diseases caused by forming free radicals is reactive to cells and tissues.

Abbreviations: FT-IR, Fourier Transform Infrared Spectroscopy; IC_{50}, Half-maximal inhibitory concentration; BW, Body Weight; ROS, Reactive Oxygen Species; Abs, Absorbance; TLC, Thin-Layer Chromatography; HSD, Honestly Significant Difference

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Therefore we need a compound that can inhibit the formation of excess free radicals in the body, namely antioxidants [1].

Antioxidants are compounds that can inhibit and prevent the oxidation process [2]. The way it works is to stop free radical reactions from metabolism in the body or the environment [3]. Antioxidants come from synthetic and natural. Conditions in which the number of free radicals in the body is greater than the number of antioxidants are called oxidative stress. Prolonged oxidative stress results in damage to cells and tissues and also continues to damage organs. Organ damage due to oxidative stress can trigger various diseases, such as stroke, heart disease, diabetes mellitus, kidney disorders and cancer.

Free radicals are molecules, atoms or groups with one or more unpaired electrons in their outer shell, making them highly reactive radicals, such as reactive oxygen species (ROS). There are many types of free radicals, but those that are most abundant in the body's biological systems are reactive oxygen species (ROS) and reactive nitrogen species (RNS) [4]. ROS formation will cause depolarisation of the beta cell membrane and an increase in Ca2+. The cytosol will activate various enzymes that cause lipid peroxidation, DNA fragmentation, and protein fragmentation. As a result, pancreatic beta cells will be destroyed, so their function for insulin synthesis and secretion decreases [5].

Antioxidants are chemical compounds that can donate one or more electrons (electron donors) to free radicals to inhibit free radical reactions. This compound has a small molecular weight but can inactivate the development of oxidation reactions by preventing the formation of radicals [6].

Natural antioxidants can protect the body against damage caused by reactive oxygen species, inhibit degenerative diseases, and inhibit lipid peroxidase in food [7]. Flavonoid compounds are known to play a role in capturing free radicals or as natural antioxidants [8].

South Sulawesi, one of the provinces in Eastern Indonesia, has various types of plants that may have pharmacological properties and effects that are very beneficial for the development of alternative medicine. Research on the pharmacological activities of plants found in South Sulawesi needs to be carried out as one of the exploration efforts for the treatment of natural ingredients so that, in this way, many plants will be identified and have complete scientific data. One of the plants originating from South Sulawesi that has the potential to contain antioxidants is Dandang gendis leaves. It was confirmed through research showing that dandang gendis leaf (*Clinacanthus nutans* L.) extract contains flavonoid compounds [9]. It was also reinforced through research which showed that dandang gendis leaf extract contained flavonoids, steroids/triterpenoids, and tannins which reduced blood glucose levels in mice with the greatest glucose tolerance method compared to other fractions [10].

This study aims to screen phytochemical tests, test antioxidant activity, analyse flavonoid compounds contained in dandang gendis leaves using FT-IR, and determine the increase in the number of cells and expression of pancreatic β due to alloxan induction.

2. Materials and methods

This research was carried out from November 2021 to September 2022 at the organic laboratory of FST UIN Alauddin Makassar, GG Makassar Clinical Laboratory and the Maros Veterinary Center. The equipment used is a microwave (kirin), rotary

vacuum evaporator (Buhci), microwave (kirin), analytical balance (kernt), pipettes, cutters, capillary pipes, aluminium foil, various sized beakers (Pyrex), various measuring pipettes. (Pyrex), micropipette (Watson), test tube, stative and clamp, small 5 mL vial (ampoule), Shimadzu UV lamp λ 254 nm and λ 366 nm, hot plate, spectrophotometer UV/Vis (Varian), spectrophotometer FT- IR (Thermo Scientific), vortex mixer (Felp Arec), histopathological examination tool for Routine Haemotoxyllin Eosin staining, binocular microscope, and camera.

The materials used in this study were dandang gendis leaves (*C. nutans*) taken from the Poltekkes Kemenkes Makassar. *C. nutans* plant voucher specimens (leaves) were collected in South Sulawesi, Indonesia, on January 15, 2024. The sampling location was at the herb garden of UIN Alauddin campus, Gowa Regency, South Sulawesi, and the plant was morphologically identified under the supervision of a botanist from the Poltekkes Kemenkes Makassar. The voucher specimen is deposited in the herbaria of the Poltekkes Kemenkes Makassar with voucher code CN-SS-1501-2024.

Organic solvent ethyl acetate p.a (E.Merck), distilled water, Liberman-Burchard reagent, Wagner, Dragendorf, 1% FeCl3, 5% FeCl3, ascorbic acid, DPPH, Folin-Ciocalteu reagent, citrate buffer pH 4.5, distilled water, aqua pro injection (Otsuka), methanol (E. Merck), alcohol (E. Merck), formalin buffer, xylol, paraffin, acetone (E. Merck), diethyl ether (E. Merck), Na.CMC, STZ ALX 350 -010 from ALEXIS Corporation, animal feed (H II-B). Histopathological material stained with Hematoxylin Eosin and immunohistochemical staining. Bovine Serum Albumin (BSA), KCI, KH2PO4, NaCl, Na2HPO4, H2O, NaOH, Tween, NaNO3 (E. Merck), antibodies, Mouse insulin.

2.1. Extraction of C. nutans leaves

500 g of sample powder was extracted by cascading maceration using a microwave with a power of 125 W for 5 minutes. Samples were macerated first with n-hexane solvent, then ethyl acetate than with ethanol. The maceration results were filtered and concentrated using a rotary vacuum evaporator to obtain a thick extract [11]. The condensed extract was then tested for phytochemicals, and antioxidants, characterised using FTIR and tested on pancreatic β cells.

2.2. Identification of active compounds in Dandang Gendis leaves based on phytochemical screening

Phytochemical screening testing uses a colour reaction with the appropriate reagent. In the alkaloid test, 2 mL of the extract solution is added to 5 mL of 2N HCl, then three drops of Dragendroff reagent are added, and an orange precipitate forms [11]. The saponin test can be detected by foam test in hot water. Stable foam will continue to appear for 5 minutes and does not disappear with adding one drop of HCl 2 N, indicating the presence of saponins [12]. Tannin test. As much as 2 mL of the extract solution is added to 3-5 drops of 1% Iron (III) Chloride solution. The changes that occur are observed. The formation of dark blue or greenish black indicates the presence of tannin compounds [13]. Flavonoid Test, As much as 2 mL of extract solution, boil for 5 minutes. Then, a little Mg powder and 1 mL of concentrated HCl was shaken. A positive test is indicated by the formation of red, yellow or orange [14]. Test Steroids. 1 ml extract + 10 ml concentrated H2SO4 will form a reddish solution [15].

2.3. Antioxidant activity test of the thick extract of C. nutans Leaves

Furthermore, the antioxidant activity was tested using DPPH with an ascorbic acid standard. The standard series of ascorbic acid used to measure antioxidant capacity are two ppm, 6 ppm, 10 ppm, 12 ppm in ethanol solvent.

The sample concentration measured was 200 ppm. In addition to measuring the antioxidant capacity, IC_{50} measurements were also carried out. The series used in the IC_{50} measurement for ethyl acetate extract is 200 ppm, 400 ppm, 600 ppm and 800 ppm. The addition of DPPH is done with a ratio of 1:3. Absorbance measurements were carried out at λ 517 nm.

The antioxidant activity test was carried out in duplicate. Free radical scavenging activity was calculated as the percentage of DPPH colour reduction. Antioxidant capacity and (per cent inhibition) to inhibit free radicals according to [16] are determined by the equation:

"% inhibition"=(control abs-sample abs)/(control abs) ×100%

Information:

Abs Control = absorbance of the control solution at a wavelength of 517 nm

Abs Sample = absorbance of the test solution or reference solution at a wavelength of 517 nm.

From the percentage of free radical scavengers, a curve is made between the per cent of free radical scavengers and the concentration of the test solution. From the linear regression equation, the IC_{50} value is determined, namely the test solution's inhibitory concentration, which can ward off 50% of free radicals. The calculation results are entered in a linear equation with the equation:

$Y = aX+b$

Information:

Y =% Inhibition

a = Gradient

X = Concentration (µg/ml)

b = Constant

The resulting linear equation is used to obtain the IC_{50} value. The IC_{50} value is the concentration obtained when the% inhibition is 50 from the equation Y=aX+b. When% Inhibition = 50, the formula for calculating the IC_{50} value becomes 50 = aX+b.

2.4. FT-IR Analysis

The purity of the ethyl acetate extract resulting from column chromatography was tested by two-dimensional thin-layer chromatography using several eluents. If the isolate still shows a single spot pattern, it can be said that it is pure [16]. We then identified pure isolates using FT-IR.

2.5. Histopathological examination of pancreatic β cells

The study used 24 mice (*Mus musculus*) which were divided into 6 groups, namely Group MH (healthy control = given Na CMC 1% b/v), Group MS (negative control = sick control = induction of alloxan 50 mg/kgBB), M50 group (alloxan induction 50 mg/kgBB + 50 mg/kgBB), M100 group (alloxan induction 50 mg/kgBB + 100 mg/kgBB), M150 group (alloxan induction 50 mg/kgBB + 150 mg/kgBB) and MG group (positive control = alloxan induction 50 mg/kgBB + glibenclamide 0.65 mg/kgBB). There were 24 mice that met the inclusion criteria, on the first day they were taken randomly and then put into 6 groups (MH, MS, M50, M100, M150, MG). Each group consisted of 4 mice and were housed according to the requirements, fed approximately 10% of their body weight and given to drink approximately 15-30 mL of water per day. Mice were acclimatized for 7 days, after 7 days the blood sugar levels and body weight of the mice were measured. Furthermore, 5 groups (MS, M50, M100, M150, MG) were induced with alloxan 50 mg/kg BW intravenously for 3 days, then incubated for 7 days. After the incubation period, all mice in all groups were fed.

Histopathological examination of pancreatic *β* cells was carried out six times in the MH, MS, M50, M100, M150, and MG groups. The MH group was healthy; glucose levels remained stable without alloxan induction. The MS, M50, M100, M150 and MG groups were induced with Alloxan at 50 mg/Kg BW. For the MS group, after being induced by Alloxan with a dose of 50 mg/Kg BW, there was no intervention with EDDG. It was carried out utilising a cervical dislocation where previously anaesthesia was carried out with dieter ether, according to the group. Pancreatic tissue was taken, preparations were made, and then a histopathological examination was carried out with Hematoxylin Eosin and staining to examine the number of pancreatic *β* cells quantitatively. Afterward, immunohistochemical staining for insulin expression examination [17].

3. Results

3.1. Extraction results of C. nutans leaves

The results of *C. nutans* leaf extraction can be seen in Table 1. Selection of 125 W power for 5 minutes based on research by Qun Yu et al. in 2017, which extracted *C. nutans* leaves using a water solvent. Several other studies support primarily low power ranging from 50-200 W with a time range of 75 seconds to 5 minutes [18]. The yield of crude extract using non-polar solvents was higher than polar and semipolar solvents.

Table 1. The yield of the crude extract of the leaves of *C. nutans*

Solvent	Power (W)	Time (Minutes)	Yield (%)
Etil Asetat	125	5	1.24

4. Discussion

4.1. The yield of the crude extract of the leaves of C. nutans

The results of *C. nutans* leaf extraction can be seen in Table 1. The selection of 125 W power for 5 minutes was based on research that extracted *C. nutans* leaves using water as a solvent [18]. Several other studies support primarily low power ranging from 50-200 W with a time range of 75 seconds to 5 minutes [18].

4.2. Phytochemical Screening

The bioactive compounds in the leaf extract of dandang gendis are flavonoids, alkaloids, steroids, saponins, terpenoids, tannins and phenolics and antioxidants based on the results of the phytochemical screening at the beginning of the study (Table 2). It follows the previous reported research that in dandang gendis leaves, there are alkaloid compounds, saponins, and essential oils [18]. The function of each of these bioactive compounds, namely flavonoids, especially in the form of glycosides, has a sugar group that acts as a hydroxyl radical binder and inhibits the action of Alloxan. Flavonoids also have the ability as antioxidants [19]. Flavonoids act as hydroxyl radicals, inhibiting glucose reabsorption in the kidneys and increasing the solubility of glucose in the blood so it can be excreted in the urine. Antioxidants can inhibit beta cell death without altering pancreatic beta cell proliferation. Antioxidants can reduce insulin resistance by scavenging free radicals [20]. Antioxidants can also increase the number of beta cells by replacing the remaining beta cell progenitor cells that differentiate and replicate into normal pancreatic beta cells [21, 22].

Table 2. Results of Phytochemical Screening

Fraction	Test Phytochemicals	Reactor	Change with reagents	Results test
Ethyl acetate extract	Flavonoids	Mg-HCl	Brownish green	+
	Alkaloids	Dragendorff	Precipitate formed.	+
	Steroids	Salkowski's test	Brown in colour, and a green steroid ring is formed.	+
	Saponins	Aquadest	Formed foam	
	Terpenoid	2 ml etanol + 2 ml $CHCl_3$ + 3 ml H_2SO_4	Green to brick red	+
	Tannins	$FeCl_3$ dan gelatin	Precipitate formed	+
	Phenolic	FeCl3	Blue-black	+

4.3. Test results of antioxidant activity with DPPH

The antioxidant activity test method using DPPH is simple and easy to evaluate the antioxidant activity of natural compounds. The antioxidant activity test was carried out by reacting the extract solution with the DPPH solution and then reading the absorbance at a wavelength of 517 nm. This method takes hydrogen atoms from antioxidant compounds by free radicals so that these free radicals capture one electron from antioxidant compounds [23-24].

This antioxidant activity test method uses UV-Vis spectrophotometry so that the value of free radical scavenging activity will be known, which is expressed by the IC_{50} (Inhibitory Concentration) value. The IC_{50} value is defined as the concentration of the test compound that can inhibit free radicals by 50%. The smaller the IC_{50} value, the higher the free radical inhibition activity. Table 3 shows the results of standard antioxidant activity measurements of ascorbic acid and dandang gendis leaf extract by spectrophotometry. Based on the calculation results, the IC_{50} value of ethyl acetate extract was obtained at 160 mg/L (Table 3).

Table 3. Test results of antioxidant activity with DPPH

No	Ingredient	IC_{50}(mg/L)
1	Ethyl acetate	160
2	Ascorbic acid	9

A substance has antioxidant properties if the IC_{50} value is 100-1000 µg/mL. Therefore, ethyl acetate extract is quite active and has the potential as an antioxidant agent [24]. The positive control used in this study was vitamin C. If the IC_{50} value of the sample is the same or close to the IC_{50} value of the vitamin C, it can be stated that the sample has the potential as a robust antioxidant alternative. From the calculation results, the IC_{50} value of vitamin C is 9 mg/L. In comparison, the IC_{50} value of the ethyl acetate extract is 160 mg/L, so the ethyl acetate extract of dandang gendis leaves has weak antioxidant activity compared to vitamin C. As for the n-hexane and ethanol have fragile antioxidant activity. Based on the phytochemical screening, the ethyl acetate extract contains phenolic compounds, which can act as compounds that inhibit free radicals through hydroxyl groups which can donate their hydrogen atoms to free radicals. The weak antioxidant activity of phenolic compounds in the ethyl acetate extract is caused by the phenolic compounds present in the ethyl acetate extract in an impure state. The phenolic compounds in the ethyl acetate extract are suspected to bind to the glycoside groups. Therefore, the glycosides bound to the phenolic samples are first hydrolysed to increase the antioxidant activity of the phenolic

compounds—the reaction between DPPH radicals and antioxidant compounds [25].

4.4. Results of the FT-IR method of flavonoid test

As much as 10 g of methanol extract was successfully separated by column chromatography using silica gel GF60 stationary phase and n-hexane: ethyl acetate mobile phase.

The results of column separation obtained 48 fractions. Then all fractions were subjected to combined TLC.

Based on the thin layer chromatography analysis results, one fraction was obtained from 48 fractions, considering that these fractions showed the same stain pattern with good separation. In addition, this fraction still shows six spots. It means that this isolate is thought to be not pure, so it needs to be separated again by preparative thin layer chromatography using silica gel as the stationary phase and n-hexane: ethyl acetate as the mobile phase. A comparison of the analysis results shows that this fraction shows one spot.

The isolates were suspected to be pure before being identified with an IR spectrophotometer (Figure 1). The eluent ratio used in this analysis is n-hexane: ethyl acetate (8:2). The analysis results show that this isolate's stain pattern is single. I indicate that this isolate is pure.

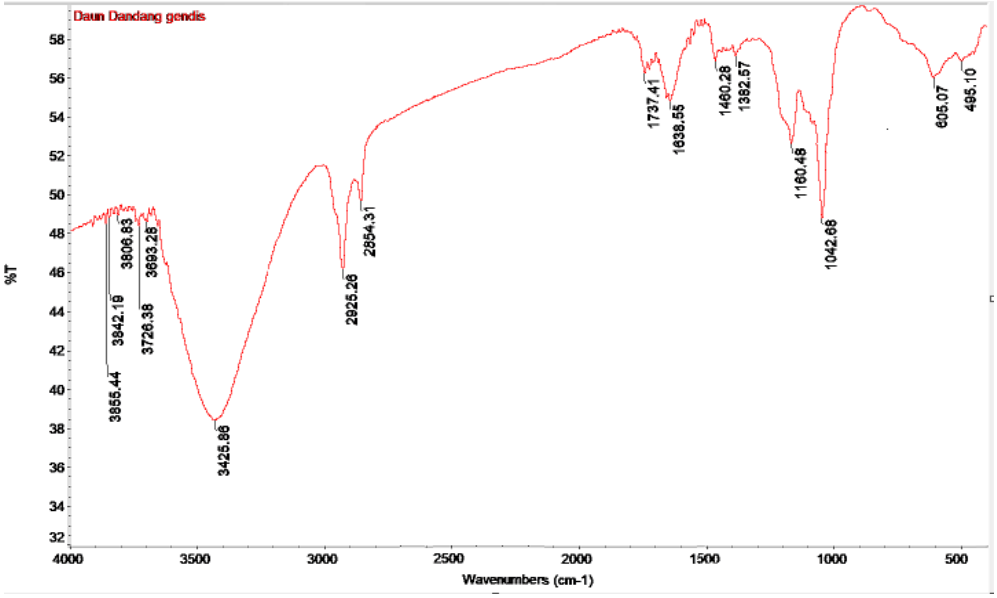

Fig. 1. Infrared Spectrophotometer

4.5. Infrared spectrophotometer results

Based on the infrared spectrum analysis in Figure 1 and Table 4 hows the presence of several functional groups. The analysis of this isolate results in a broad absorption with weak intensity in the wave number region of 3425.86 cm-1, which is suspected to be stretching absorption from the O-H group. Sharp and weak aliphatic C-H stretching absorptions appear in wave numbers 2925.26 cm-1 and 2854.31 cm-1. The study's results support that absorption at wave number 2927.36 cm-1 shows C-H stretching vibrations in aliphatic C-H groups [9]. A carbonyl group (C=O), a general characteristic of flavonoid compounds [26], is indicated by absorption in the wave number region of 1737.41 cm-1 and 1638.55 cm-1. C=C aromatic stretching absorption appears in wave

numbers 1460.28 cm-1 and 1382.57 cm-1. Then the C-O stretching vibrations in phenol compounds produce strong bands in the 1260-1000 cm-1 region [27], and in this isolate, C-O absorption appears in the wave number region 1160.48 cm-1 with a weak band and 1042.68 cm-1 with a band sharp and robust.

Meanwhile, at wave number 605.07 cm-1, an aromatic C-H group is out of a plane. The presence of aliphatic OH, CH, aromatic C=C and C-O functional groups indicates that this isolate is a flavonoid compound. It was strengthened based on the results of research conducted by the results of the infrared spectrum for the presence of O-H, C=O, CO, aromatic C=C, and aliphatic C-H functional groups, which support that the isolate is positive for a flavonoid compound [9].

4.6. The result is an increase in the number of pancreatic β cells

Calculating the number of pancreatic beta cells in the 6 sample groups of mice (Table 5) and Figure 2 & 3) after being treated showed the results in table 6. The healthy control group (MH group) in this group were not given any treatment, and the average number of cells in his pancreatic *β* increased from the first day (H0) to the 14th day (D14) with an average value of 95.94 and a standard deviation of 4.36. For the positive control group, in which this group was only given Alloxan (MS group) without being given dandang gendis leaf extract, the average number of pancreatic *β* cells decreased from the first day (D0) to the 14th day (D14) with an average value of 55, 99 and a standard deviation of 14.87. Based on these conditions, it can be said that the administration of Alloxan to mice reduced the number of pancreatic *β* cells in these mice.

Table 4. Infrared Spectrophotometer Results

No	Wave Number (cm^{-1}) Isolat	Ribbon Shape	Intensity	Possible Functional Groups
1.	3425.86	Widened	Weak	Help O-H
2.	2925.26	Sharp	Weak	Help C-H
	2954.31		Weak	Aliphatic
3.	1737.41	Sharp	Weak	Help C=O
	1638.55	Sharp	Weak	
4.	1460.28	Sharp	Weak	Help C=C
	1382.57	Sharp	Weak	Aromatic
	1160.48	Sharp	Weak	C-O alcohol
5.	1042.68	Sharp	Weak	
6.	605.07	Sharp	Weak	C-H aromatics Ex. field

Table 5. Mean pancreatic *β*-cell insulin levels

	Mean pancreatic *β*-cell insulin levels					
	MH	**MS**	**M50**	**M100**	**M150**	**MG**
H0	92.16	70.15	68.17	70.70	69.30	70.77
H3	93.76	65.86	75.89	80.60	76.76	81.66
H7	95.73	50.19	80.73	85.73	80.73	87.73
H14	102.11	37.77	85.71	98.76	90.16	95.66

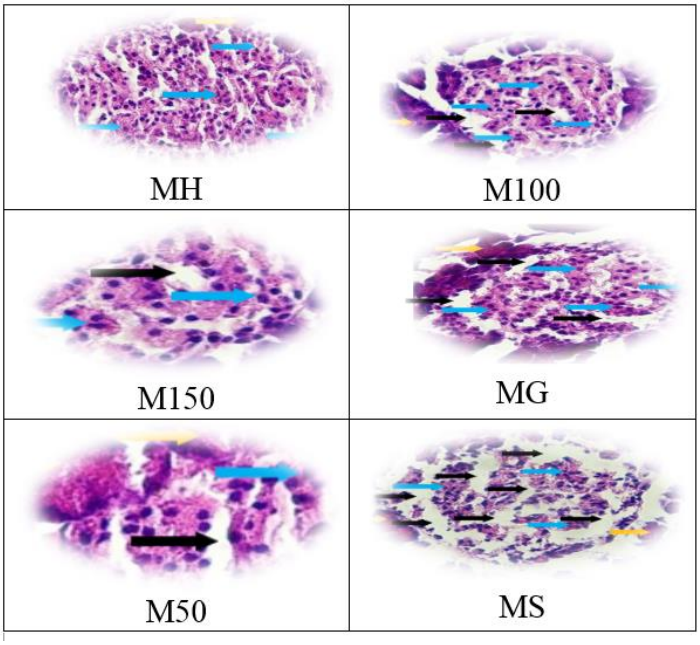

Fig. 2. Pancreatic β cell expression results

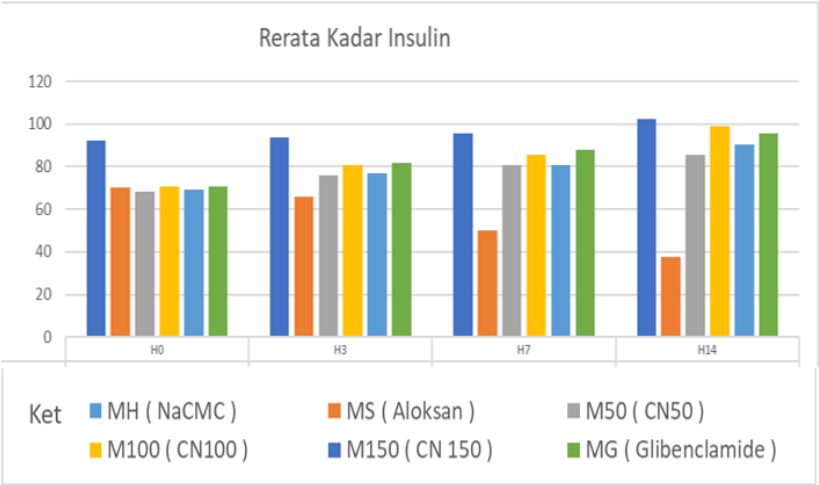

Fig. 3. Graph of average insulin levels

Table 6. The average of the calculation results of the number of pancreatic β cells with a standard deviation value

Group	Average	Standard Deviation
MH	95.94	4.36
MS	55.99	14.87
M50	77.62	7.47
M100	83.94	11.68
M150	79.23	8.68
MG	83.95	10.49

For the group of mice that were given Alloxan and supplemented with dandang gendis leaf extract at a dose of 50 mg/kg BW mice (group M50), there was an average increase in the number of pancreatic β cells that was not significant from day three (D3) to day 14 (H14)

with an average value of 77.62 and a standard deviation of 7.47. For the group of mice that were given Alloxan and supplemented with dandang gendis leaf extract at a dose of 100 mg/kg BW of mice (group M100), there was an average increase in the number of pancreatic β cells significantly from the third day (D3) to the 14th day (D14). With an average value of 83.94 and a standard deviation of 11.68.

For the group of mice that were given Alloxan and supplemented with dandang gendis leaf extract at a dose of 150 mg/kg BW mice (group M150), there was an average increase in the number of pancreatic β cells significantly from day three (D3) to day 14 (D14). With an average value of 79.23 and a standard deviation of 8.68. For the group of mice that were given Alloxan and added glibenclamide at a dose of 0.65 mg/kg, BB mice (MG group) experienced a significant increase in the average number of pancreatic β cells starting from the third day (D3) to the 14th day (D14) with an average value of 83.95 and a standard deviation of 10.49.

Alloxan induction at a dose of 50 mg/kg BW reduced the number of pancreatic β cells in all mice except the healthy control group, which was not given Alloxan. The decrease in the number of pancreatic β cells in mice is by the theory that administration of Alloxan for more than 1.5 minutes can cause diabetogenic [28]. The decrease in the number of pancreatic β cells in mice was evidenced by the decrease in the number of pancreatic β cells in the positive control group after being induced by Alloxan with a dose of 50 mg/kg BW repeatedly. However, an average number of pancreatic β cells in mice approached normal mice in the M100 and MG groups. Alloxan can produce animal models that slowly damage pancreatic beta cells, which will cause chronic DM [28]. Chronic hyperglycemia tends to increase the formation of free radicals (ROS) through glucose metabolism pathways such as glucose auto-oxidation, metabolism of methylglyoxal formation, and oxidative phosphorylation [29]. The formation of ROS can increase the modification of lipids, DNA, and proteins in various tissues [30]. This excess ROS increases the incidence of oxidative stress and can lead to beta cell dysfunction [29]. Dysfunction of pancreatic beta cells is caused by decreased growth in the number of pancreatic beta cells, increased apoptosis in pancreatic beta cells, and decreased regeneration and function of pancreatic beta cells, accompanied by genetic disorders of pancreatic beta cell mitochondria [28].

Advanced Diabetes is characterised by a decrease in the number of beta cells, accumulation of amyloid in the islets of Langerhans, and accumulation of fat [31]. Meanwhile, according to previous research, in diabetic mice, the number of beta cells was reduced by 50% compared to prediabetes [32]. In my previous research [28], repeated induction of low doses of Alloxan can reduce the number of pancreatic β cells in mice. Giving dandang gendis leaf extract as an antioxidant in preventing further damage to pancreatic β cells has increased the number of alloxan-induced pancreatic β cells in mice.

4.7. The effect of dandang gendis leaf extracts on pancreatic β cell expression

The results of the preparations of the pancreas organs of mice divided into six groups (Fig. 2) showed changes in the histopathological preparations of mice treated with dandang gendis leaf extract. The group of healthy mice (MH) had the best morphological arrangement of the islets of Langerhans because Alloxan did not induce them. The group of mice as negative control (MS) not given dandang gendis leaf

extract therapy had an irregular cell morphology arrangement, and a lot of cell degeneration occurred. The mice given dandang gendis leaf extract therapy at a dose of 100 mg/kg BW (M100) were given dandang gendis leaf extract at a dose of 150 mg/kg BW (M150). They were given glibenclamide therapy (positive control) at a dose of 0.65 mg/kg. Kg BW (MG) looks almost the same where the shape of the cell nucleus is round, the morphological shape is balanced, and there are almost no cavities or gaps in the Langerhans islands, indicating cell regeneration.

Based on the results of the post test, the mean body weight of mice between the MH group and the MS group was significantly different with a value of $P < 0.05$ (Table 7). Between the MH group and the M50, M100, M150, and MG groups were not significantly different with a value of $P > 0.05$. Between the MS group and the M50, M100, M150, and MG groups were not significantly different with a value of $P > 0.05$. Between the M50 group and the MS, M100, M150, and MG groups were not significantly different with a value of $P > 0.05$. Between the M100 group and the MS, M50, M150, and MG groups were not significantly different with a value of $P > 0.05$. Between the M150 group and the MS, M50, M100, and MG groups were not significantly different with a value of $P > 0.05$. Between the MG group and the MS, M50, M100, and M150 groups, there was no significant difference with a value of $P > 0.05$. From the SPSS data, it shows that EDDG intervention in the MH group has an effect on increasing the body weight of mice induced with alloxan and for groups M50, M100, M150 and MG has no effect on increasing the body weight of mice induced with alloxan.

The group of mice that were given dandang gendis leaf extract therapy at a dose of 50 mg/kg BW (M50) showed improvements, but the improvements that occurred were not as good as in the M100, M150, and MG groups. There were still gaps or cavities in the islets of Langerhans but not as many as in the MS group. Giving dandang gendis leaf extract to mice can increase the expression of pancreatic β cells, thereby improving pancreatic histopathology. It is presumably due to the presence of active antioxidant substances in the form of flavonoids, alkaloids, steroids, saponins, terpenoids, tannins, and phenolics in dandang gendis leaf extract. Giving dandang gendis leaf extract, which contains antioxidant compounds, can repair pancreatic organ cells damaged by alloxan administration. The cells will experience regeneration, although not at a fast time. The results of observations of pancreatic histopathological preparations in mice showed that the administration of dandang gendis leaf extract inhibited damage to the islets of Langerhans cells in the pancreas but could not significantly repair pancreatic damage in a short time. Antioxidants will work to neutralise free radicals present in mice by completing the electron deficiency possessed by free radicals [31]. Antioxidants also play a role in repairing cell damage caused by free radicals. Antioxidants will act as reducing compounds and reduce oxidising agents before damaging cells so that cell damage can be inhibited. Antioxidants can protect some pancreatic β cells from remaining normal so that they can stimulate the process of cell regeneration, increasing the number of pancreatic β cells [32].

Table 7. Results of the multiple comparison test using the Tukey HSD to compare the mean and significance values of the test groups (Statistical results)

Method	Sample	Sample	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	MH (NaCMC)	MS (Aloksan)	7.80500*	1.76863	0.004
		M50 (CN50)	4.63750	1.76863	0.142
		M100 (CN100)	2.79500	1.76863	0.621
		M150 (CN 150)	4.10750	1.76863	0.236
		MG (Glibenclamide)	4.26000	1.76863	0.205
	MS (Aloksan)	MH (NaCMC)	-7.80500*	1.76863	0.004
		M50 (CN50)	-3.16750	1.76863	0.495
		M100 (CN100)	-5.01000	1.76863	0.097
		M150 (CN 150)	-3.69750	1.76863	0.335
		MG (Glibenclamide)	-3.54500	1.76863	0.378
	M50 (CN50)	MH (NaCMC)	-4.63750	1.76863	0.142
		MS (Aloksan)	3.16750	1.76863	0.495
		M100 (CN100)	-1.84250	1.76863	0.897
		M150 (CN 150)	-0.53000	1.76863	1.000
		MG (Glibenclamide)	-0.37750	1.76863	1.000
	M100 (CN100)	MH (NaCMC)	-2.79500	1.76863	0.621
		MS (Aloksan)	5.01000	1.76863	0.097
		M50 (CN50)	1.84250	1.76863	0.897
		M150 (CN 150)	1.31250	1.76863	0.974
		MG (Glibenclamide)	1.46500	1.76863	0.958
	M150 (CN 150)	MH (NaCMC)	-4.10750	1.76863	0.236
		MS (Aloksan)	3.69750	1.76863	0.335
		M50 (CN50)	0.53000	1.76863	1.000
		M100 (CN100)	-1.31250	1.76863	0.974
		MG (Glibenclamide)	0.15250	1.76863	1.000
	MG (Glibenclamide)	MH (NaCMC)	-4.26000	1.76863	0.205
		MS (Aloksan)	3.54500	1.76863	0.378
		M50 (CN50)	0.37750	1.76863	1.000
		M100 (CN100)	-1.46500	1.76863	0.958
		M150 (CN 150)	-0.15250	1.76863	1.000

5. Conclusion

The results showed that the phytochemical screening of dandang gendis leaf extract with ethyl acetate solvent contained flavonoids, alkaloids, steroids, saponins, terpenoids, tannins, and phenolics. Antioxidant activity is moderately active, with an IC_{50} value of 160 mg/L. FT-IR analysis containing the functional groups OH, CH aliphatic, C=C aromatic and C-O indicated that this isolate is a flavonoid compound. At a concentration of 100 mg/kg BW of mice, dandang gendis leaf extract worked optimally in increasing the number and expression of pancreatic β cells.

Ethical Clearence

This study has gone through ethical approval procedures in accordance with applicable scientific research standards. Prior to the implementation of the experimental test, all procedures of this study were approved by the Research Ethics Committee registered at Research Center of Poltekkes Kemenkes Makassar (No.: 0330/O/KEPK-PTKMS/III/2023). The approval process was conducted based on the consideration that this research will not cause significant harm or loss to the subjects used, both in terms of their health and well-being.

Conflict of Interest

The authors declare no conflict of interest in this study.

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Author contributions

Artati Artati: Study design, data analysis, and manuscript preparation; Syahida Djasang: Methodology and data analysis.

References

1. Wollney E, Sovich K, LaBarre B, Maixner SM, Paulson HL, Manning C, Fields JA, Lunde A, Boeve BF, Galvin JE, Taylor AS, Li Z, Fechtel HJ and Armstrong MJ. End-of-life experiences in individuals with dementia with Lewy bodies and their caregivers: A mixed-methods analysis. *PLoS One.* 2024; 19(8): e0309530. doi: 10.1371/journal.pone.0309530.

2. Mackova V, Raudenska M, Polanska HH, Jakubek M and Masarik M. Navigating the redox landscape: reactive oxygen species in regulation of cell cycle. *Redox Rep*. 2024; 29(1): 2371173. doi: 10.1080/ 13510002. 2024.2371173.

3. Wu Q and Huang F. Targeting ferroptosis as a prospective therapeutic approach for diabetic nephropathy. *Ann. Med.* 2024; 56(1): 2346543. doi: 10.1080/07853890.2024.2346543.

4. Akhlaghi M and Bandy B. Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. *J. Mol. Cell. Cardiol.* 2009; 46(3): 309-317. doi: 10.1016/j.yjmcc.2008.12.003.

5. Rohilla A and Ali S. Alloxan induced diabetes : mechanisms and effects. *Int. J. Res. Pharm. Biomed. Sci.* 2012; 3(2): 819-823.

6. Le DD, Kim YR, Yu S, Dang T, Lee KT and Lee M. Phytochemical and bioactivities of promising flavonoid glycosides and their content from unmatured fruits of *Vicia bungei* with their bioactivity. *Food Chem*. 2024; 460(Pt 3): 140541. doi: 10.1016/ j.foodchem.2024.140541.

7. Azam MNK, Biswas P, Khandker A, Tareq MMI, Tauhida SJ, Shishir TA, Bibi S, Alam MA, Zilani MNH, Albekairi NA, Alshammari A, Rahman MS and Hasan MN. Profiling of antioxidant properties and identification of potential analgesic inhibitory activities of *Allophylus villosus* and *Mycetia sinensis* employing in vivo, in vitro, and computational techniques. *J Ethnopharmacol*. 2025; 336: 118695. doi: 10.1016/j.jep.2024.118695.

8. Amić D, Davidović-Amić D, Bešlo D and Trinajstić N. Structure-radical scavenging activity relationships of flavonoids. *Croat. Chem. Acta*. 2003; 76(1): 55-61.

9. Hong S and Liu S. Targeted acylation for all the hydroxyls of (+)-catechin and evaluation of their individual contribution to radical scavenging activity. *Food Chem*. 2016; 197(Pt A): 415-21. doi: 10.1016/ j.foodchem. 2015.10.134.

10. Nurulita Y, Dhanutirto H and Soemardji AA. Penapisan aktivitas dan senyawa antidiabetes ekstrak air daun dandang gendis (*C. nutans*). *J. Natur Indones.* 2012; 10(2): 98. doi: 10.31258/jnat.10.2.98-103.

11. Thalib A, Masadah R, Prihartono P, Hamid F, Haidir M, Hasan H, Keliwawa S and Labulawa I. Antioxidant activity of *Laportea decumana* (Roxb) Wedd ethanol and n-hexane extracts. *Open Access Maced. J. Med. Sci*. 2022; 10(G): 590-594. Doi: 10.3889/oamjms. 2022.839.

12. Susanto Y, Khaerati K and Widodo A. Physicochemical, phytochemical, and evaluation of angiotensin-converting enzyme inhibitory activity of Tamoenju (*Hibiscus surattensis* L.) leaves extract. *Rasayan J. Chem*. 2023; 16(02): 565-572. doi: 10.31788/RJC.2023.1628199.

13. Lumowa SVT and Bardin S. Uji fitokimia kulit pisang kepok (*Musa paradisiaca* L.) bahan

alam sebagai pestisida nabati berpotensi menekan serangan serangga hama tanaman umur pendek. *J. Sains dan Kesehat.* 2018; 1(9): 465-469. doi: 10.25026/jsk.v1i9.87.

14. Illing I, Safitri W and Erfiana. Uji fitokimia ekstrak buah dengen. *J. Din.* 2017; 8(1): 66-84.

15. Fermanasari D, Zahara TA and Wibowo MA. Uji total fenol, aktivitas antioksidan dan sitotoksitas daun akar bambak (*Ipomoea* sp.). *Jkk.* 2016; 5(4): 68-73.

16. Herath A, Tiozon RJ, Kretzschmar T, Sreenivasulu N, Mahon P and Butardo V Jr. Machine learning approach for high-throughput phenolic antioxidant screening in black Rice germplasm collection based on surface FTIR. *Food Chem*. 2024; 460(Pt 3): 140728. doi: 10.1016/j.foodchem.2024.140728.

17. Banda M, Nyirenda J, Muzandu K, Sijumbila G and Mudenda S. Antihyperglycemic and antihyperlipidemic effects of aqueous extracts of *Lannea edulis* in Alloxan-induced diabetic rats. *Front Pharmacol*. 2018; 9: 1099. doi: 10.3389/fphar.2018.01099.

18. Yu Q, Duan W, Liu B, Duan Z and Shang F. Microwave-assisted extraction of bioactive substance from *C. nutans*. 2016. doi: 10.2991/icseee-15.2016.128.

19. Nur Fitriyani Sukamto D, Hanifwati A and Yunita S. Pengaruh ekstrak tempe kedelai hitam (*Glycine soja*) terhadap jumlah sel pankreas tikus putih jantan (*Rattus novergicus* strain sistar) model dm tipe 2. *Saintika Med.* 2017; 8(1): 2012. doi: 10.22219/sm.v8i1.4099.

20. Molyneux P. The use of stable free radical diphenyl-picrylhydrazyl (DPPH) for estimating antioxidant activity. *J. Sci. Technol.* 2004; 26(2): 211-219.

21. Dehpour AA, Ebrahimzadeh MA, Fazel NS and Mohammad NS. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas y Aceites.* 2009; 60(4): 405-412. doi: 10.3989/gya.010109.

22. Sukadana IM. Aktivitas antibakteri senyawa flafonoid dari kulit akar awar (*Ficus septica* Burm F). *Journal Kimia* 2010; 4(1): 63-70.

23. Silverstein RM, Webster, Kiembel. Spectrometric identification of organic compounds. 7th ed., USA: John Wiley and Sons; 2005.

24. Szkudelski T. The mechanism of alloxan and streptozotocin action in b cells of the rat pancreas. *Physiol. Res.* 2001; 50(6): 537-546.

25. Robertson RP, Harmon J, Tran POT and Poitout V. Beta-cell glucose toxicity, lipotoxicity, chronic oxidative stress type 2 diabetes. *Diabetes* 2004; 53: 119-124. doi: 10.2337/diabetes.53.2007.s119.

26. Purnomo S. Oksidan, antioksidan, dan radikal bebas. Buku naska; 2000.

27. Nugent DA, Smith DM and Jones HB. A review of islet of langerhans degeneration in rodent models of type 2 diabetes. *Toxicol. Pathol.* 2008; 36(4): 529-551. doi: 10.1177/0192623308318209.

28. Finegood DT, MC Arthur M, Kojwang D, Thomas MJ, Topp BG, Leonard T and Buckingham RE. Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazion prevent the rise of net cell death. *Diabetes.* 2001; 50(5): 1021-1029. doi: 10.2337/diabetes.50.5.1021.

29. Lusiana. Potensi antioksidasi ekstrak etanol jamur tiram putih (*Pleurotus ostreatus*). *J. Gradien*. 2015; 11(1): 1066-1069.

30. Li F, Li Q, Gao D and Peng Y. The optimal extraction parameters and anti-diabetic activity of flavonoids from *Ipomoea batatas* leaf. *Afr. J. Tradit. Complement. Altern. Med.* 2009; 6(2): 195-202. doi: 10.4314/ajtcam.v6i2.57091.

31. Anwer T, Alkarbi ZA, Hassan Najmi A, Alshahrani S, Siddiqui R, Khan G and Firoz

Alam M. Modulatory effect of zingerone against STZ-nicotinamide induced type-2 diabetes mellitus in rats. *Arch. Physiol. Biochem.* 2021; 127(4): 304-310. doi: 10.1080/ 13813455.2019. 1637436.

32. Wang GG, Lu XH, Li W, Zhao X, Zhang C. Protective effects of luteolin on diabetic nephropathy in STZ-induced diabetic rats. *Evidence-based Complement. Altern. Med.* 2011; 2011(1): 323171. doi: 10.1155/2011/323171.

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