PHARMACIA

Pharmacia 70(1): 39–47
DOI 10.3897/pharmacia.70.e94899

Research Article

Antidiabetic activity of *Anredera cordifolia* (Ten.) Stennis extracts with different ethanol percentages: an evaluation based on *in vitro*, *in vivo*, and molecular studies

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Received 16 September 2022 ♦ **Accepted** 3 November 2022 ♦ **Published** 9 January 2023

Citation: Sulfianti A, Firdausi N, Nurhadi N, Ngatinem N, Agustini K, Ningsih S (2023) Antidiabetic activity of *Anredera cordifolia* (Ten.) Stennis extracts with different ethanol percentages: an evaluation based on *in vitro*, *in vivo*, and molecular studies. Pharmacia 70(1): 39–47. https://doi.org/10.3897/pharmacia.70.e94899

Abstract

Anredera cordifolia (Ten.) Stennis, also known as Binahong (B), is an Indonesian plant used to treat diabetes. The purpose of this study was to determine the best extragent for preparing Binahong extract as an antidiabetic agent using different concentrations of ethanol (50%, 70%, and 96%), labelled as BE50%, BE70%, and BE96%. An alpha-glucosidase inhibiting assay was used to assess the activity. The most active extract was tested *in vivo* assay using an oral glucose tolerance test (OGTT) and alloxan-high feed diet (alloxan-HFD)-induced diabetes in rats, with glucose level and beta cell Langerhans repair as parameters. A molecular assay was also performed to look into the expression of homeostasis regulator genes on 3T3-L1 adipose cells. The results showed that 96% ethanol extract (BE96%) inhibited alpha-glucosidase the most effectively (IC_{50} 119.78± 11.14 µg/mL). The *in vivo* assay revealed that the treatment BE96% at 250 mg/kg BW for 21 consecutive days significantly reduced plasma glucose levels in Type 2 DM rats compared to the control group ($p \leq .05$) with improved of Langerhans beta cells. BE96% also significantly reduced postprandial glucose levels. At the cellular level, Oil-Red-O staining revealed that differentiated adipocytes treated with BE96% had the highest lipid absorbance ($p \leq .05$), compared to the control. BE96% significantly increased the expression of Glucose Transporter Isoform 4 (GLUT4) at the molecular level. It could be concluded that BE96% exhibited the best antidiabetic properties.

Keywords

Alloxan-HFD-induced diabetic rat, alpha-glucosidase inhibition activity, Binahong, glucose tolerance test, GLUT4 expression

Introduction

A metabolic condition known as diabetes mellitus (DM) is characterized by chronically high blood sugar levels (hyperglycemia) and blood insulin levels (hyperinsulinemia) (Westman 2021). In the majority of the developed and emerging nations, DM is a serious issue. According to estimates, there would be approximately 463 million cases

of DM worldwide in 2019 and 700 million cases by 2045 (Shrestha et al. 2022). There are two types of DM: Type 1 DM (T1DM), which is defined by insulin deficiency caused by the destruction of Langerhans beta cells of the pancreas, and Type 2 DM (T2DM), which is caused by the failure of cells' response to this hormone. T2DM accounts for nearly 90% of all diabetes cases worldwide (Westman 2021). However, the complexity of T2DM due to the interaction of multiple genes and environmental factors makes it difficult for researchers worldwide to find the most effective antidiabetic agent (Felisbino et al. 2021).

One of the additional resources for treating diabetes is medicinal plants (Alam et al. 2022). Due to Indonesia's untapped mega-biodiversity wealth, research into possible antidiabetic herbs is still a promising subject. One of the Indonesian medicinal plants frequently used to treat DM as well as other ailments is *A. cordifolia* (Ten.) Stennis, also known as *Heartleaf maderavine madevine*, or (Binahong), belongs to the Basellaceae family. Previous studies reported that ethyl acetate extract of *A. cordifolia* demonstrated inhibitory activity toward alpha-glycosidase enzyme (IC_{50} 81.23 µg/mL), and an isolated compound, 8-glucosylapigenin, had IC_{50} 20.23 µg/mL (Djamil et al. 2017). In addition, other plant-derived substances include vitexin (Mulia et al. 2017) and Boussingoside A_1 Saponin (Espada et al. 1990) also exhibited antidiabetic properties.

Numerous studies have been conducted to identify an active sugar-lowering candidate agent in *A. cordifolia*. In general, the antidiabetic effect of a new compound is usually evaluated *in vivo* on a diabetogenic-compound-induced DM animal model using alloxan or streptozotocin (Kottaisamy et al. 2021), or with a combination of a high-fat diet (HFD) (Tang et al. 2006; Murakami et al. 2021). Alloxan, also known as 5,5-dihydroxyl pyrimidine-2,4,6-trione, is an organic compound derived from urea and a glucose analogue. This diabetogenic agent is frequently used in the development of animal models for the screening of novel antidiabetic compounds. Alloxan causes diabetes by causing the formation of reactive oxygen species (ROS), which causes pancreatic B cell necrosis and selective inhibition of insulin secretion (Ighodaro et al. 2017). On the other hand, Oral Glucose Tolerance Test (OGTT) is another appropriate method to measure the capacity of a new drug in lowering postprandial glucose (Hamza et al. 2015; Yusoff et al. 2015). This test is a widely used to evaluate the secretion function of beta pancreas and the sensitivity of tissues to insulin (Stumvoll et al. 2000). The glucose area under the curve (AUC), which is an indicator of total glucose excursion following glucose loading, also has been widely used for computing the glycemic index to evaluate the efficacy of medications for postprandial hyperglycemia (Sakaguchi et al. 2016). Furthermore, the *in vitro* evaluation of enzyme activity of alpha-glucosidase and alpha-amylase activity, which contribute to the metabolism of carbohydrates in the digestive tract are additional tests that could be performed to investigate a new antidiabetic agent (Mechchate et al. 2021).

However, evaluation at molecular levels is also critical to support the pharmacodynamic of *A. cardifolia*. One gene that critically regulates T2DM is GLUT4, which is expressed mainly in insulin-sensitive tissues, such as the adipose tissue, liver tissue, and skeletal muscle tissue. Increased GLUT4 expression has been shown to reduce blood glucose and improve glucose transport at a cellular level (Jitrangsri et al. 2020). Previous studies demonstrated that several compounds that increased GLUT4 expression could potentially be used in treating diabetes (Lv et al. 2021).

In this study, we determined the best ethanol concentration for preparing *A. cordifolia* extract to be used as an antidiabetic agent. *A. cordifolia* extracts with 50, 70, and 96% ethanol (BE50%, BE70%, and BE96%) were evaluated for *in vitro* and *in vivo* activity, as well as their capability to regulate GLUT4 expression. To begin, all extracts (BE50%, BE70%, and BE96%) were tested *in vitro* to determine which extract was the most active using an alpha-glucosidase enzyme inhibition assay. The selected *A. cordifolia* extract was then tested for hypoglycemic activity using the OGTT and long-term treatment with alloxan-HFD-induced diabetic rats. The extracts were also administered during 3T3-L1 adipose cell differentiation to investigate the effect of plant on GLUT4 expression. The choice of solvent type in this study became one of the most critical points in extraction to obtain an appropriate extract containing the desired metabolites and suitable for the intended purpose. The application of solvents in the industrial-scale extraction process is regulated by the Indonesian Food and Drug Administration (BPOM). Due to safety concerns, ethanol or a mixture of ethanol and water is preferred over other organic solvents (BPOM RI 2017). Choosing the right solvent combination affects polarity, including the solubility of bioactive compounds and the bioactivities of the extract (Jitrangsri et al. 2020).

Materials and methods

Plant extraction

Plant collection and authentication

Fresh *A. cordifolia* leaves were collected from Yogyakarta, Indonesia, and authenticated by Biology Research Centre, **LIPI** (Indonesian Institute of Science) (No. B-104/ IV/ DI.01/I/2021).

Preparation of extracts

A. cordifolia leaves were cleaned, washed, and dried in a 50 °C oven before being milled into coarse powder. One hundred grams of powder were extracted with 1 L 96% food grade ethanol in a shaking incubator at room temperature for 24 hours. The extraction process was repeated twice after filtering. Furthermore, the filtrate was collected and evaporated using a rotary evaporator at 50 °C. The obtained semisolid mass then stored in an amber bottle at -80 °C for future experiments. The same extraction was carried out with 50% and 70% ethanol. Finally, the extracts were labelled with the percentages BE96%, BE70%, and BE50%. The yield of each extract was 11,72%, 10,54%, and 9.77%, respectively.

In vitro assays

Alpha-glucosidase inhibitory assay

The inhibitory activity of alpha-glucosidase was conducted based on a previous study (Assefa et al. 2020). Ten milligrams of each sample were dissolved in 1 mL of 5% DMSO, sonicated at room temperature for 15 minutes, and centrifuged at 5000 rpm for 5 minutes. The supernatant was as the stock solution. By diluting the stock with 0.5% DMSO, a range concentration of each sample was prepared (40, 80, 160, 320, 640 ppm, as final concentrations). Enzyme solution was made by combining 10 mg of rat intestinal acetone powder alpha-glucosidase (Sigma-Aldrich, USA) with 50 mL 0.1 M phosphate buffer (pH 7), which was then sonicated in an ice water batch for 10 minutes before being centrifugated at 5000 rpm for 5 minutes at 4 °C to separate the supernatant. One hundred microliters of each sample concentration were mixed with 250 µL 0.02 M *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) (Sigma-Aldrich, USA) and 50 µL of phosphate buffer, then incubated at 37 °C for 5 minutes. After incubation, the solution was reacted with 150 µL enzyme solution, followed by 15 minutes of incubation. The reaction was stopped by adding 1000 µL of 0.2 M Na_2CO_3 solution (Merck, Germany), and the absorbance was read at 405 nm. As positive control, Acarbose (Sigma-Aldrich, USA) was diluted in 5% DMSO for the final concentration of 1, 2, 4, and 8 ppm, then it was used to replace the administration of samples. All experiment was conducted in triplicate. Furthermore, the enzyme inhibition rate was determined as a percentage of inhibition using the equation below:

$$\%\text{Inhibition}=\frac{\text{Abs(control} - \text{blank)} - \text{Abs(sample} - \text{blank)}}{\text{Abs(control} - \text{blank)}}\times 100\%$$

The IC_{50} value was derived from a linear regression equation of the curve that plotted each concentration toward the % inhibition value using Microsoft Excel. The sample that demonstrated the lowest IC_{50} was then chosen for further experiments.

In vivo assays

Animals

The experiment was conducted at the animal laboratory of the National Research and Innovation Agency (**BRIN**), Indonesia. 31 male Sprague Dawley rats (10–12 weeks of age, 150–200 BW) were used for efficacy test. 15 male and 15 female rats (6–8 weeks, 80–100 BW) were used for toxicity test. All animals were procured from the Central Animal House of **BPOM**. The animals were placed in polycarbonate cages (32×24×16 cm) with husk as bedding material and kept at a controlled temperature (21–25 °C), humidity (55±5%), and a 12-light/dark circle. Animals were regularly fed and watered *ad libitum*. All animals were acclimatized for seven days before the experiment. The Health Research Ethical Committee, Faculty of Medicine, the University of Indonesia had approved all these *in vivo* experiments (Ethical approval: No. KET- 411/UN2.F1/ETIK/PPM.00.02/2021).

Acute toxicity test

The acute toxicity test of selected *A. cordifolia* extract followed WHO protocol guideline (WHO 2000). The 15 male and 15 female rats were distributed into three groups, namely, namely Group 1 (BE96% 1.00 g/kg BW), Group 2 (BE96% 4.25 g/kg BW), and Control Group (vehicle carboxymethyl cellulose 0.5%), consisting of five pairs each. The chosen doses were following the guidelines of BPOM (Indonesian FDA) (BPOM RI 2014). After overnight fasting, rats were treated *p.o.* (2 mL/200 g BW). Observations for the appearance of toxic effects such as sedation, convulsion, tremor, diarrheal, constipation, and abnormal urination were performed at 0.5, 1, 2, and 3 h after gavage and then continued once every day for the next 14 days at the same time. In addition, the body weight of rats was recorded before and twice a week after the treatment. Dead rats during the experiment were autopsied immediately. At the end of the study, the number of mortalities was investigated to observe the toxicity category of the sample.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance activity of BE96% was determined based on a previous study (Nagy and Einwallner 2018). After overnight fasting (water was given), 15 male rats were divided into three groups, consisting of five rats each: Group 1 (BE96% 250 mg/kg BW + 3 g/kg BW Glucose), Group 2 (carboxymethyl cellulose 0.5% + 3 g/kg BW Glucose) and Control Group (carboxymethyl cellulose 0.5% + water). The used doses of extracts were selected based on our previous study. Extract or vehicle was gavage *p.o.* (1 mL/200 g BW). Groups 1 and 2 received 1 mL/200 g BW of glucose 30 minutes after sample administration. Blood was collected at 30, 90, and 150 minutes after glucose loading from sinus orbitalis, and blood glucose postprandial was determined using GOD-PAP commercial reagent kit diagnostic (Diasys, Germany). The postprandial blood glucose (PBG) level was served as a glucose-time graphic. The blood glucose was calculated using the equation below:

$$\text{Glucose}\ \left(\%\ \text{toward}\ \text{M}_0\right)=\frac{\text{Glucose level M}i}{\text{Glucose level M0}}\times 100\%$$

In addition, as other lowering glucose parameter, Area Under the Curve (AUC) was calculated from the glucose-time graphic using a trapezoidal mathematic equation (Sakaguchi et al. 2016).

Hypoglycemic test on an alloxan-HFD-induced Type 2 diabetic rat

The hypoglycemic activity of the selected extract was evaluated in alloxan-HFD-induced Type 2 diabetic rat was based on the method of Tang et al. (2006). Briefly, rats were treated with an HFD (consisted of standard laboratory chow (Indofeed, Bogor, Indonesia) 1000 g, tallow 500 mL, skimmed milk 250 g, dry powder of cassava 40 g) for 30 days. After fasting overnight, rats were intraperitoneally injected with 100 mg/kg BW alloxan (Sigma-Aldrich, USA) solution in cold saline. Four days after induction,

all rats were fasted overnight (water provided *ad libitum*), and blood was collected from sinus orbitalis. FBGL (fasting blood glucose level) of the plasma EDTA was measured using GOD-PAP commercial diagnostic reagent kit (Diasys, Germany). Rats with FBGL above 200 mg/dL were considered diabetic and randomly divided into three groups, namely, Group 1 (HFD + BE96% 250 mg/kg BW), Group 2 (HFD + glibenclamide 0.18 g/kg BW), Group 3 (HFD + carboxymethyl cellulose 0.5%), Control group (Normal diet + carboxymethyl cellulose 0.5%), four rats each. The selected doses of the extract were referred to the OGTT. Samples were gavage *p.o.* (1 ml/200 g BW) for 21 consecutive days. After overnight fasting (10–12 h), blood was collected from sinus orbitalis after 14 and 21 days of sample treatments, and FBGL was measured using a GOD-PAP reagent kit. Animals were kept on HFD for the experiment. On day 21, all animals were euthanized, and the pancreases were taken. Pancreas histological slides were prepared by hematoxylin-eosin staining using standard procedures (Bancroft and Cook 1994), and the number of beta cells of Langerhans islet was counted using a confocal microscope (Carl ZEISS Axio).

Molecular assays

3T3-L1 differentiation and sample treatment

The 3T3-L1 pre-adipocyte cells were obtained from the collection of LAPTIAB National Research and Innovation Agency (**BRIN**), Indonesia. The cells were maintained in DMEM High Glucose (Sigma-Aldrich, USA), supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, USA), 100 U/mL penicillin (Gibco, USA), 100 g/mL streptomycin (Sigma-Aldrich, USA), 0.5 percent amphotericin (Sigma-Aldrich, USA) (DMEM Hg + FBS Complete) and incubated at 37 °C in a humidified incubator with 5% CO_2. The adipocyte differentiation procedure followed a previous protocol (Zebisch et al. 2012). The media was replaced with DMEM +10% FBS on the seventh day, and the cells serum-starved for three hours before being treated with all A. *cordifolia* extracts in DMSO 1 M at IC_{25} concentrations (1500 ppm; 188 ppm; and 500 ppm for BE50%, BE70%, and BE96%, respectively). The selection of IC_{25} was based on preliminary study which showed the effectiveness of the samples on cells without causing cell death (data did not published). The cells were incubated for 24 hours after the sample was given. The cell was rinsed with PBS 1×, and RNA was ready to be extracted. All the experiments were carried out in triplicate.

GLUT4 expression assay

Total RNA of adipocytes treated was isolated using Genezol (Geneaid, Taiwan) reagent and quantified using a Nano-Drop UV-vis spectrophotometer (SensiFAST SYBR No-ROX). One-Step Kit (Bioline, USA) was used to perform a direct quantitative real-time PCR according to the manufacturer's instructions. The primer sequences were seen on Table 1. The expression values for GLUT4 and Beta-actin expression are shown as Ct (threshold cycle). Furthermore, the mean of Beta-actin expression values was utilized to standardize the relative expression levels of GLUT4 using the "Delta-Delta Ct" formula ($2^{-\Delta\Delta CT}$) as follows:

$$\Delta CT: CT \text{ (target gene)} - CT \text{ (house keeping gene)}$$
$$\Delta\Delta CT: \Delta CT \text{ (target gene)} - \Delta ct \text{ (reference gene)}$$

Table 1. Primer sequences for Glucose Transporter Isoform 4 (GLUT4) and Beta-actin genes.

No	Gene	Primer Sequences
1	GLUT4	Forward: 5' -GATTCTGCTGCCCTTC TGTC-3'
		Reverse: 5'-ATTGGACGCTCTCTCTCCAA-3'
2	Beta-actin	Forward: 5'-CTCTGGCTCCTAGCACCATGAAGA-3'
		Reverse: 5'-GTAAAACGCAGCTC AGTAACAGTCCG-3'

Statistical analysis

Statistical analysis was performed by GraphPad Prism 8.0. The difference between groups was compared using a One-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison Test *post hoc*. Statistical significance was defined as a p value of less than 0.05 ($p \leq .05$).

Results

Effect of *A. cordifolia* extracts on the alpha-glucosidase inhibition assay

As a preliminary study, *A. cordifolia* extracts were assessed for their activity by alpha-glucosidase inhibitory assay. Our experiment showed that among three extracts, BE96% demonstrated the highest inhibitory activity (IC_{50} 119.78 ± 11.14 µg/mL). Thus, this extract (BE96%) was selected as sample for *in vivo* experiments. However, its activity was lower than that of acarbose (IC_{50} 3.34 ± 0.95 µg/mL) (Table 2).

Table 2. The alpha-glucosidase inhibitory activity of *A. cordifolia* extracts.

Samples	Binahong 50% (BE50%)	Binahong 70% (BE70%)	Binahong 96% (BE96%)	Acarbose
IC_{50} (x±SD) (µg/mL)	271.17 ± 1.97*	253.52 ± 1.57*	119.78 ± 11.14*	3.34 ± 0.95

Data are presented as mean ± SD, triplicate. *Significantly different from acarbose ($p \leq .05$).

Activity of BE96% on *in vivo* studies

The acute toxicity test of BE96% showed no mortality in both sexes until the dose of 4.25 g/kg BW. Additionally, no symptoms such as drowsiness, convulsion, tremor, diarrheal, constipation, or polyuria were observed in all groups. A closer examination of the animal body weights revealed that all rats gained weight during the research.

Based on OGTT (Fig. 1), the ingestion of glucose (3 g/kg BW) *p.o.* was able to raise the proportion of postprandial

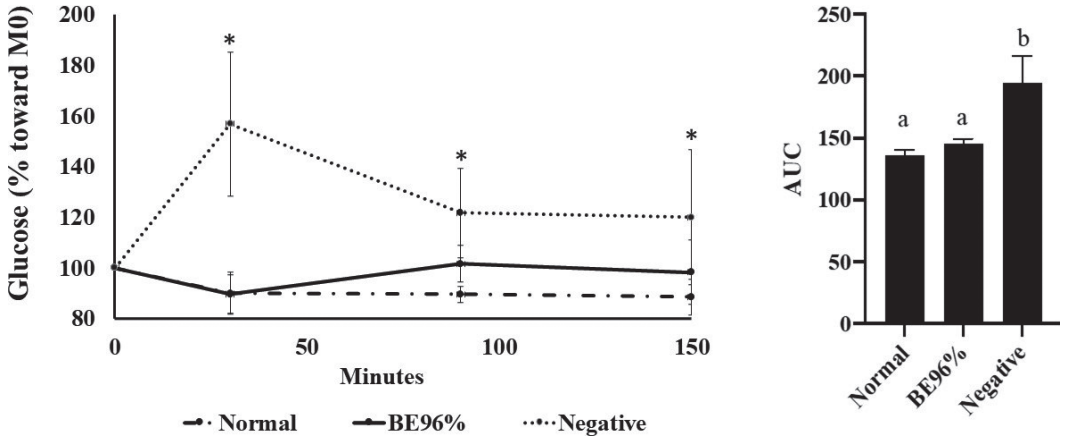

Figure 1. Percent of plasma glucose level (left) and AUC value of each group (right) during Oral glucose tolerance test (OGTT). *: Significantly different from Normal ($p \leq .05$). Different letters (a, b) indicated significant differences ($p \leq .05$). Glucose (% toward M_0) = (glucose level M_i/glucose level M_0) × 100%.

blood glucose (PBG) levels in normal rats. The group treated with 250 mg/kg of BE96% and loaded with glucose had lower PBG after 30 minutes of glucose consumption, which was significantly different from the negative group ($p \leq .05$). Until the completion of the study, the PBG levels in the treatment group did not differ significantly from the normal group ($p > .05$). The PBG levels each group were delivered as the area under curve (AUC), state as relation between glucose levels toward the time from each measurement. The AUC values of BE96% group was significantly lower than the negative group ($p \leq .05$) (Fig. 1).

Following that, the potency of BE96% was also examined in an alloxan-HFD-induced diabetic rats (Table 3). Our study found that induction of the combined alloxan *i.p.* and long-term HFD *p.o*. raised the FBGL more than 200 mg/dL and kept persistent until the end of the experiment. After 14- and 21-days treatment, both groups treated with BE96% and glibenclamide demonstrated significantly lower FBGL than the negative group ($p \leq .05$). The study on beta-cell Langerhans showed that BE96% improved the damaged pancreatic beta cells caused by alloxan induction (Fig. 2). It was demonstrated that the induction of the combined alloxan *i.p.* and long-term HFD *p.o*. caused destroyed beta cells. BE96% at the dose of 250 mg/kg BW for 21 consecutive days restored the damaged pancreatic beta cells caused by alloxan induction, significantly compared to the negative group ($p \leq .05$). On the other hand, the diabetic rats given glibenclamide did not show the improvement of the b-cells damage compared to the negative group ($p > .05$).

Table 3. FGBL on the Alloxan-HFD-induced diabetic rat assay.

Groups	FBGL (mg/dL) at day of measurement		
	D0	D14	D21
Normal	122.93±9.84[a]	119.54±6.95[c]	92.05±4.83[e]
BE96%	277.65±23.41[b]	135.94±7.29[c]	104.20±6.51[e]
Glibenclamide	266.15±29.66[a]	113.61±24.09[c]	85.56±11.98[e]
Negative	233.98±27.11[a]	245.89±16.48[d]	221.18±27.56[f]

FBGL before (D0), after 14- (D14), and 21-day (D21) treatment. The different letters (a, b, c) indicated significant differences at the point of measurement ($p \leq .05$).

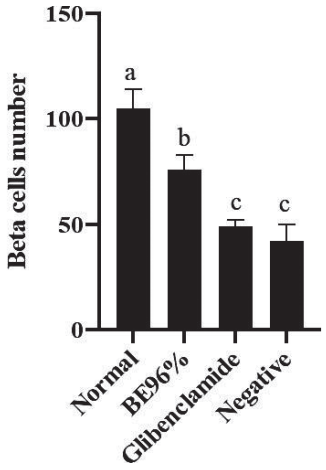

Figure 2. The average number of Langerhans beta cells on the Alloxan-HFD-induced diabetic rat assay. Different letters (a, b, c) indicated significant differences at the point of measurement ($p \leq .05$).

Activity of *A. cordifolia* extracts on the cellular levels and the expression of glucose homeostasis gene markers

Based on the cellular study, *A. cordifolia* expedited the differentiation of 3T3-L1 fibroblast cells into an adipocyte-like phenotype. It can be seen microscopically by changes in pre-adipocyte cell shape, clonal growth, and an increase in the storage of lipids (Abozed et al. 2014; Hamza et al. 2015) (Fig. 3). In addition, evaluation with Oil-Red-O staining displayed those adipocytes treated with BE96% had the highest lipid absorption in all group significant different to the control ($p \leq .05$), followed by BE70% and BE50%. Both these last extracts were not different to the control ($p > .05$) (Fig. 4A). By further assessment the molecular activity of *A. cordifolia* on glucose uptake, we observed the level of mRNA GLUT4 (Fig. 4B) showed that during the differentiation stage, adipocytes treated with BE96% could upregulate GLUT4 as well as BE70% and BE50% but not significantly different compared to the control ($p > .05$).

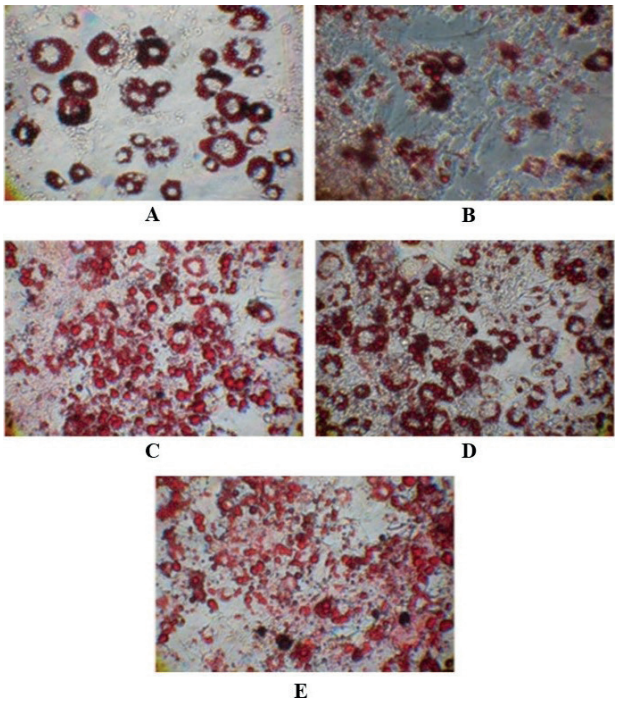

Figure 3. Stained fat droplet by Red-Oil O-Staining after differentiation. **A** Negative control with only dimethyl sulfoxide (DMSO) treatment; **B** Control positive with rosiglitazone treatment; adipocyte treatment with **C** BE50%; **D** BE70%; and **E** BE96%.

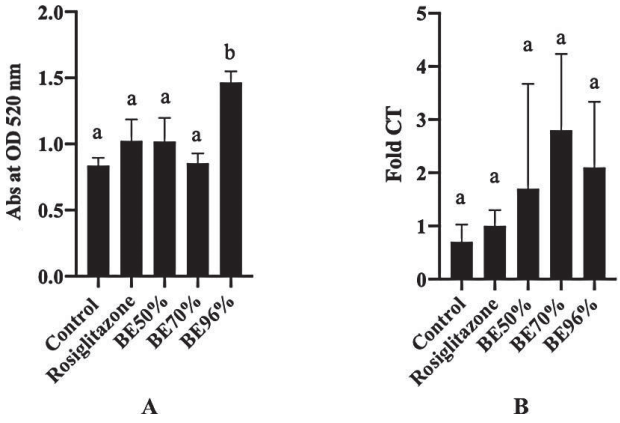

Figure 4. **A** The level of 3T3-L1 adipocyte differentiation; **B** mRNA GLUT4 expression after *A. cordifolia* extracts treatment. Different letters (a, b) indicated significant differences ($p \leq .05$).

Discussions

The ability of *A. cordifolia* extract to inhibit alpha-glucosidase enzyme was demonstrated *in vitro*. It was seen by the highest inhibition of BE96% compared to the two other *A. cordifolia* extracts. It was shown by the IC_{50} value of BE96% which was the lowest among the other extracts. Theoretically, the lower the level of IC_{50} demonstrated, the higher the quality of enzymatic inhibition (Hamza et al. 2015). However, when comparing this extract to acarbose as the positive control, the level of IC_{50} of BE96% was relatively high than acarbose. It is because acarbose is a single compound, while BE96% was a crude extract which consisted of complex compounds.

Furthermore, the polarity of solvent that used in extraction process influenced the chemical compounds in the extract. The polarity of solvent is inversely proportional to ethanol content, the higher the concentration of ethanol, the lower the polarity of the solvent. Our results showed that extract produced using the higher percentage ethanol solvent demonstrated a more potent inhibitory activity of the alpha-glucosidase enzyme. It was most likely due to the higher ethanol content of the solvent, increasing the active compounds concentration of inhibitor alpha-glucosidase enzyme, although it remained to be measured. Martinez-Gonzalez et al. (2017) reported that the content of phenolic compounds in the *Limnophila aromatica* extract decreased in the following order: 100 percent ethanol > 75 percent ethanol > 50 percent ethanol > water and correlated with the strength of biological properties.

It was reported that several compounds in *A. cordifolia* that be claimed to play a role in antidiabetic activity were Boussingoside A1 Saponin (Espada et al. 1990), and flavonoid such as 8-glucosylapigenin (orientoside) (Djamil et al. 2017), and vitexin (Mulia et al. 2017). The structure and substitution pattern of flavonoid hydroxyl groups determined the degree of pharmacological activity (Abozed et al. 2014). It was studied that the total number and configuration of hydroxyl groups on flavonoid compounds increased flavonoids' antioxidant and antidiabetic properties. A pair of the hydroxyl group at the position of C3' and C4' enhanced the radical scavenging, alpha-glucosidase, and DPP4 inhibitory activities. Conversely, the methyl and acetate groups decreased flavonoid antioxidant and antidiabetic effects. The absence of the C2–C3 double bond and the ketonic group at the C4 in ring C reduced the alpha-glucosidase and DPP-4 inhibitory activities (Sarian et al. 2017).

Further evaluation on BE96% also was carried out by *in vivo* assay. The acute toxicity assay was firstly performed to investigate the extract safety profile used in the efficacy trials. According to the results, the BE96% was categorized as practically non-toxic. It was indicated that until the dose of 4.25 g/kg BW, there was no mortality in both sexes, and no acute symptoms were observed in all groups. Additionally, all rats gained weight during the research, indicating that the dose bellowed of the LD_{50} threshold was safe to be used in further *in vivo* research.

The efficacy study indicated that BE96% (250 mg/kg BW) reduced glucose levels in both glucose-loaded rats and alloxan-HFD-induced diabetic animals. Activity BE96% reducing the postprandial glucose (PBG) after exposure to glucose solution supported the *in vitro* experiment on the alpha-glucosidase inhibitory assay. The potency BE96% as a hypoglycemic agent was also demonstrated in an alloxan-HFD-induced diabetic rats. The lowering FBGL after BE96% treatment was due to its ability to repair the damaged pancreatic beta cells generated by alloxan induction. Previous research have shown that *A. cordifolia* extract and its isolated compounds have hypoglycemic activity. The 96% ethanolic extract of *A. cordifolia* at the dose of 25–100 mg/kg BW lowered BGL in HFD-induced diabetic rats after 21 days of treatment compared to the negative group significantly ($p \leq .05$) (Dwitiyanti et al. 2021). The 8-glucosylapigenin, also known as orientoside, inhibited

the alpha-glucosidase enzyme *in vitro* (IC_{50} 20.23 ug/mL) and reduced blood glucose in alloxan-induced DM rats (Djamil et al. 2017).

Induction of the combined alloxan *i.p*. and long-term HFD *p.o*. raised FBGL. The combination of HFD and alloxan could likely hinder the occurrence of auto-reversion to normal circumstances, as described if alloxan was administered in the ranges of 90–140 mg/kg BW, *i.p.*, without HFD (Jain and Arya 2011). Overeating HFD on a regular basis causes meta-inflammation, altering peripheral insulin receptor-associated signalling and decreasing sensitivity to insulin-mediated glucose clearance. It resulted in higher fasting glucose and insulin levels, as well as worse glucose tolerance, both of which are essential indications of insulin resistance (Mosa et al. 2015). The combination of HFD and Alloxan causes abnormalities of lipid metabolism, characterized by increased triglyceride, total cholesterol, and LDL cholesterol, followed by decreased HDL cholesterol. This condition is like Type 2 diabetes in humans (Zhang et al. 2018).

Further research showed that the bioactive ingredient in *A. cordifolia* engaged in controlling the balance of lipid and glucose in the 3T3-L1 adipocyte cell culture. Treatment with three different *A. cordifolia* extracts (BE50%, BE70%, and BE96%) on 3T3-L1 cells showed that *A. cordifolia* enhanced the differentiation of 3T3-L1 fibroblast cells into an adipocyte-like phenotype. It can be seen microscopically by changes in pre-adipocyte cell shape, clonal growth, and an increase in the storage of lipids (Ruiz-Ojeda et al. 2016). Oil-Red-O staining revealed that adipocytes treated with BE96% had the most excellent lipid absorbance, followed by BE70% and BE50%. These studies could enlighten the effect of this plant on improved insulin sensitivity through regulation of glucose uptake GLUT4, a facilitative diffusion which has been found a majority in skeletal muscle, heart, and adipocytes. This protein is translocated from intracellular storage vesicles to the plasma membrane in adipocytes and muscle in response to increased insulin secretion after eating. In adipocytes of both humans and rodents with obesity or T2D, the GLUT4 is downregulated. This condition is one of the earliest events in the pathogenesis of insulin resistance and T2D (Moraes-Vieira et al. 2016; Cignarelli et al. 2019). At the molecular level, all *A. cordifolia* extracts increased the level of mRNA GLUT4 compared to cells treated with Rosiglitazone as the positive control and cells not treated.

A. cordifolia functioned as an insulin mimic, controlling the anabolic response in adipose tissue by increasing glucose uptake. As a result, fatty acid synthesis from glucose was generated in adipocytes. This activity is most likely due to bioactive compounds in *A. cordifolia*, such as flavonoids, which can stimulate glucose uptake. The other study showed that flavonoids influence peripheral insulin sensitivity (Semaan et al. 2018). Insulin resistance is triggered by the presence of radical compounds caused by hyperglycemia. It was proven that both extract and compounds *A. cordifolia* (3,5,3',4'-tetrahydroxyflavone and the flavonoid 8-glucopyranosyl-4',5,7-trihydroxyflavone (vitexin)) exhibited antioxidant activity (Alba et al. 2020). Being a radical scavenger, *A. cordifolia* can effectively prevent and manage diabetes mellitus. According to our findings, *A. cordifolia* extracts are anti-diabetic on multiple levels, including *in vitro*, *in vivo*, and molecular.

Conclusions

To summarize, the results of this study demonstrated that the higher the ethanol content of the solvent in *A. cordifolia* extracts, the greater the efficacy of plant extract as an anti-diabetic agent. It was demonstrated by in vitro, in vivo, and molecular studies that *A. cordifolia* has a promising inhibitory activity against alpha-glucosidase enzyme, significantly reduced postprandial hyperglycemia ($p \leq .05$), reduced fasting blood glucose level ($p \leq .05$), and potentially promote the improvement of damaged beta cells, and finally regulated glucose level in cell and molecular level by upregulating the GLUT4 mRNA level. However, further studies needed to be conducted to elaborate on the compound in BE96% that played in lowering blood glucose.

Ethical approval

Animal experiment was conducted at the animal laboratory, National Research, and Innovation Agency (BRIN), Indonesia. Male and female Sprague Dawley rats were procured from the Central Animal House of BPOM (Indonesian FDA). The animals were placed in polycarbonate cages (32×24×16 cm) three animals each with husk as bedding material and kept at a controlled temperature (21–25 °C), humidity (55±5%), and a 12-light/dark circle. Animals were regularly fed and watered *ad libitum*. All animals were acclimatized for seven days before the experiment. At the end of the experiments, all animals were euthanized for pancreas collection. The Health Research Ethics Committee, Faculty of Medicine, the University of Indonesia had approved all these in vivo experiments (Ethical approval: No. KET- 411/UN2.F1/ETIK/PPM.00.02/2021).

Declarations of interest

All authors declared that they have no known conflict of financial interest or personal relationships that could have appeared to influence the outcome of this research.

Acknowledgements

This work was supported by Insinas Research Grant, First Term of 2019, from the Ministry of Research, Technology and Higher Education, Republic of Indonesia (No: 4/E/KPT/2019). The authors thank all technicians in mammalian cell culture and animal laboratory, National Research and Innovation Agency (BRIN) Republic of Indonesia, for their support in this study.

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