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Enhancing antidiabetic bioactivity of purslane leaf extract (*Portulaca oleracea* L.) through synthesis of nanoemulsion: *In vitro* and *in vivo* evidence

Andin Tasyalia BUDAYA [1], Putri Ramadhani KAHARUDDIN [1], Aisyah Aulia SAID [1], Siti Nur JANNAH [2], Nanda NICOLA [3], BAHRUN [1], Herlina RASYID [1]*

1 Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia
2 Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia
3 Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia
* Corresponding Author. E-mail: herlinarasyid@unhas.ac.id (H.R.); Tel. +62 852-5560-6583

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ABSTRACT: Purslane plant (*Portulaca oleracea*) is a type of herbal plant that can be used in traditional diabetes treatment because it has the highest content of the quercetin found in the leaves which plays a role in inhibiting the α-amylase enzyme. However, it is less active due to limitations in drug solubility and delivery. Therefore, we developed a nanoemulsion of purslane leaf extract as an antidiabetic *in vitro* through α-amylase enzyme inhibition and *in vivo* through alloxan induction. The nanoemulsion was made using virgin coconut oil as the oil phase, polysorbate as the surfactant, propylene glycol as the cosurfactant, and phosphate buffer as the water phase with purslane leaf extract masses of 0.005, 0.01, 0.02, and 0.04 grams. The physical stability tests of nanoemulsions carried out were organoleptic tests, percent transmittance tests, viscosity tests, pH tests, solubility tests, emulsification time tests, cycling tests, particle size tests, and zeta potential. The smallest nanoemulsion particle size in the F2 formulation was 10.53±0.20 nm. However, the results of antidiabetic activity obtained an IC_{50} value of inhibition of purslane leaf extract nanoemulsion against α-amylase enzyme which was better than purslane leaf extract, which was 1225.38 ppm, while the IC_{50} value of purslane leaf extract was 7904.61 ppm. *In vivo* analysis reveals purslane leaf extract nanoemulsion significantly reduces the sugar blood level on alloxan-induced mice models compare to the extract. This result indicates that nanoemulsion enhancing the antidiabetic activity and potentially applied for drug development.

KEYWORDS: Purslane; quercetin; diabetes; α-amylase; alloxan.

1. INTRODUCTION

Today's human lifestyle has largely ignored health aspects such as a diet high in fat, sugar, and an unhealthy lifestyle. This is one of the main factors in the development of degenerative diseases in the community. One example of such a degenerative disease is diabetes mellitus [1]. Diabetes mellitus is a group of metabolic disorders with hyperglycemia conditions caused by work disorders and insulin secretion [2]. The number of diabetics worldwide is predicted to reach 700 million by 2045. Indonesia ranks fourth in the world after India, China, and the United States with the highest prevalence of diabetes in the world [3]. Based on data from *the International Diabetes Federation* as of 2021, the number of people with diabetes in Indonesia is 19.47 million with a prevalence of 10.6%. The number of diabetics in the country is estimated to continue to increase from year to year. WHO data estimates that the number of people with diabetes mellitus in Indonesia will increase significantly to 21.3 million people by 2030 [4]. Therefore, one way to overcome diabetes mellitus caused by excessive blood glucose levels is to inhibit the enzyme α-amylase [5].

α-Amylase enzymes belong to the group of hydrolytic enzymes because they convert starch into glucose in the hydrolysis process in the body. This hydrolytic enzyme can be inhibited in diabetics by using synthetic drugs [6]. However, if taken for a long time, these drugs cause bad side effects. One example is the acarbose drug, which has side effects, namely gastrointestinal disorders [7]. This chooses traditional medicines such as herbal plants as an alternative for diabetics because they have the same performance and produce low side effects. One of these herbal plants is purslane (*Portulaca oleracea*) [8].

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Purslane (*Portulaca oleracea*) is a herbal plant that has wide range biactivity. Ethanol extract from Purslane extract is able to reduce LPS-induced synthesis of NO release [9]. Aqueous extract from the Purslane plant has antioxidant activity and shows strong scavenging activity against superoxide radical [10]. Methanol and ethanol extracts from Purslane also show antioxidant activity and hypoglycemia effects [11]. Some of these studies show that polar extracts from this plant contain active compounds.

The total phenolic content of methanol and ethanol extracts for fresh leaves of Purslane is more than dried leaves, and contain apigenin, kaempferol, luteolin, quercetin, isorhamnetin, kaempferol-3-O-glucoside, rutin, caffeic acid, p-coumaric acid, and ferulic acid [11]. Other compounds found in Purslane oil are phytosterols, α-tocopherols, and squalene which make Purslane oil have angiogenic activity [12]. Purslane extract also contain antibacterial compounds such as portulaceramide A - D [13].

Traditional used of Purslane (*Portulaca oleracea*) by local society is for diabetes treatment because it contains the compound quercetin which effectively inhibits the enzyme α-amylase [14]. The highest content of quercetin compounds in purslane plants is found in the leaves [15]. Based on research by Sicari et al. (2018) [11], purslane leaf extract has the potential to have antidiabetic activity and can inhibit the enzyme α-amylase, but is still less active. This is due to limitations in terms of solubility and absorption.

Nanoemulsion preparations are one of the preparations selected to overcome the solubility of purslane leaf extract in terms of solubility [16]. The selection of this preparation is due to the stable drug delivery system and better solubility properties [17]. However, further research on nanoemulsion preparations of purslane leaf extract as an antidiabetic has not been conducted. Based on this, research was carried out on the synthesis of nanoemulsion of purslane leaf extract by conducting physical stability testing, as well as testing antidiabetic activity *in vitro* through α-amylase enzyme inhibition and *in vivo* through alloxane induction to see the pharmacological effects on living organisms.

2. RESULTS AND DISCUSSION

2.1 Purslane Leaf Extraction Results

Based on the results of extraction, the percentage of yield of purslane leaf thick extract to simplicia powder was 8.15%. The yield is larger when compared to the results obtained by Sari (2023) [18] where 70% ethanol extract only produces a yield of 7.95%. These results prove that the selection of solvents affects the amount of yield obtained from extraction.

2.2 Toxicity Test Results

The toxicity test was carried out using *the Brine Shrimp Lethality Test* (BSLT) method. This toxicity test aims to determine the presence of bioactivity in purslane leaf extract. According to Walean et al. (2021), this test can be used to determine various biological activities in plant extracts, one of which is enzyme inhibition. The results of the toxicity test of the BSLT method were stated with *Lethal Concentration* 50 (LC_{50}), which is the optimum concentration of the extract that is able to kill 50% of the shrimp larval population. Lower LC_{50} values indicate higher cytotoxicity effects. A compound can be said to have bioactivity if the LC_{50} value is <1000 ppm [19]. Testing of BSLT results showed that purslane leaf extract had an LC_{50} toxicity of 482 ppm obtained from the regression equation of the relationship between the extract concentration log and the probit value. This shows that purslane leaf extract has the potential for bioactivity. The potential of bioactivity cannot be separated from the phytochemical content in it.

2.3 Test Results for Determination of Cuercetin Levels

In this research, the quercetin level was determined with a standard solution of quercetin in a series of concentrations of 2, 4, 6, 8, and 10 ppm. The standard maximum wavelength of quercetin is 439 nm which is used to measure uptake from purslane leaf extract samples. The absorbance value of the consecutive standard series of quercetin is 0.009; 0.026; 0.042; 0.067; and 0.081 which is then plotted into a curve between its concentration and absorption which can be seen in Figure 1.

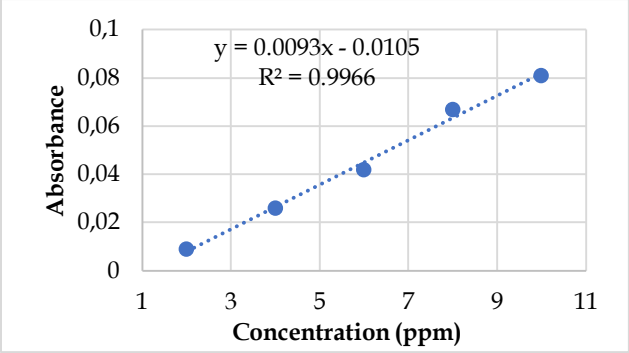

Figure 1. Linear curve of quercetin concentration

From the curve above, a linear regression equation is obtained, namely y = 0.0093x - 0.0105 with an R^2 value obtained of 0.9966. The quercetin calibration curve equation can be used as a comparison to determine the concentration of quercetin in the sample extract because it has a regression value of ≥ 0.995 [18]. The results of this research obtained quercetin levels of purslane leaf extract of 6.106% extract which can be seen in Table 1.

Table 1. Results of determination of quercetin levels of purslane leaf extract

Sample	Replication	Initial rate (mg/mL)	Total levels (mg QE/g extract)	Average (g QE/g extract)	Up to Quercetin (%)
Purslane Leaf Extract	1	0.00758	75.8	0.06106	6.106
	2	0.00682	68.2		
	3	0.00392	39.2		

2.4 Nanoemulsion Stability Test Results

2.4.1 Organoleptic Test

Table 2. Organoleptic test results

Formula NEDK	Organoleptic Test Parameters			
	Colour	Aroma	Homogeneity	Texture
F1	Yellow	Typical Polysorbate	Homogeneous	Fall
F2	Yellow	Typical Polysorbate	Homogeneous	Fall
F3	Dark Yellow	Typical Polysorbate	Homogeneous	Fall
F4	Yellow Brownish	Typical Polysorbate	Homogeneous	Thick

The results of the nanoemulsion organoleptis test in Table 2 show that all formulas have a distinctive polysorbate aroma and are homogeneous.

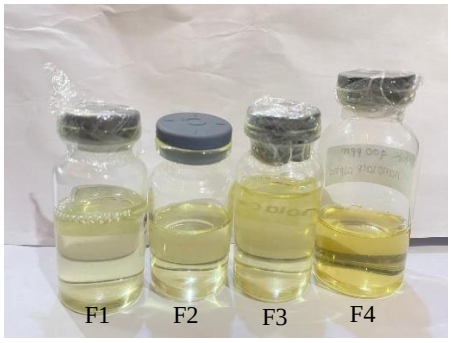

Figure 2. Organoleptic test results

All formulas are yellow, but have different color intensities which can be seen in Figure 2. Then F1, F2, and F3 have a liquid texture and F4 has a slightly thick texture. This is in accordance with the characteristics of

good nanoemulsion preparations, namely the texture is liquid and not too thick, yellowish in color, and does not smell rancid [20].

2.4.2 Percent Transmittance Test Results

Table 3. Test results of transmittance, viscosity, pH, and emulsification time

Formula NEDK	Transmittance (%)	Viscosity (cP)	pH	Emulsification Time (s)
F1	98.33±0.85	8.83±0.28	6.33±0.05	7.07±1.55
F2	99.03±0.32	10.83±1.04	6.3±0.0	9.60±2.70
F3	98.4±0.60	28.3±0.28	6.33±0.05	11.43±3.20
F4	95.23±3.08	28.83±0.28	6.43±0.05	18.32±3.61

The results of the percent transmittance test measurement can be seen in Table 3. The results showed that all purslane leaf extract nanoemulsion preparations (NEDK) had a transmittance percent value almost close to 100%. The formula meets the transmission percentage requirement when the transmission percentage value reaches 90 to 100%. So that the four NEDK variations have met the transmission percent requirements [21].

2.4.3 Viscosity Test Results

The results of viscosity test measurements can be seen in Table 3 which shows that all NEDK formulas are ideal nanoemulsion preparations because the viscosity values meet the requirements, namely in the range of 1 to 100 cP [22].

2.4.4 pH Test

The results of the pH test can be seen in Table 3 which shows that formula 1, formula 2, and formula 3 have a pH value of 6.3, and formula 4 has a pH value of 6.4.

2.4.5 Emulsification Time Test Results

The emulsification time test of all NEDK formulas can be seen in Table 4 showing that all NEDK formulas have an emulsification time of less than 1 minute. This qualifies for a good nanoemulsion because it indicates a clear emulsion system, so that the nanoemulsion formula is able to form an emulsion after direct contact with gastric liquids [23].

2.4.6 Solubility Test Results

The results of the solubility test in the research aim to compare the solubility of nanoemulsions. Based on Figure 3, all nanoemulsion formulations are perfectly soluble in ethanol and insoluble in ethyl acetate as well as insoluble in *n*-hexane. This shows that there is no change in the properties of purslane leaf extract when it becomes a nanoemulsion [17].

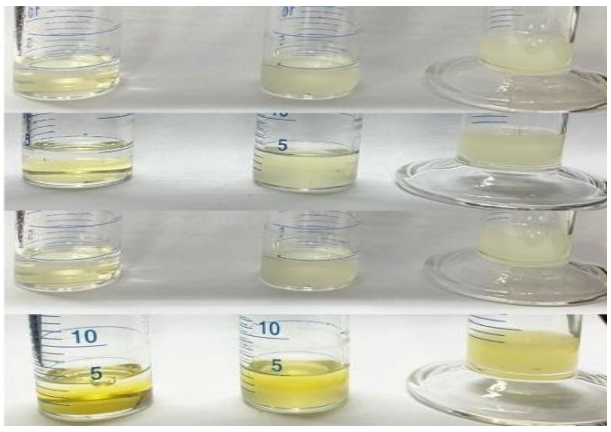

Figure 3. Solubility results of nanoemulsions in *n*-hexane (left), ethyl acetate (middle), and ethanol (right)

2.4.7 Cycling Test Results

Table 4. Cycling *test results*

Formula NEDK	Stability of Nanoemulsions		
	1 cycle	2 cycle	3 cycle
F1	stable	stable	stable
F2	stable	stable	stable
F3	unstable	unstable	unstable
F4	stable	stable	stable

The results of the cycling test in Table 4 show that the NEDK in F1, F2, and F4 is stable because there is no color change and 1 phase is still formed. However, in F3 it is unstable because there is a color change to cloudy as shown in Figure 4. The preparation that is said to be stable after a cycling test does not change color and does not change the aroma of nanoemulsion [24].

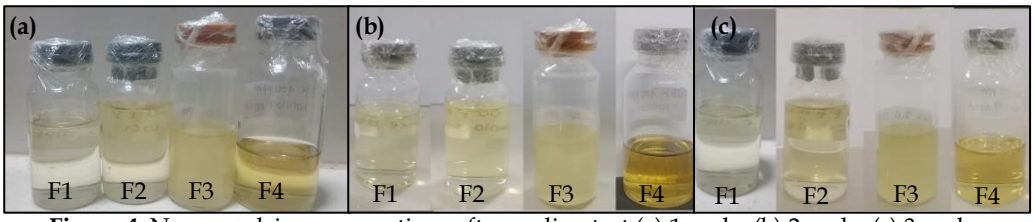

Figure 4. Nanoemulsion preparations after cycling test (a) 1 cycle; (b) 2 cycle; (c) 3 cycle

2.4.8 Particle Size and Zeta Potential Test Results

Table 5. Particle size and polydispersity index value

Formula NEDK	Particle size (nm)	Polydispersity Index
F1	10.96±0.47	0.204±0.10
F2	10.53±0.20	0.30±0.38
F3	11.23±0.11	0.23±0.07
F4	10.76±0.15	0.05±0.03

The particle size test results in Table 5 show that all nanoemulsion formulas have the appropriate size, namely in the range of 10 to 100 nm. All polydsipersity index values obtained < 0.5. This shows that the distribution of nanoemulsion particles is uniform and homogeneous [25].

Table 6. Zeta potential value

Formula NEDK	Zeta Potential (mV)
F1	-23.9±0.34
F4	-12.03±0.56

The measured zeta value of nanoemulsion potential is in F1 which has the lowest concentration of EDK and F4 which has the highest concentration of EDK. Both formulas have good zeta potential values in the range of -30 mV to +30 mV, indicating that the nanoemulsion preparations are stable [25].

2.5 Antidiabetic Activity

2.5.1 a-Amylase Enzyme Inhibition (In vitro)

The α-amylase enzyme inhibition test is obtained by calculating the IC_{50} value, the smaller the IC_{50} value indicates higher inhibition activity [26]. The highest IC_{50} value was successively obtained in purslane leaf extract at 7904.61 ppm; NEDK at 1225.38 ppm; and acarbosa at 32.04 ppm. The IC_{50} value of NEDK has a high α-amylase enzyme inhibition ability compared to the inhibition ability of purslane leaf extract. Acarbose has a smaller IC_{50} than NEDK due to the presence of other compounds that inhibit the α-amylase enzyme activity. The inhibitory activity is displayed in table 7.

Table 7. α-Amylase Enzyme Inhibition

Sample	IC_{50} (ppm)		
	Simplo	Duplo	Average
Purslane Leaf Extract	7900.07	7909.15	7904.61
NEDK	1235.41	1215.25	1225.38

2.5.2 Alloxan Induction (In vivo)

Blood sugar measurements were taken four times within 135 minutes, and the blood sugar levels of mice were measured at 45-minute intervals. Based on Figure 5, there are differences in the decrease in blood glucose levels in the four treatment groups. The highest decrease in blood glucose levels was found in the NEDK preparation group. The NEDK preparation group was higher than the positive control administration in this case was acarbose and purslane leaf extract administration. The decrease in blood glucose levels is good for NEDK because NEDK has a more stable delivery system and better solubility properties [25].

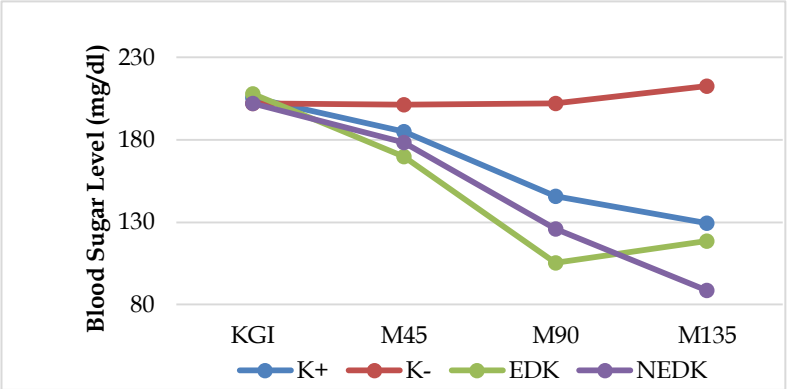

Figure 5. Blood Sugar Level Analysis

3. CONCLUSION

A nanoemulsion based on purslane leaves (*Portulaca oleracea*) extract has been successfully synthesized with nano-sized emulsion characteristics. The broad solubility of the nanoemulsion used in this investigation is another crucial feature. The ideal characteristics for nanoemulsion have been fulfilled, and more importantly, this product has better α-amylase enzyme inhibitory activity compared with purslane leaf extract. In addition, its potential utilization as an antidiabetic agent is also evident from the ability of the nanoemulsion to significantly reduce blood sugar levels in alloxan-induced mice.

4. MATERIALS AND METHODS

4.1 Materials

The materials used in this research are aquades, aloxans, pH 7 phosphate buffer, butylated hydroxytoluene (BHT), purslane leaves (*Portulaca oleracea*), α-amylase enzymes, ethanol p.a, HCl 1N, filter paper, starch solution (1%), propylene glycol, sorbitan monooleate, polysorbate, and virgin coconut oil (VCO).

4.2 Purslane Leaf Extraction

Extraction is carried out by the maceration method, previously dried purslane leaves are made into powder using a blender to obtain smooth simplicia. A total of 170 grams of purslane leaf powder macerated with ethanol p.a were put into a container, tightly closed, then left for 1×24 h at room temperature and stirred every 6 h. Samples are filtered using filter paper (Whatman No. 42). The maceration was carried out three times with the addition of ethanol solvent p.a and left for 1×24 h. The filtrate obtained is mixed, then evaporated and concentrated with a rotary evaporator (Hahnvapor HS 2000NS by Hahn Shin Scientific Co., South Korea) at a temperature of 45°C [22].

4.3 Purslane Leaf Extract Toxicity Test

The toxicity test was carried out using the BSLT method, and the sample was weighed at as much as 24 mg and then dissolved with 12 mL DMSO. The broodstock solution of purslane leaf extract was diluted with seawater followed by the addition of 2 mL of seawater containing 15 shrimp larvae that had been aged for 48 h to obtain an extract solution concentration of 125, 62.5, 31.25, and 15.625 ppm. The extract solution that has contained the shrimp larvae is then incubated for 24 h under an incandescent lamp. Then, the mortality rate (%) of shrimp larvae was calculated to calculate the LC_{50} value [27].

4.4 Test for Determination of Cuercetin Levels of Purslane Leaf Extract

Test to determine quercetin levels by making a standard solution of quercetin. The standard solution of quercetin is made with standard series concentrations of 2, 4, 6, 8, and 10 ppm, as well as a solution of 100 ppm purslane leaf extract which is sufficient with ethanol p.a. Each is pipetted 1 mL into a test tube. After that, 3 mL of ethanol p.a, 0.2 mL of $AlCl_3$ and 1 M of potassium acetate, as well as 5.6 mL of aquaades were added and incubated for 30 minutes at room temperature and their absorption was measured on a UV-Vis spectrophotometer (T60 UV-Visble Spectrophotometer by PG Instruments, United States) with a wavelength of 439 nm [18].

4.5 Preparation of Formulation of Nanoemulsion Preparations of Purslane Leaf Extract

Preparation of modified nanoemulsion preparations from Zulfa et al. (2023) [21] made with variations of purslane leaf extract presented in Table 8. The nanoemulsion is made using a magnetic stirrer at a speed of 890 rpm for 1 h. It starts by mixing sorbitan monooleate into polysorbates. Next, dissolve purslane leaf extract that has been dissolved in VCO until homogeneous. Then add BHT until homogeneous and add propylene glycol. Then, a phosphate buffer is added little by little and continued with sonication for 1.5 h.

Table 8. Purslane Leaf Extract Nanoemulsion Formula

Material (g)	Concentration (%b/b)			
	F1	**F2**	**F3**	**F4**
Purslane Leaf Extract	0.005	0.01	0.02	0.04
VCO	3	3	3	3
Polysorbate	27	27	27	27
Sorbitan monooleat	1	1	1	1
Propylene Glycol	5	5	5	5
BHT	0.1	0.1	0.1	0.1
Phosphate Buffer	ad 100	ad 100	ad 100	ad 100

4.6 Nanoemulsion Stability Test

4.6.1 Organoleptic Test

The organoleptic test was carried out by identifying aroma, color, texture, and homogeneity [28].

4.6.2 Transmittance Percent Test

The percentage transmittance of nanoemulsion preparations was measured by a UV-Vis spectrophotometer (T60 UV-Visble Spectrophotometer by PG Instruments, United States) at a maximum wavelength of 650 nm [29].

4.6.3 Viscosity Test

The viscosity test was carried out using *a Brookfield* viscometer with three repetitions [17].

4.6.4 pH Test

The pH measurement of nanoemulsions was carried out with a pH meter with 3 repetitions [28].

4.6.5 Nanoemulsion Solubility Test

The solubility test was carried out by mixing nanoemulsion with organic solvents in a ratio of 1:1 from various levels of polarity, namely *n*-hexane, ethyl acetate, and ethanol, then observing their solubility [17].

4.6.6 Nanoemulsion Emulsification Time Test

The emulsification time and nanoemulsion type test was carried out by dissolving the nanoemulsion into an aqueous with a ratio of 1:50 and then stirring at a speed of 400 rpm using a magnetic stirrer [29].

4.6.7 Cycling Test

Nanoemulsions are tested for stability over three cycles. One cycle consists of nanoemulsions placed at a temperature of 4°C for 24 h, then placed at a temperature of 40°C for 24 h Then the changes that occur are seen [25].

4.6.8 Particle Size and Zeta Potential Test

Particle size analysis was performed on all purslane leaf extract nanoemulsion formulas by using particle size analyzer (Nano Particle Analyzer Horiba SZ-100, Japan). Then a zeta potential analysis was carried out on the nanoemulsion of purslane leaf extract at the lowest concentration (F1) and the highest concentration (F2). The analysis was carried out using a particle size analyzer [30].

4.7 Antidiabetic Activity Test Test

4.7.1 a-Amylase Enzyme Inhibition (In vitro)

The inhibition activity test of α-amylase enzyme was carried out by utilizing starch as a substrate using the dinitrosalicylic acid (DNS) method. The concentration series of purslane leaf extract and carbose root 1000, 2000, 4000, and 8000 ppm, as well as the nanoemulsion of purslane leaf extract in formulas 1, 2, 3, and 4, as much as 0.1 mL were reacted with 0.2 mL α-amylase enzyme. Then 0.1 mL of phosphate buffer pH 6.9 was added and incubated for 20 minutes, then 0.1 mL of 1% starch was added and incubated for 5 minutes, then 0.5 mL of DNS was added, then heated for 10 minutes, then cooled. After that, the absorption is measured at the maximum wavelength (λ_{max}) [31].
Antidiabetic activity is calculated using the following equation:

$$\text{Percent Inhibition (\%)} = \frac{\text{Control absorbance - Sampel absorbance}}{\text{Control absorbance}} \times 100\%$$

The IC_{50} value is calculated based on a linear equation obtained from the graph between the concentration and the percentage of inhibition, so the equation for calculating IC_{50} is as follows:

$$x = \frac{y-b}{a}$$

$$x = \frac{50-b}{a}$$

Information:
y: percent inhibition (to calculate IC_{50} then y = 50
x: concentration (IC_{50})

4.7.2 Alloxan Induction Test (In vivo)

This exam is carried out under the permission and guidelines of the Research Ethics Commission of Hasanuddin University number 518/UN4.6.4.5.31/PP36/2024. First, a solution of aloxan 3.5 mg/20 g was given as a trigger for diabetes in mice that had been fasted for 8 h. After 3 days of induction, the blood was drawn. Then it was continued by providing treatment to four groups of mice, each of which consisted of five mice (Group I positive control (acarbose), group II negative control, Group III purslane leaf extract and group IV nanoemulsion purslane leaf extract with a dose of 5.6 mg/20 g). Experimental animals were measured for 135 minutes at 45-minute intervals using a *glucometer test* [32].

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