The Effect Of Nanoparticle Standardized Extracts Of Bay Leaves (Syzygium polyanthum (Wight) Walp.) In Lowering Blood Glucose Levels Of White Rats (Rattus norvegicus)

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Abstract

Objective: This research aimed to develop the potential of nanoparticles derived from the standardized extract of bay leaves (*Syzygium polyanthum* (Wight) Walp) to be a herbal medicine with fine quality and efficacious in lowering blood glucose levels.

Methods: The preparation of nanoparticles of standardized extract of bay leaves was done using ionic gelation method with chitosan-tripolyphosphate base. The experimental designs used in this study were pre and post test-controlled group designs. Wistar white rats were divided into 6 groups containing 5 rats in each group. The group was divided into negative control, normal control, and positive control (Glibenclamide) groups and a control group that was given the test dosages. Measurement of fasting blood glucose levels was carried out using the enzymatic glucometer (GlucoDr) method by taking blood samples from the tails of rats. Analysis of the area under the curve (AUC) was carried out based on the trapezoidal formula and was statistically analyzed using the Kruskal-Wallis and Mann-Whitney tests with a 95% confidence level.

Results: The results shows that the simplicia and the ethanolic extracts of bay leaves meets the quality requirements and the secondary metabolites contained in it are alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, glycosides, and essential oils. Total flavonoid content in 96%, 70%, 50% ethanolic extracts of bay leaves and in the nanoparticles of standardized extract are 1.48%; 1.62%; 1.50%; and 0.03%, respectively. From the evaluation of the nanoparticles, it is found that the average particle size is 549.2 nm, and the zeta potential is -40.2 mV. Administration of the nanoparticles standardized extract at a dose of 426.80 mg/kgBB; 213.40 mg/kgBW; and 106.70 mg/kgBW shows a decrease in blood glucose levels which has no significant difference (P>0.005) when compared to positive controls. The smallest dose of nanoparticles extract that can reduce blood glucose levels is at a dose of 106.70 mg/kgBW.

Conclusion: The nanoparticles standardized extract of bay leaves (Syzygium polyanthum (Wight) Walp.) meet quality requirements and can reduce blood glucose levels.

Keywords: Blood glucose levels, ethanolic extract, *in vivo Syzygium polyanthum* (Wight) Walp., nanoparticle

INTRODUCTION

According to the International Diabetes Federation report, in 2019, there were 463 million adults suffering from diabetes. This number is expected to increase to 578 million in 2030 and

to 700 million in 2045 with the increase rate of 51%. In Southeast Asia, the increase was even greater (74%), where in 2019 it was 88 million, and is expected to increase to 115 million in 2030 and 153 million in 2045. Indonesia is included in the top 10 countries with the highest number of diabetics in the world. In 2019, the number of people with diabetes in Indonesia was 10.7 million, and it is estimated that it will increase to 13.7 million in 2030 and 16.6 million in 2045 [1]. Various efforts to prevent, treat, and reduce the risk of side effects arising from the use of chemically synthesized drugs continue to be carried out, including by developing natural materials by taking into account the requirements for its quality, safety and benefits. An ethnomedicine study conducted on the Sasak tribe, Mataram showed that bay leaves were used to lower blood sugar levels [2].

The chemical content contained in bay leaves are essential oils, tannins, and flavonoids [3]. In previous studies, the active compounds of flavonoids and tannins of the ethanolic extract of bay leaves were proven to increase the levels of GLUT 4 in adipose tissue of experimental animals because it is suspected that these two compounds had a synergistic effect in increasing glucose uptake (glucose reabsorption). In addition, one of the biological activities of flavonoids associated with diabetes mellitus is as an antioxidant, in which one of the functions of antioxidants is to protect lipids from the effects of oxidation by various mechanisms [4]. Tannins stimulate the metabolism of glucose and fat so that the accumulation of these two sources of calories in the blood can be avoided. Tannins have antioxidant activity and hypoglycemic activity by increasing glycogenesis. Tannins also function as a chelating agent (astringent) that can shrink the epithelial membrane of the small intestine thereby reducing the absorption of food essences. As a result, it inhibits sugar intake and the rate of increase in blood sugar becomes stable [5].

However, herbal plants and their active substances are mostly less water soluble and have poor bioavailability leading to poor absorption in the gut, for instance the flavonoid compounds which are the most abundant in the plant kingdom [6]. Therefore, it is necessary to modify the performance physically or chemically to overcome these deficiencies and to increase the solubility of the active substances. One of the physical modifications that can be taken is a modification in terms of formulation. Nanoparticle is one of the formulations that can overcome these problems because nanoparticle is able to improve the solubility of herbal medicines and it can reach specific targets. A nanoparticle refers to colloidal systems with particle sizes ranging from 10 to 1000 nm. Some of the advantages of nanoparticles are increased solubility and absorption of herbal medicines, increased bioavailability, and efficacy, reduced dose, and better drug safety. Reducing the therapeutic dose can improve patient compliance [7].

MATERIALS AND METHODS

Plant material

The fresh bay leaves were obtained from the Garden of the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), Bogor. The plants were determined at the Herbarium Bogoriense, The Research Center for Biology, Indonesian Institute of Sciences (LIPI). It shows that the plant is included in the species of *Syzygium polyanthum* (Wight) Walp. from the *Myrtaceae* family (No: B-116/IV/DI.01/1/2021).

Chemical and reagent

Ethanols of 96%, 70%, and 50 %, aquadest, hydrochloric acid, ammonia, ammonia hydroxide, chloroform, Bouchadart's reagent, Dragendorff's reagent, Mayer's reagent, magnesium plate, 25% hydrochloric acid, amyl alcohol, 1% solution of iron(III) chloride, Stiasny's reagent, sodium acetate, ether, acetic anhydrous acid , concentrated sulfuric acid, 1N sodium hydroxide, phenolphthalein, petroleum ether, chitosan, sodium tripolyphosphate (NaTPP), propylene glycol, DMSO, silica gel 60 F_{254}, ethyl acetate, formic acid, 5% solution of

citroborate, maltodextrin, male white rats (*Rattus norvegicus*) wistar strain, alloxan, sterile distilled water for injection, glibenclamide (Indofarma), Na CMC, quercetin comparison standard.

Making extract powder derived from bay leaves

The bay leaves were determined at the Research Center for Biology, Indonesian Institute of Sciences (LIPI); the dried bay leaves were determined as foreign organic matter (BOA). The leaves were blended and the degree of smoothness of the simplicia was measured.

Making ethanolic extract of bay leaves

Dried bay leaves powder was macerated with 96%, 70%, and 50% of ethanols. The obtained macerate was collected and concentrated with a vacuum rotary evaporator then the DER-Native and the extract yield were calculated.

Examination of the extract quality parameters

a. Specific Parameters
    1) Organoleptic
    2) Levels of dissolved compounds in ethanol
    3) Levels of dissolved compound in water
b. Non-specific Parameters
    1) Water content using Karl Fischer
    2) Total ash content using gravimeter
    3) Acid insoluble ash content using gravimeter
    4) Density using densitometer
    5) Heavy metal contamination using Atomic Absorption Spectrophotometry (AAS)
    6) Microbiological contamination by figuring out the Total Plate Counts (TPC), the Total Yeast and Mold Counts (TYMC), and the pathogenic bacteria

Total flavonoid levels determination and identification of thin layer chromatography (TLC)

Total flavonoid levels determination

Test Solution:

Measure carefully a 0,2 grams of extract, put it into the erlenmeyer, add 25 mL ethanol P, extract for 1 hour on a magnetic stirrer. Strain into a 25 mL measuring flask, rinse the filter paper with ethanol P and add ethanol P until it reaches the mark.

Comparison Solution:

Measure carefully a 10 mg of quercetin, put into a 10 mL measuring flask, dissolve and add ethanol P until it reaches the mark. Make a series of dilutions of comparison solution with levels of 80, 70, 60, 50, and 40 µg/mL, respectively.

Identification of Thin Layer Chromatography (TLC)

Stationary phase: Silica Gel 60 F_{254}, 10 x 7 cm; thickness: 0,20 mm.
Mobile phase: Toluen: Aceton (10 : 10 + 3 drops of acetic acid).
Test solution: 0.5 grams of each ethanol extract (96%, 70%, and 50%), and the nanoparticles of bay leaves extract were hydrolyzed by the hexamine method in 5 cc of ethyl acetate.
Comparison solution: quercetin 0,01 % in methanol
Spotting volume: 25 µL of test solution and 15 µL of comparison solution
Detection: 5 % of citroborate solution in ethanol. Plate heated at at the temperature of 100^{o} C for 5-10 minutes at UV_{366}.

Making nanoparticles extract by ionic gelation method

Every 1 gram of viscous extract was dissolved in 50 mL of 1% DMSO + 50 mL of PPG + 50 mL of 70% ethanol + 50 mL of aquadest + 50 mL of 1% chitosan on a magnetic stirrer then dripped with 4 mL of 0.2% NaTPP which is soluble in aquadest. The addition of 0.2% NaTPP solution was carried out by dripping at a speed of 1 drop/3 seconds using a burette and stirring with a magnetic stirrer until nanoparticles were formed which were characterized by homogeneous turbidity. Leave for 15 minutes and then observe the stability of the nanoparticle suspension including the color, turbidity and sediment for 5 days.

Spray drying

The drying of the nanoparticle suspension used additional maltodextrin filler with an inlet temperature of 210^{o}C and outlet 90^{o}C. The examination results of the water content in the nanoparticle powder of bay leaves was 5.10%.

Test of blood glucose lowering activities

This research was conducted based on the ethical approval letter of Health Research Ethics Commission (KEPK), UHAMKA University in Jakarta number 03/21.02/0862. The test animals used were white rats (*Rattus norvegicus*) wistar strain aged 8-10 weeks and body weight of 170-200 grams. All rats were acclimatized for (approximately) 7 days which were randomly divided into 6 groups, each consisting of 5 rats and induced alloxan at a dose of 150 mg/kg BW intraperitoneally. Measurements of body weight and blood glucose levels were carried out on the day 0, the 8th day (hyperglycemic condition) until blood glucose levels 200 mg/dl, the 15th day, and the 22nd day. The rats must be fasted for approximately 10 hours each time their blood glucose levels are measured. The measurement of blood glucose levels by glucometer (Gluco Dr) and the blood samples were taken through the tail in the perpendicularis channel on the surface using a syringe. Each test dosage was administered orally. After completing the experiment, the rats are sacrificed. In principle, test animals are sacrificed according to the rules of the Declaration of Helsinki (2008) by neck dislocation.

Preliminary test

Group I : Normal group
Group II : **Negative control group (alloxan-induced)**
Group II : **Positive control group (Glibenclamide at a dose of 0.45mg/kg BW)**
Group IV : 96% ethanolic extract of bay leaves at a dose of 625 mg/kg BW
Group V : 70% ethanolic extract of bay leaves at a dose of 625 mg/kg BW
Group VI : 50% ethanolic extract of bay leaves at a dose of 625 mg/kg BW

Advanced test

Group I : Normal group
Group II : **Negative control group (alloxan-induced)**
Group III : **Negative control group (**Glibenclamide at a dose of 0.45mg/kg BW)
Group IV : Nanoparticle of bay leaves standardized extract at a dose of 426.80 mg/kg BW
Group V : Nanoparticle of bay leaves standardized extract at a dose of 213.40 mg/kg BW
Group VI : Nanoparticle of bay leaves standardized extract at a dose of 106.70 mg/kg BW

Analysis Area Under Curve (AUC) after administration of test dosages

The area of AUC was analyzed using data obtained by calculating the area of AUC from each rat blood glucose level after alloxan was induced and administered with the test dosage from the 8th day, 15th day, and 22nd day. The AUC is calculated using trapezoid formula as follows:

$$\text{Area}_1 = \frac{(BGL\ on\ the\ 8th\ day) + (BGL\ on\ the\ 15th\ day)}{2}\ X\ day\ difference$$

$$\text{Area}_2 = \frac{(BGL\ on\ the\ 15th\ day) + (BGL\ on\ the\ 22nd\ day)}{2}\ X\ day\ difference$$

Explanation:
BGL = Blood Glucose Level
The 8th day = Hyperglycemia on blood glucose levels
The 15th day = Blood glucose levels after 7 days of treatment
The 22nd day = Blood glucose levels after 14 days of treatment
Wherein, in order to get the total area of AUC in each rat, the area of the trapezoid is summed up ($Area_1$+$Area_2$). Calculation of the AUC area was then continued to get the percentage of decrease in blood glucose levels.

Percentage of decrease in blood glucose level
The percentage of the blood glucose levels of rats shows the ability of the test to lower the blood glucose levels of rats.
The formula:

$$P = \frac{AUC\ of\ Control - AUC\ of\ dosage\ test}{AUC}\ X\ 100\%$$

Explanation:
P = Percentage of decrease in blood glucose level
AUC = Area Under Curve
Control = Negative group

Test dosage =
- Positive group (Glibenclamide)
- Nanoparticles of bay leaves extract at a dose of 426.80 mg/kg BW
- Nanoparticles of bay leaves extract at a dose of 213.40 mg/kg BW
- Nanoparticles of bay leaves extract at a dose of 106.70 mg/kg BW

RESULTS AND DISCUSSION

Making powder from bay leaves extract

The foreign organic matter (BOA) of the simplicia of bay leaves was 0.17%. It was then powdered and from the results obtained a fine degree of 4/18 meaning that the powder can pass through the number 4 sieve by 100% and the powder that can pass through the number 18 sieve is not more than 40%.

Making bay leaves ethanolic extract

After making the extract, the DER-native and extract rendemen were determined. The results can be seen in Table 1.

Table I. DER-native and rendemen results

Extracts	DER-*Native*	Rendemen	Rendemen requirements*	Examination results
96% Ethanolic Extract	5,2071	19,21%	Not less than 18,2%	Fulfilled
70% Ethanolic Extract	2,9756	33,61%		Fulfilled
50% Ethanolic Extract	2,3008	43,46%		Fulfilled

***** *Indonesian Herbal Pharmacopoeia standard, Second Edition, 2017*

Phytochemical screening and extracts quality examination

The results of the phytochemical screening on simplicia powder of 96%, 70% and 50% ethanolic extracts of bay leaves showed that it has secondary metabolites of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, glycosides, and essential oils.

Table II. Examination results of the quality of viscous ethanolic extracts of bay leaves

Parameters	**Examination Results**			**Requirements**	**Examination Results**
	96% Ethanolic Extract	**70% Ethanolic Extract**	**50% Ethanolic Extract**		
Latin name	*Syzygium polyanthum* (Wight) Walp.	*Syzygium polyanthum* (Wight) Walp.	*Syzygium polyanthum* (Wight) Walp.	-	-
Organoleptic	Extract is viscous, brownish green, weak aromatic smell	Extract is viscous, brownish green, weak aromatic smell	Extract is viscous, brownish green, weak aromatic smell	Extract is viscous, brownish green, weak aromatic smell	Qualified
Levels of dissolved compounds in water	15.65%	25.38%	25.33%	Not less than 14,8%*	Qualified
Levels of dissolved compounds in ethanol	35.90%	24.09%	35.90%	Not less than 19,9%*	Qualified
Water content	8.51%	8.92%	8.88%	Not more than 10%*	Qualified
Total ash content	0.49%	1.,65%	2.28%	Not more than 2.5%*	Qualified
Acid insoluble ash content	0.05%	0.38%	0.17%	Not more than 0,2%*	Qualified
Density	0.9934 g/cm^3	1.0078 g/cm^3	1.0083 g/cm^3	-	Qualified
Metal contamination	- Pb = ≤ 10 mg/kg - Cd = ≤ 0.3 mg/kg - As = ≤ 5 mg/kg - Hg = ≤ 0.5 mg/kg	- Pb = ≤ 10 mg/kg - Cd = ≤ 0.3 mg/kg - As = ≤ 5 mg/kg - Hg = ≤ 0.5 mg/kg	- Pb = ≤ 10 mg/kg - Cd = ≤ 0.3 mg/kg - As = ≤ 5 mg/kg - Hg = ≤ 0.5 mg/kg	- Timbal (Pb) ≤ 10 mg/kg - Kadmium (Cd) ≤ 0,3 mg/kg - Arsen (As) ≤ 5 mg/kg - Timbal (Hg) ≤ 0.5 mg/kg**	Qualified
Microbiological contamination	- ALT = <10 Cfu/g - AKK = <10 Cfu/g - E. coli = <10 Cfu/g - *Staphyloccus aureus* = Negative	- ALT = 70 Cfu/g - AKK = 10 Cfu/g - E. coli = <10 Cfu/g - *Staphyloccus aureus* = Negative	- ALT = <10 Cfu/g - AKK = <10 Cfu/g - E. coli = <10 Cfu/g - *Staphyloccus aureus* = Negative	- ALT ≤ 10^5 koloni/g - AKK ≤ 10^3 koloni/g - *Escherichia coli* negative/g - *Staphylococcus aureus* negatif/g	Qualified

- *Salmonella* sp. = Negative - *Pseudomonas aeruginosa* = Negative	- *Salmonella* sp. = Negative - *Pseudomonas aeruginosa* = Negative	- *Salmonella* sp. = Negative - *Pseudomonas aeruginosa* = Negative	- *Salmonella* negative/g - *Pseudomonas* negative/g**

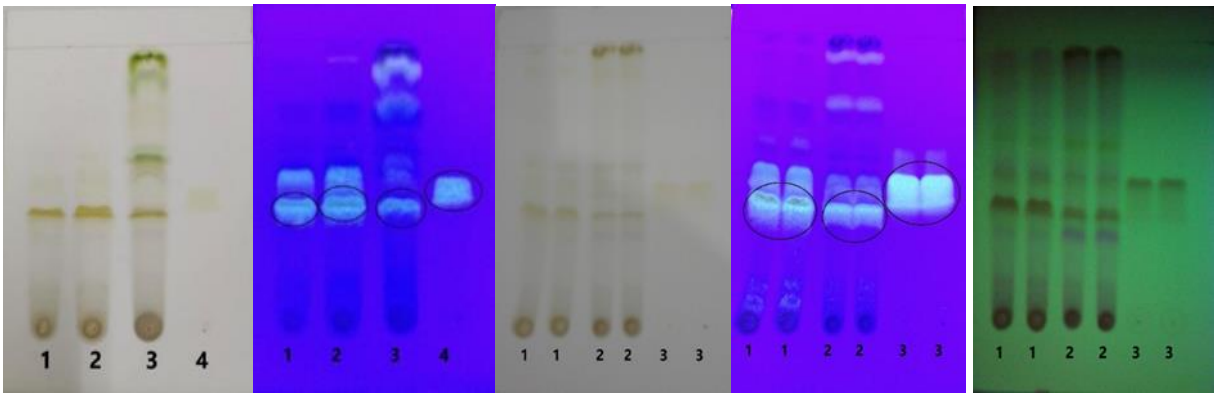
** Indonesian Herbal Pharmacopoeia standard, Second Edition, 2017*
*** National Agency of Drug and Food Control of Indonesia No. 32 of 2019*

Total flavonoid content determination using spectrophotometer

Table III. Determination results of total flavonoid content

Bay leaves extract samples	Total flavonoid content (as quercetin)
96% ethanolic extract	1,4775%
70% ethanolic extract	1,6165%
50% ethanolic extract	1,4960%
Nanoparticles powder	0,030%

TLC Identification



A **B** **C** **D** **E**

Figure 1. TLC plates of 50%, 70%, 96% ethanolic extracts of bay leaves and quercetin comparison (A); at UV366 (B); 50% ethanolic extract, nanoparticles of ethanolic extract and quercetin comparison (C); at UV_{366} (D); at UV_{254} (E)

The TLC test results on 96%, 70%, and 50% ethanolic extracts of bay leaves showed that the extracts contained quercetin marked by spots on the plate when irradiated at UV_{366} and had an Rf value of 0.68 (Figure A dan B). Figure C, D, E show that the nanoparticle powder and the 50% ethanolic extract contain quercetin marked by spots on the plate when irradiated at UV_{366} and have an Rf value of 0,68 to 0,70. Therefore, it can be concluded that the content between the nanoparticle powder and the 50% ethanolic extract of bay leaf remains the same even though it has been through the spray drying process.

Evaluation results of nanoparticles suspension: Examination on particle size, particle distribution, and zeta potential

Table IV. Evaluation results of nanoparticles suspension

Parameters	Results
Average particle size	549.2 nm
Polydispersity index	0.378
Zeta potential	-40.2 mV

The suspension of a nanoparticle is said to be nano-sized if the particle diameter is 10-1000 nm. Table 4 shows that the nanoparticle suspension of the extract has a particle size that meets the requirements as a nanoparticle suspension. Particle size distribution is expressed in terms of polydispersity index. A high polydispersity index indicates a high surface tension between the organic phase and the aqueous phase, while a low polydispersity index indicates a small surface tension which causes the formation of smaller particle sizes. The polydispersity index ranges from 0 to 1. A polydispersity index value close to 0 indicates a homogeneous dispersion while the polydispersity index with a value of more than 0.5 indicates high heterogeneity. The result of the examination of the polydispersity index of the nanoparticles of bay leaves ethanolic extract is 0.3 - 0.4 thereby the dispersion is relatively homogeneous.
The greater the repulsive force between the particles, the smaller the possibility of the particles joining and forming aggregates. This is related to the binding of the anionic group by the long amino group of chitosan to a high electrical value to prevent the formation of aggregation. Nanoparticles with a zeta potential value of more than +/- 30 mV have been shown to be stable and able to prevent aggregation. The zeta potential of the nanoparticles suspension of bay leaves extract has a value of -40.2, which means that the residue from the amino group in chitosan is not able to bind all the negatively charged particles in the extract so that the zeta potential value becomes negative. This can prevent particle aggregation between the nanoparticles.

Particle morphology of nanoparticles

Test on particle morphology of nanoparticles using SEM (*Scanning Electron Microscope*).

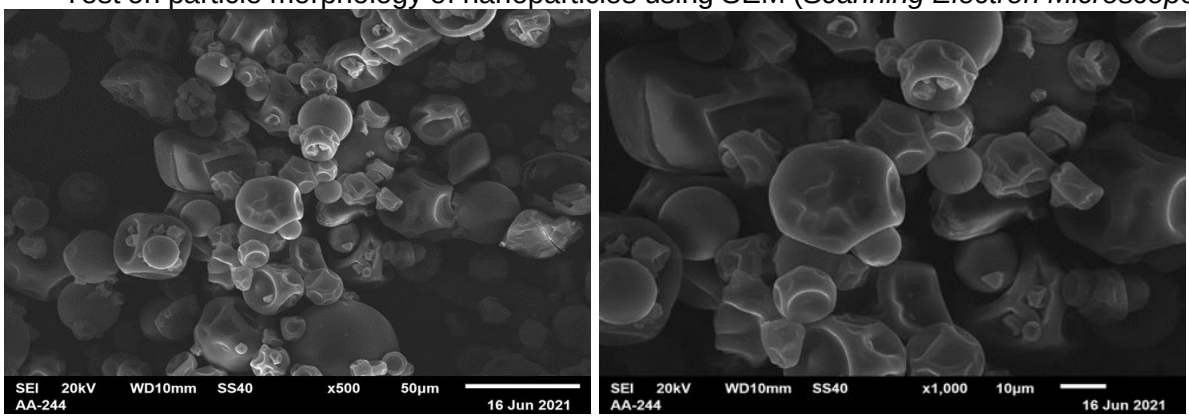


Figure 2. Particle morphology of bay leaves nanoparticles

The results of the SEM test of nanoparticles powders showed that the surface morphology was round, shriveled, rough surface, and the shape tended to be non-uniform. It can be caused by the addition of maltodextrin fillers that do not completely coat the extract, or because the high drying temperature causes the particle shape to become shriveled and not uniform.

Results of blood glucose lowering activity test by in vivo

In the preliminary test, the calculation results of the percentage of decrease levels of the rats blood glucose using 70% bay leaves ethanolic extract at a dose of 625 mg/kgBW had the highest ability to reduce the blood glucose levels by 29.59%, followed by the group of 50% ethanolic extract at a dose of 625 mg/kg BW by 27.33%, and the group of 96% ethanolic extract at a dose of 625 mg/kg BW by 26.51%. Nevertheless, statistically, the three groups of test preparations did not have a significant difference, so that 50% ethanol extract of bay leaves was selected to be processed into powdered extract nanoparticles. The nanoparticle powder was then tested for its activity in lowering the blood glucose levels of rats. The reason for choosing a small ethanol concentration is that in the future, the manufacture of bay leaf extract nanoparticles can be implemented on an industrial scale because the use of small concentrations of ethanol can reduce production costs but does not reduce the effectiveness of the extract.

In the advanced test, the treatment group was given test dosages of nanoparticles extract powder at a dose of 426.80 mg/kg BW, 213.40 mg/kg BW, and 106.70 mg/kg BW for 14 days. The dose selection was based on the calculation of the equivalent dose of 625 mg/kg BW with the dry extract powder contained in the nanoparticles (426.80 mg/kg BW), then reduced by half to 213.40 mg/kg BW. Then, it was reduced again by half to 106.70 mg/kg BW. The dose reduction aims to see the smallest dose of bay leaf extract nanoparticles that can still have the effect of reducing blood glucose levels.

Table V shows that on the 8th day (hyperglycemic conditions), groups II, III, IV, V and VI experienced weight loss, but group I experienced weight gain. Weight loss occurs according to the theory that one of the typical symptoms in diabetics is the occurrence of weight loss even though their appetite is very good. The weight loss of rats was due to the body's energy requirements from glucose metabolism being unable to be met, resulting in an overhaul of fat and protein [8].

On the 22nd day, after being given the test dosage for 14 days, all groups experienced an increase in body weight. In group I, weight gain was caused by the food consumed every day and as the rats got older, their body weight also increased. The results can be seen in Figure 3.

Table V. Rats weighing results

Treatment Groups	Body Weight (gram)			
	0	8th day (Hyperglycemic conditions)	15th day	22nd day
Normal	181.60 ± 5,24	184.40 ± 6,18	186,60 ± 6,28	188.60 ± 5.92
Negative	182.00 ± 8,49	179.20 ± 8,08	179.80 ± 8,16	181.60 ± 6,77
Positive	183.40 ± 6.10	180.80 ± 6,90	183.20 ± 6,70	185.20 ± 6.70
Nanoparticles at a dose of 426.80 mg/kg BW	182.60 ± 7,36	178.80 ± 7,86	182.20 ± 8,21	186.60 ± 7.42
Nanoparticles at a dose of 213,40 mg/kg BW	183.40 ± 6.50	179.80 ± 5.91	182.20 ± 5.95	186.60 ± 6.62
Nanoparticles at a dose of 106.70 mg/kg BW	183.60 ± 6.41	180.80 ± 6.79	182.20 ± 6.05	184.60 ± 5.68

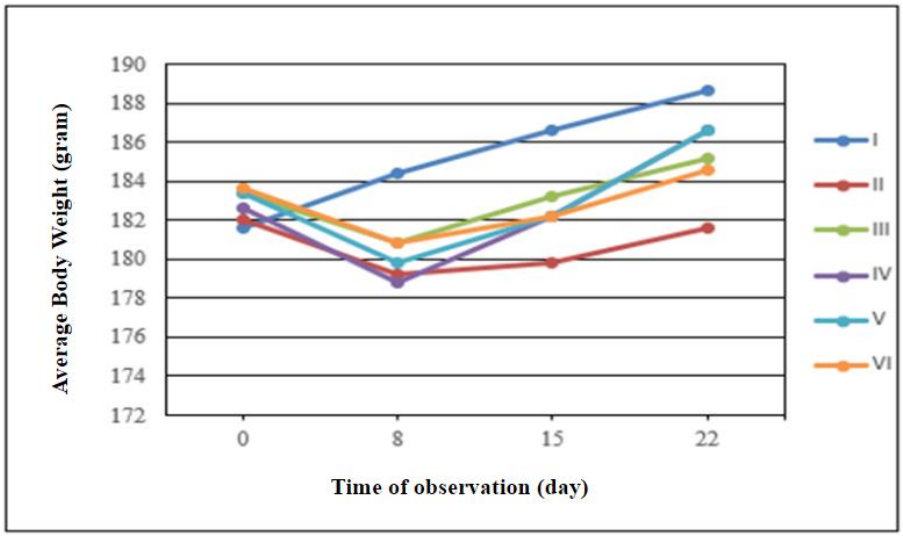


Figure 3. Observation of average body weight

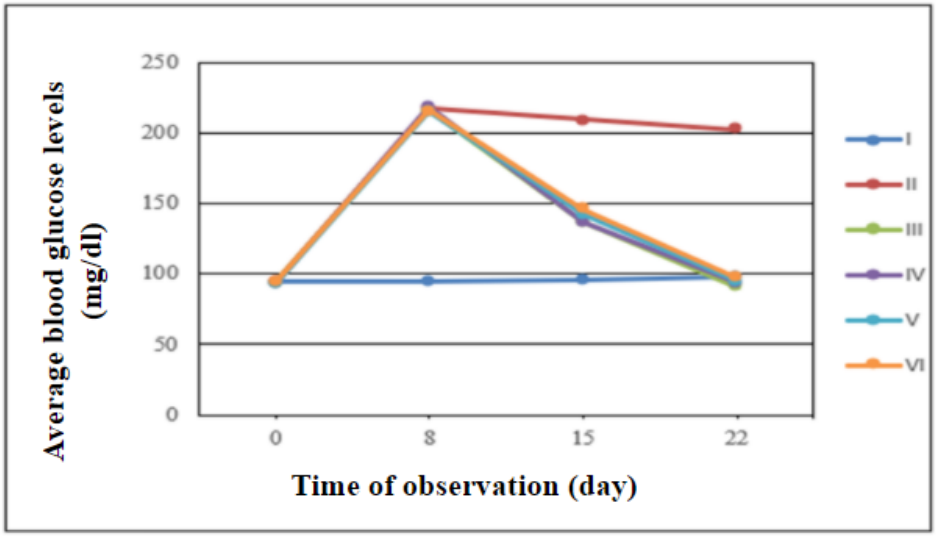


Figure 4. Observation of average blood glucose levels

On day 0 (beginning), measurement of blood glucose levels was carried out to ensure the uniformity of the initial blood glucose levels in each rat as well as the amount of food and drink in the initial conditions had not increased because they were still in normal conditions.

The group II was used as a negative control and as a comparison to the group that was given the test dosage (Groups IV, V, and VI) in order to show a decrease in the blood glucose levels of rats after the administration. If the rat has an average glucose level above 200 mg/dL, the rat has hyperglycemia. The reason for giving alloxan to group II was for the regeneration and neogenesis of pancreatic beta cells so that after induction there would be an increase in blood glucose and the condition of the rats could return to normal within a few months. Group III was used as a positive control to show that the test method used was valid in the presence of a decrease in blood glucose levels of rats after glibenclamide administration..

On the 22th day, the blood glucose levels of groups IV, V and VI were statistically analyzed to determine whether the administration of test dosage including the ethanolic extracts of bay leaves and the nanoparticles extract could reduce blood glucose levels to normal when

compared to group I (normal). The results of statistical analysis showed that there were no significant differences in groups IV, V, and VI. The results can be seen in Table 6.

Table VI. Examination on blood glucose levels of the rats

Treatment Groups	Blood Glucose Levels (mg/dL)			
	Day 0	8th day (hyperglycemic conditions)	15th day	22nd day
Normal	94.40 ± 5,75	94.80 ± 7.19	96,00 ± 6.32	98.00 ± 7.16
Negative	94,80 ± 9,20	217.60 ± 16.98	209.80 ± 18.32	202.40 ± 15.50
Positive	94.40 ± 9.13	216.40 ± 18.47	136.80 ± 4.53	90.80 ± 8.13
Nanoparticles at a dose of 426.80 mg/kg BW	93.40 ± 6.22	218.80 ± 10.98	137.00 ± 5.44	93.60 ± 6,34
Nanoparticles at a dose of 213.40 mg/kg BW	93.60 ± 9.93	216.00 ± 18.73	142.40 ± 13.54	94.80 ± 10.36
Nanoparticles at a dose of 106.70 mg/kg BW	94.60 ± 9.75	216,60 ± 8.80	146.00 ± 9,90	97.60 ± 9.56

The area under the curve (AUC) was analyzed on the 8th day to the 22nd day in each group. The results can be seen in Table 7. The percentage of decrease in blood glucose levels of the rats from each test dosage was calculated and the results showed that the nanoparticles extract at a dose of 426.80 mg/kgBW had the highest ability to reduce blood glucose levels in rats by 29.35%, followed by the nanoparticles extract with a dose of 213.40 mg/kgBW by 28.24%, and the nanoparticles extract with a dose of 106.70 mg/kgBW by 26.96%. Nevertheless, statistically, the results of the three test preparation groups did not have a significant difference. The results can be seen in Table 8.

Table VII. Analysis results of AUC after administration of test dosages

Treatment Groups	AUC (mg x day/dL)
Normal	1346.80 ± 94.26
Negative	2905,00 ± 122.59
Positive	2032.80 ± 110.98
Nanoparticles at a dose of 426.80 mg/kg BW	2052.40 ± 59.34
Nanoparticles at a dose of 213.40 mg/kg BW	2084.60 ± 183.10
Nanoparticles at a dose of 106.70 mg/kg BW	2121.70 ± 101,09

Table VIII. Percentage of decrease in blood glucose levels

Test dosages	Percentage of decrease (%)
Glibenclamide	30.02
Nanoparticles at a dose of 426.80 mg/kg BW	29.35
Nanoparticles at a dose of 213.40 mg/kg BW	28.24
Nanoparticles at a dose of 426.80 mg/kg BW	26.96

The ability of the ethanolic extract derived from bay leaves in lowering blood glucose levels of rats which are induced by alloxan is related to the content of secondary metabolites in it which synergize with each other. It was mentioned in previous research that the active compounds are flavonoids and tannins [4]. In addition, the nanoparticle has the capabilities in reaching therapeutic organs, penetration capabilities and an extended contact time. Moreover, chitosan as a carrier in nanoparticles can protect drugs from degradation that occurs due to gastric acid therebay the bioavailability of drugs in the blood is better without chitosan.

CONCLUSION

The results shows that the simplicia and ethanolic extracts of bay leaves meets the quality requirements and the secondary metabolites contained in it are alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, glycosides, and essential oils. Total flavonoid content in 96%, 70%, 50% ethanolic extracts of bay leaves and in the nanoparticles of standardized extract are 1.48%; 1.62%; 1.50%; and 0.03%, respectively. From the evaluation of nanoparticles, it is found that the average particle size is 549.2 nm and the zeta potential is -40.2 mV. Administration of the nanoparticles standardized extract at a dose of 426.80 mg/kg BW; 213.40 mg/kg BW; and 106.70 mg/kg BW shows a decrease in blood glucose levels which has no significant difference (P>0.005) when compared to positive controls. The smallest dose of nanoparticles extract that can reduce blood glucose levels is at a dose of 106.70 mg/kg BW.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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