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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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ARTICLE HISTORY

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Abstract

Several studies revealed that *Syzygium polyanthum* (Wight) Walp, known as bay leaves, has the potential to be used as herbal medicine due to its active compounds including flavonoids. Herbal medicines are chosen to replace synthetic drugs which have side effects on health. However, some active substances of herbal medicines including bay leaves are less soluble in water and have low bioavailability to be absorbed by the intestine is low. A formulation into nanoparticles will provide more effective results. This study aimed to develop a standardized herbal medicine from the bay leaves extract in nanoparticle form with fine quality and efficacious in lowering blood glucose levels. The nanoparticle formulation was conducted using the ionic gelation method with chitosan- tripolyphosphate base and was designed into pre- and post-test-controlled group designs. Wistar white rats (*Rattus norvegicus*) were used as the experimental animal and grouped into a negative control group, normal control group, positive control(Glibenclamide) groups, and test dosages-induced control group. Fasting blood glucose levels were measured using an enzymatic glucometer and the AUC was analyzed based on the trapezoidal formula statistically using the Kruskal-Wallis and Mann-Whitney tests.The results showed that the bay leaves contained secondary metabolite including flavonoid with concentrations at 96%, 70%, 50%, respectively. The nanoparticles sizes were 1.48%; 1.62%; 1.50%; and 0.03%, respectively. The average particle size was 549.2 nm, and the zeta potential was -40.2 mV. Nanoparticle administration at a dose of 426.80 mg/kg BW; 213.40 mg/kg BW; and 106.70 mg/kg BW showed decreasing blood glucose levels when compared to the positive control group but not significant (P>0.005). The smallest dose of nanoparticle extract that lowered blood glucose levels was at a dose of 106.70 mg/kg BW. It can be concluded the nanoparticles form of bay leaves extract can lower blood glucose levels and meets the quality requirements.

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

Introduction

According to the International Diabetes Federation report, in 2019, 463 million adults suffer from diabetes. This number is expected to increase to 578 million in 2030 and to 700 million in 2045 with an increased rate of 51%. In Southeast Asia, the increase waseven 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 withthe highest number of diabetics in the world. In 2019, the number of people with diabetesin this country 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] On the other side, various efforts to prevent and treat diabetes disease and at the same time reduce the risk of side effects arising from the use of chemically synthesized drugs continue to be carried out including the use of natural ingredients while considering the requirements for quality, safety, and efficacy.

Syzygium polyanthum (Wight) Walp or known as bay leaves is a plant widely spread in Indonesia.[2] The tree has a lush title with leaves that are elongated and pointed at the base. The leaves and flowers have a fragrant aroma.[3] Bay leaves have been widely usedas a traditional medicine to treat various diseases, especially the disease that is a risk factor for the emergence of other diseases, namely diabetes mellitus. Many regions in Indonesia traditionally used bay leaves as herbal medicine.[2] This is due to the chemicalcontent of bay leaves, namely essential oils, tannins, and flavonoids which have medicinal properties.[3]

A previous study[4] revealed that the active compounds of flavonoids and tannins of the ethanolic extract of bay leaves were proven to increase the levels of GLUT 4 in the 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 mellitusis as an antioxidant, in which one of the functions of antioxidants is to protect lipids fromthe effects of oxidation by various mechanisms.[4] The content of other compounds in thisplant, namely tannins, stimulates 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 and hypoglycemic activities by increasing glycogenesis which functions as an astringent. Astringents can shrink the epithelial membrane of the small intestine, thereby reducing the absorption of food essence, inhibiting sugar intake, and stabilizingthe rate of increase in blood sugar.[5]

However, the active substances of bay leaves including the flavonoid compounds when used as medicine are mostly less water soluble and have poor bioavailability. These disadvantages lead to poor absorption in the intestine of the body.[6] Therefore, it is necessary to improve the efficacy of the herbal medicine by modifying the performanceof the medicine physically or chemically so that even using small doses can still improvethe water soluble and bioavailability of the medicines in curing the disease. One of the physical modifications that can be taken is a modification in terms of formulation called nanoparticle technology. Nanoparticle is one of the formulations that can overcome theseproblems because nanoparticle is able to improve the solubility of herbal medicines andit can reach specific targets. A nanoparticle refers to colloidal systems with particle sizesranging 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 patientcompliance.[7] This study aimed to develop a standardized herbal medicine from the bay leaves (*Syzygium polyanthum* (Wight) Walp) extract in nanoparticle form with fine qualityand efficacious in lowering blood glucose levels. It is hoped that through the formulationin the form of nanoparticles, bay leaf extract can be absorbed properly by the body so that even small doses have good pharmacological effects compared to extract doses that were usually used before. It is also hoped that even though there is a decrease in the dosage used, the

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extract will still be more efficacious in lowering blood sugar levels in diabetic patients.

Methods

Plant materials

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 %, distilled water (aquadest), hydrochloric acid, ammonia, ammonia hydroxide, chloroform, Bouchardat 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 (PT. Indofarma Global Medika), Na CMC, quercetin comparison standard.

Making extract powder derived from bay leaves

Prosedure of making bay leaves extract powder followed Silalahi[8]. 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

Methods of making bay leaves ethanolic extract followed Brylianto[9] with modification. Dried bay leaves powder was macerated with 96%, 70%, and 50% of ethanol. 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

The examination of the extract quality was carried out at PT. Phytochemindo Reksa withthe following parameters.

a. Specific Parameters
   1. Organoleptic
   2. Levels of the 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 a gravimeter
   3. Acid insoluble ash content using a gravimeter
   4. Density using a 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.

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

Total flavonoid levels determination

The examination of the total flavonoids and TLC follows methods that have been validated by PT. Phytochemindo Reksa as follows.

Test Solution:

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

Comparison Solution:

Measure carefully 10 mg of quercetin, put it 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 the test solution and 15 µL of comparison solution
Detection: 5 % of citroborate solution in ethanol. Plate heated at the temperature of 100^{o}C for 5-10 minutes at UV_{366}.

Making nanoparticles extract by ionic gelation method

Prosedure of making nanoparticles extract followed Silalahi[8] with modification. 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 distilled water + 50 mL of 1% chitosan on a magnetic stirrer then dripped with 4 mL of 0.2% NaTPP which is soluble in distilled water. 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 characterized by homogeneous turbidity. Leave for 15 minutes and then observe for five days the stability of the nanoparticle suspension including the color, turbidity, and sediment.

Spray drying

The drying of the nanoparticle suspension used additional maltodextrin filler with an inlettemperature of 210^{o}C and outlet 90^{o}C. The examination result of the water content in thenanoparticle 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 bodyweight of 170-200 grams. All rats were acclimatized for (approximately) 7 days which wererandomly divided into 6 groups, each consisting of 5 rats and induced alloxan at a dose of150 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

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were taken through the tailin the perpendiculars 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***
Group III = **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*
***Alloxan-induced**

Advanced test

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

Both in the Preliminary Test and the Advanced Test, groups II, III, IV, V and VI were induced with alloxan. The difference treatment between the Preliminary Test and the Advanced Test was the formulation of bay leaves ethanolic extract. At the Advanced Test, all ethanolic extracts were formulated into nanoparticles form, whereas in the Preliminary Test, all ethanolic extracts was in the original form.

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 fromeach blood glucose level of the rats after alloxan was induced and administered with thetest dosage from the 8th day, 15th day, and 22nd day.[9] The AUC is calculated using the 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

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Where the area of the trapezoid is summed up ($Area_1$+$Area_2$) to get the total area of AUC in each rat. 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 dosage to lower the blood glucose levels of rats.[9]

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

Making powder from bay leaves extract

The foreign organic matter 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 the yield were determined. The results can be seen in Table 1.

Table 1. DER-native and Yield Results

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

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

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Phytochemical screening and extracts quality examination

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

Table 2. Examination Results of the Quality of Viscous Ethanolic Extracts of Bay Leaves

Parameters	96% Ethanolic Extract	70% Ethanolic Extract	50% Ethanolic Extract	Requirements	Examination Results
Latin name	*Syzygium polyanthum* (Wight) Walp.	*Syzygium polyanthum* (Wight)Walp.	*Syzygium polyanthum* (Wight)Walp.	*Syzygium polyanthum* (Wight) Walp	Qualified
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

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Table 2. (Extension)

Parameters	96% Ethanolic Extract	70% Ethanolic Extract	50% Ethanolic Extract	Requirements	Examination Results
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**	Pb = ≤ 10 mg/kg Cd = ≤ 0.3 mg/kg As = ≤ 5 mg/kg Hg = ≤ 0.5 mg/kg**	Qualified
Microbiological contamination	ALT = <10 CFU/g AKK = <10 CFU/g E. coli = <10 CFU/g *Staphylococcus aureus* = Negative *Salmonella* sp. = Negative *Pseudomonas aeruginosa* = Negative**	ALT = 70 CFU/g AKK = 10 CFU/g E. coli = <10 CFU/g *Staphylococcus aureus* = Negative *Salmonella* sp. = Negative *Pseudomonas aeruginosa* = Negative**	ALT = <10 CFU/g AKK = <10 CFU/g E. coli = <10 CFU/g *Staphylococcus aureus* = Negative *Salmonella* sp. = Negative *Pseudomonas aeruginosa* = Negative**	ALT ≤ 10^5 CFU/g AKK ≤ 10^3 CFU/g E. coli = <10 CFU/g *Staphylococcus aureus* = Negative *Salmonella* sp. = Negative *Pseudomonas aeruginosa* = Negative**	Qualified

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

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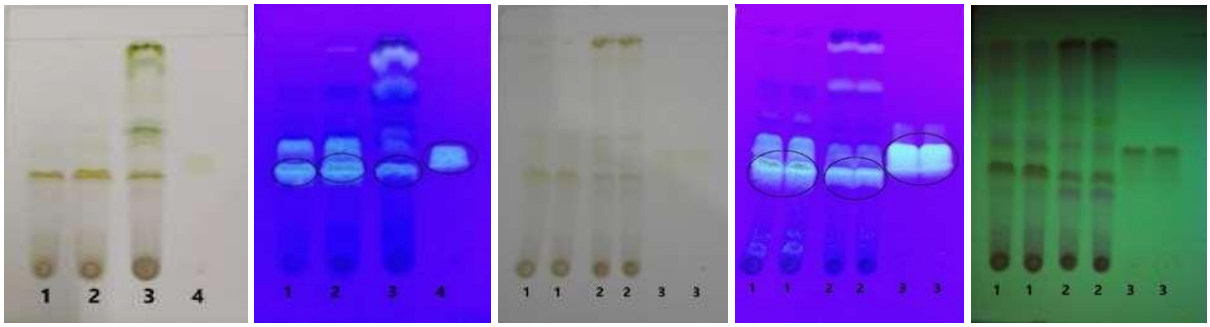
Total flavonoid content determination using spectrophotometer

Table 3. Determination results of the total flavonoid content

Bay Leaves Extract Samples	Total Flavonoid Content (Asquercecin)
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)

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

Table 4. Evaluation Results of Nanoparticles Suspension

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

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Particle morphology of nanoparticles

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

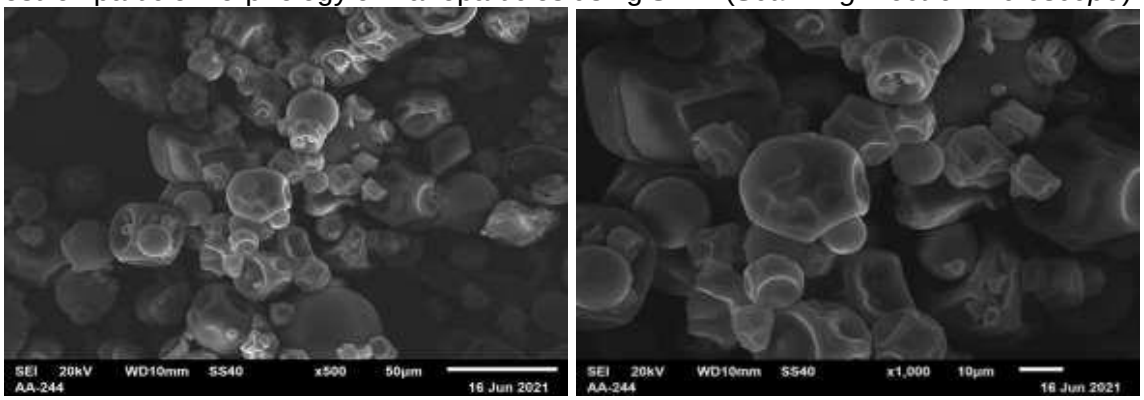

Figure 2. Particle morphology of bay leaves nanoparticles

Results of blood glucose lowering activity test by in vivo

Table 5. Rats Average Weighing Results

Treatment Groups	Average 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

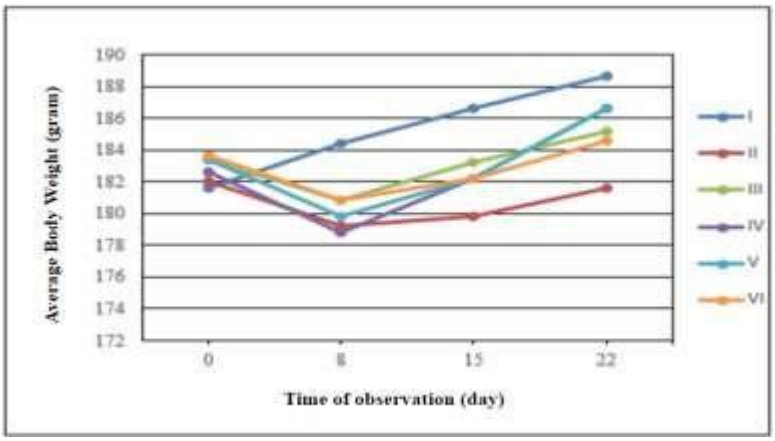

Figure 3. Observation of average body weight

Group I = Normal group

Group II = **Negative control group***

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Group III = **Positive 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*
***Alloxan-induced**

Table 6. Examination on Blood Glucose Levels of the Rats

Treatment Groups	Average 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

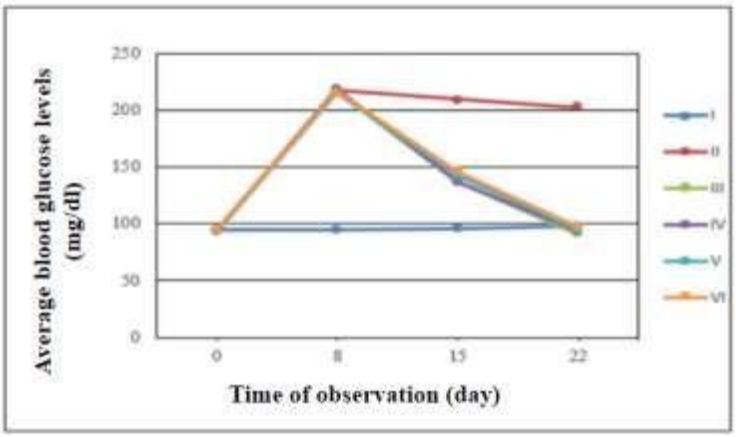

Figure 4. Observation of average blood glucose levels
Group I = Normal group
Group II = **Negative control group***
Group III = **Positive control group (Glibenclamide at a dose of 0.45mg/kg BW)***
Group IV = Nanoparticle of bay leaves standardized extract at a dose of 426.80 mg/kg BW*
Group V = Nanoparticle 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*
***Alloxan-induced**

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Table 7. 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 8. 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

Discussion

Making powder from bay leaves extract

Determination of foreign organic matter wascarried out with the aim of separating other parts that were not included in the simplicia description, such as the roots and stems because it could affect the quality of the extract.The purpose of determining the fine degree of simplicia was to produce an optimal extractin the extraction process.

Total flavonoid content determination using spectrophotometer

Test on the total levels of flavonoids in bay leaves ethanolic extract were carried out with the results as presented in Table 3 to determine whether the flavonoids contained in the ethanol extract could increase GLUT 4 levels in the adipose tissue of the test animals. GLUT 4 is the main glucose transporter located in muscle cells and fatcells. Increased levels of GLUT 4 cause an increase in glucose reabsorption.[4]

TLC identification

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 byspots 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 throughthe spray drying process.

The purpose of carrying out the identification process using TLC is to see the separation of samples in the form of a typical chromatogram pattern in the extract basedon the difference in polarity between the sample and the solvent (eluent) where the results can provide an initial picture of the composition of the chemical constituents based on the chromatogram pattern.[10]

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

The suspension of a nanoparticle is said to be nano-sized if the particle diameter is 10-1000 nm.[11] Table 4 shows that the nanoparticle suspension of the extract has a

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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.[12,13]

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 meansthat 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.[13,14]

Particle morphology of 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. In addition, the influence of inlet temperature and pressure from the spray drying process, the ratio of the combination of coating materials used, and the evaporation flow rate also affect this surface morphology.[15,16,17]

Results of blood glucose lowering activity test by in vivo

In the preliminary test, the calculation results of the percentage of lowered levels ofblood glucose of rats using 70% bay leaves ethanolic extract at a dose of 625 mg/kg BW had the highest ability to lower the blood glucose levels by 29.59%, followed by the groupof 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, thereby 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 implementedin industrial scale because the use of small concentrations of ethanol can reduce production costs but, at the same time, maintain 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 BWfor 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/kgBW), 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 5 shows that on the 8th day (hyperglycemic conditions), groups II, III, IV, V, and VI experienced weight loss, but the group I experienced weight gain. Weight loss occurs according to the theory that one of the typical symptoms of 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 unableto be

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met, resulting in an overhaul of fat and protein.[18] On the 22nd day, after being giventhe test dosage for 14 days, all groups experienced an increase in body weight. In groupI, weight gain was caused by the food consumed daily and as the rats got older, their body weight also increased. The result can be seen in Figure 3.

As presented in Table 6, on day 0 (initial day), 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.

Group II was used as a negative control 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, III, IV, V, IV 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 ratsafter Glibenclamide administration. In addition, group III was used as a comparasion group IV, V, and VI.

On the 22nd 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 lower blood glucose levels when compared to group III (positive). The results of statistical analysis showed that there were no significant differences in groups IV, V, and VI.

Based on Figure 4, it appears that there was change in blood glucose levels in group I (normal control) but not significant. In group II, III, IV, V, VI the rats were inducedby Alloxan so the rat pancreas was damaged and experienced hyperglycemia. After experiencing this condition, groups III, IV, V, VI were given treatment, namely group IIIwas given an oral antidiabetic drug (Glibenclamide) and the other three groups (groupsIV, V, and VI) were given bay leaf extract nanoparticles for 14 days. From each treatment group, the result was that group II continued to experience hyperglycemia until the end of the experiment because group II was not given Glibenclamide or given bay leaf extract nanoparticles, while in the other groups, there was a decrease in bloodglucose levels until the 22nd day. In this study, Group III, i.e., positive control group wasused as a comparison for groups IV, V, and VI to see if there were significant differencesin the groups that were given bay leaf extract nanoparticles.

After measuring blood glucose levels, the next step was analyzing the Area Under Curve (AUC) to determine the decreased percentage levels of blood glucose. 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/kg BW had the highest ability to reduce blood glucose levels in rats by 29.35%, followed by the nanoparticles extract with a doseof 213.40 mg/kg BW by 28.24%, and the nanoparticles extract with a dose of 106.70 mg/kg BW by 26.96%. Nevertheless, statistically, the results of the three test preparationgroups did not have a significant difference. The results can be seen in Table 8.

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.[4] In addition, the nanoparticle has the capabilities in reaching therapeutic organs, penetration capabilities and an extended contact time.[19] 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.[20]

Conclusion

The results of the study revealed that the standardized herbal medicines from the bay leaves (Syzygium polyanthum (Wight) Walp) extract in nanoparticle form have fine quality and efficacious in lowering blood glucose levels. The results show that the bay leaves extract contain secondary metabolites including flavonoid with concentrations at 96%, 70%, and 50%, respectively. The nanoparticles sizes are 1.48%; 1.62%; 1.50%; and 0.03%, respectively where the average particle size is 549.2 nm, and the zeta potential is -40.2 mV. Administration of the nanoparticles at a dose of 426.80 mg/kg BW; 213.40 mg/kg BW; and 106.70 mg/kg BW shows decreasing blood glucose levels whencompared to the positive control group but not significant (P>0.005). The smallest doseof nanoparticles extract that can lower blood glucose levels is at a dose of 106.70 mg/kgBW. It can be concluded that the nanoparticles from bay leaves extract can lower bloodglucose levels and meets the quality requirements.

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