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Original Article

FORMULATION OF NANOPARTICLE CONTAINING KENIKIR LEAF ETHANOL EXTRACTS (*COSMOS CAUDATUS* KUNTH.) AND ANTIDIABETIC ACTIVITY IN RATS

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ABSTRACT

Objective: This study aimed to formulate the ethanol extract of kenikir leaf into nanoparticles. The ethanol extract of kenikir leaves loading into nanoparticles can enhanced the stability and effectiveness of antidiabetic activity.

Methods: The nanoparticles were prepared using the ionic gelation method with chitosan and variation in sodium tripolyphosphate. The nanoparticle formula was characterized by efficiency encapsulation using spectrophotometry methods and particle size, zeta potential, and polydispersity index using dynamic light scattering (DLS). An antidiabetic activity test was initiated by inducing a high-fat and fructose diet. The parameters tested were decreasing blood glucose levels in rats.

Results: The result of the characterization of the nanoparticle was the percent of efficiency encapsulation, particle size, PDI, zeta potential, and pH were carried out to get the best formula. The best formula obtained was the percent of efficiency encapsulation of 96.20±0.0278%, the particle size of 144.6±7.800 nm, zeta potential of+15.32±0.9550 mEv, PDI of 0.48±0.070, and pH of 4.255±0.0035. The decrease in blood glucose levels in the nanoparticles of kenikir leaves extract was not significantly ($p>0.05$) different from the positive group (metformin) compared to the kenikir leaves extract, which decreased not really significantly.

Conclusion: Nanoparticle containing kenikir leaf ethanol extract successfully prepared into nanoparticles and the potential to increase antidiabetic activity.

Keywords: Kenikir leaves ethanol extract, Nanoparticles, Antidiabetic activity, Flavonoid, Rats

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INTRODUCTION

Diabetes is defined as a type of chronic disease either when the pancreas is unable to produce enough insulin or when the body cannot use the insulin it produces effectively [1]. The prevalence of diabetes, according to the International Diabetes Federation (IDF) in 2021, states that Indonesia is in the top 5 category with the most diabetic in the world, namely 19.8 million people. It is estimated that the number of people with diabetes will increase by 28.6 million in Indonesia in 2045 [2].

The most common type of diabetes mellitus is type 2 diabetes mellitus. Type 2 diabetes mellitus can be treated pharmacologically by administering oral antidiabetic drugs [3]. Administration of oral antidiabetic drugs is only given if the dietary arrangements made by the patient are still unable to control blood sugar levels. The use of conventional oral antidiabetic drugs has side effects that cause discomfort to patients, such as hypoglycemia, hematology, digestive disorders, lactic acidosis, abdominal etc [4].

Kenikir (*Cosmos caudatus* Kunth.) has secondary metabolites are flavonoid compounds such as quercetin, myricetin, luteonin, apigenin, and kaempferol [5]. Quercetin is able to protect the function of pancreatic β cells and reduce blood glucose levels that show a decrease in blood glucose levels in Wistar rats that were previously induced by streptozotin with a dose of 40 mg/kg body weight intraperitoneally [6]. Kenikir leaf extract has antidiabetic activity at a concentration of 333 mg/kg body weight in rats [7]. This is supported by flavonoids as antioxidants that are able to bind free radicals, which, when associated with diabetic prevention, is the prevention of oxidative damage to pancreatic β cells [8]. Kenikir leaf extract is known to have a good inhibition profile of modulating carbohydrate enzymes such as α-glucosidase, which is related to glucose absorption in the intestinal organs [6].

One route of administration for antidiabetic therapy is oral. Oral administration has challenges such as poor solubility and problems in bioavailability so antidiabetic activity is not optimal. In the form of plant extract such as flavonoid, it has a weakness such as low solubility in water so it reduces its bioavailability and is environmentally unstable [9]. The problem of bioavailability and stability can be overcome with using nanotechnology. Nanoparticles are a delivery system that makes it possible to increase the availability and stability of active substances through an encapsulation process, thereby protecting compounds from natural ingredients from being easily degraded by the desired therapeutic target. In addition, the nanoparticle system has better diffusion and penetration capabilities in the body [10].

One polymer that has been widely used is chitosan with the cross-linking agent sodium tripolyphosphate using the ionic gelation method. Chitosan has a positive charge in the form of an amine group that will interact with a negative charge in the form of a phosphate group to form a complex with nanometer size. The advantage of using chitosan is that it can regulate the release and protect the active substance [11]. The use of chitosan and sodium tripolyphosphate produces an average particle size of<300 nm, PDI in the range of 0.1-0.7, and %EE of>82% [12]. Based on the description above, in this study, kenikir leaf ethanol extract will be used active substance in the formulation of nanoparticles using chitosan and variation concentration of sodium tripolyphosphate using ionic gelation methods. Characterizations of nanoparticles are particle size diameter, polydispersity index, zeta potential, and efficiency encapsulation. The antidiabetic activity was evaluated to decrease blood glucose levels. The novelty of the research in the formulation and characterization of kenikir leaf ethanol extract in nanoparticles where the antidiabetic activity is enhanced.

MATERIALS AND METHODS

Plant material

The plant material used for the research was leaves of kenikir (*Cosmos caudatus* Kunth.) obtained from Gandus, Palembang, South Sumatra, Indonesia. The kenikir leaves were identified at the Department of Biology, Faculty of Mathematics and Natural Sciences,

University of Andalas, Padang, West Sumatera, with No. 223/K-ID/ANDA/III/2023.

Chemical and reagent

Chitosan (Sigma Aldrich), sodium tripolyphosphate (Sigma Aldrich), quercetin (Sigma Aldrich), ethanol 70% (Brataco®), citric acid (Brataco®), Na CMC (Brataco®), distilled water (Merck®), 0.9% NaCl (Merck®).

The extraction of kenikir leaves (*Cosmos caudatus* Kunth.)

The dry powder of kenikir leaves of as much as 650 g was macerated using 70% ethanol with a ratio of 1:10. The first maceration process was carried out for 2 x 24 h using 4,5 L of 70% ethanol until the powder was completely submerged. The filtrate resulting from maceration is collected and stored. Remacertion is carried out on the remaining dregs using 2 L of 70% ethanol for 1x24 h (second maceration process). The macerate obtained is separated by filtering using filter paper. The filtrate obtained was concentrated by evaporating the solvent using a rotary evaporator at a temperature of 55 °C to produce a thick extract. The thick extract of kenikir leaves was screened for phytochemicals and measurement of total flavonoid concentration.

Preparation of nanoparticle kenikir leaf extract

Preparation of nanoparticle formulas of chitosan and sodium tripolyphosphate loading kenikir leaves ethanol extract was used for variation in the concentration of sodium tripolyphosphate as in table 1 [13-15].

Table 1: Formulation of nanoparticle containing kenikir leaves ethanol extract

Parameters	F1	F2	F3
Kenikir leaves extract	333 mg	333 mg	333 mg
Chitosan	50 mg	50 mg	50 mg
Sodium-TPP	115 mg	50 mg	5 mg
Tween 80	5 mg	5 mg	5 mg

Preparation of nanoparticles of kenikir leaf extract was carried out using the ionic gelation method. The 333 mg of kenikir leaf extract was dissolved in 5 ml of distilled water until it was mixed, and add tween 80 stir until homogeneously. The 25 ml of chitosan solution in 2% citric acid was added to the kenikir leaves ethanol extract solution drop by drop using a micropipette. The solution was stirred constantly using a magnetic stirrer at 750 rpm for 30 min at room temperature until it was homogeneously dispersed. Then add the sodium tripolyphosphate crosslinker drop by drop into the chitosan-kenikir leaves extract solution with constant stirring. Stirring was carried out using a magnetic stirrer and homogenized for 1 hour at 750 rpm and then homogenized using an ultraturrax and a sonicator bath [13].

Characterization of formulation nanoparticle kenikir leaves extract

Organoleptic

The organoleptic determination of the resulting nanoparticles was tested organoleptically of the preparations formed. Other parameters are considered, namely in terms of precipitate and the ability of the preparations to be dispersed. The resulting preparations are expected to be perfectly dispersed or easy to redisperse into the solvent [13].

pH test

The test of the pH of the preparation was carried out using a pH meter into nanoparticle preparations of kenikir leaves extract. Preparations are determined with a pH meter [13].

Calibration curve

The standard calibration curve was made by as much as 10 mg into 100 ml ethanol that was obtained with a concentration of 100 ppm. The standard curve solution will be made in series concentrations, namely 20,30, 40, 50, and 60 ppm. A total of 0.5 ml of each solution was added with 0.1 ml $AlCl_3$ 10%, 0.1 ml sodium acetate 1M, and 2.8 ml water. The measurement of the absorbance of the standard solution was carried out at a wavelength of 400-800 nm using a UV-Vis spectrophotometer.

Determination of percent encapsulation efficiency (% EE)

Nanoparticle purification of chitosan encapsulated kenikir leaves extract was taken by 2 ml of the nanoparticle solutions inserted into Vivaspin 300 kDa the centrifuged at 15000 rpm for 30 min to separate kenikir leaves extract unencapsulated by the nanoparticle. Determination of the % EE nanoparticles kenikir leaves extract using UV-Vis spectrophotometer with a wavelength of 436 nm. The calibration curve was made in the variation concentration from quercetin as a standard.

$$\% \text{ EE} = \frac{\sum \text{AIF} - \sum \text{AIS}}{\sum \text{AIF}} \text{ x } 100\%$$

Noted: AIF: active ingredient of the formula; AIS: active formula of supernatant

Characterization of nanoparticles kenikir leaf extract

The nanoparticles characterized were diameter, particle distribution, and zeta potential using the PSA (Zetasizer Advance Pro Blue Malvern, Inggris) tool through the DLS method (Dynamic Light Scattering). A total of 50 µl of kenikir leaves ethanol extract into nanoparticle was added to the cuvette.

Measurement of the best formula

The measurement of the best formula was processed using One way ANOVA method in the SPSS®25 program by first carrying out a normality test to find out whether the data was normally distributed or not normally distributed. One-way ANOVA analysis was carried out to determine the significant value and observe the influence of the factors on the response of the significant value (p-value). Furthermore, Duncan's post hoc follow-up test was carried out to test the differences between all treatment pairs.

Antidiabetic activity test

The experimental protocol was approved by the Ethics Committee of Universitas Ahmad Dahlan No: 022302011. The test animals used were male white rats of the Wistar strain aged 2-3 mo and weighing 150-200 g. The test animals were divided into 5 animals in each group of 5 test groups. The animals were fed a high-fructose fat diet with a composition of 1,8 g/kg BW of fructose and 15 g/kg BW of high fat consisting of 80% standard feed, 15% used oil, and 5% duck egg yolk, which was given continuously. The normal group was administered at standard diet, negative groups were administered at a high-fructose fat diet and base of nanosuspension, positive groups were administered at a high-fructose fat diet and metformin 150 mg/kg BW, The treatment I groups were administered at high-fructose fat diet and suspension of kenikir leaves ethanol extract of 333 mg/kg BW, and treatment II groups were administered high-fructose fat diet and nanoparticle of kenikir leaves ethanol extract (best formula). The rats have diabetic condition with blood glucose levels was given treatments for 15 d. Rat blood glucose measurements were carried out on days 35, 40, and 45 by means of the test animals being fasted for 12 h before the test was carried out (preprandial glucose levels). Measuring fasting blood glucose levels of rats using a glucometer [16].

Data analysis

The data obtained from the calculation of decreased blood glucose levels was analyzed quantitatively by comparing data on decreased blood glucose levels in the treatment group with the positive control group. Data on decreased blood glucose levels were analyzed using the descriptive normality test (Shapiro-Wilk) to determine the

distribution of the data. If the data proves to be normally distributed, parametric statistical analysis is performed using one-way ANOVA with a 95% confidence level. If from the data obtained, it is proven that there is a significant difference, then the LSD post hoc test is continued. The data were not normally distributed, so the Kruskal-Wallis non-parametric statistical method was used. The Mann-Whitney test can be performed if the Kruskal-Wallis test results prove that there is a significant difference. The program used for data processing is SPSS®.

RESULTS AND DISCUSSION

Plant determination

Based on identification letter No. 223/K-ID/ANDA/III/2023, the result of the identification shows that the Latin name of the kenikir leaves is *Cosmos caudatus* Kunth. with family Asteraceae. The identification of kenikir leaf was carried out at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Padang, West Sumatera. This identification is intended to ensure that the plants used in the research.

The extraction of kenikir leaves

Based on the result of screening kenikir leaves extract show that kenikir leaves extract contains flavonoid, saponin, and phenolic. A total of 650 g of kenikir leaves has been obtained as much as 115.57 g of an extract with a yield of 17.78%. The total flavonoid content of the kenikir leaf extract was 104.795 mg/g.

Characterization of nanoparticle containing kenikir leaves ethanol extract

The nanoparticle formulation consists of chitosan, sodium tripolyphosphate, and tween 80 with an ionic gelation method. The ionic gelation method applies a cross-linking process between the electrolyte and its ion pairs. The ionic gelation method is used because the ion pairs used are more suitable and can avoid excessive stirring, too high temperatures, and use of organic solvents [17]. The process of nanoparticle preparations using chitosan as a polymer and selecting the crosslinker sodium tripolyphosphate because it can form a strong bond with chitosan, namely ionic cross-linking between chitosan molecules so that it can be used as an adsorbent. The groups have a positive charge as amine from the chitosan polymer and the groups from sodium tripolyphosphate have a negative charge so that particle size are formed through the ionic gelation method [18]. The addition of a surfactant stabilizes the suspension preparation by preventing agglomeration between particles so that the chitosan particles in the solution will be stabilized.

Table 2: Characterization of kenikir leaf ethanol extract on nanoparticle

Characterization	F1	F2	F3
%EE	96.20±0.0278	95.44±0.0752	95.28±0.0557
Particle size (nm)	144.6±7.8000	167.9±33.1500	227.63±168.66
PDI	0.4878±0.070	0.4637±0.1140	0.5458±0.3260
Zeta Potential (mV)	15.32±0.9550	19.27±0.3950	18.56±0.7700
pH	4.255±0.0035	4.296±0.0040	4.656±0.060

All data showed as mean±SD (n=3), where n is the number of observations

Percent of efficiency encapsulation

The measurements were made by separating the adsorbed and non-adsorbed phases in the nanoparticle system using a centrifuge at 15.000 rpm for 30 min. The centrifuge process will produce separate phases due to the rotation of the particles at high speed. This process makes particles with weight settle more quickly along with the force of gravity. The extract of kenikir, which is not adsorbed, has a lower molecular weight compared to the nanoparticle matrix, which is a combination of carrier and active substance so that the particles with heavier molecular weight will be at the bottom while those that are lighter at the top and efficiency encapsulation measure with UV-Vis spectrophotometer with callibartion curve (fig. 1). The higher the %EE which is close to 100%, it means that the less amount of free substance in the preparation is undergoing a degradation process. The results of the percent encapsulation efficiency on the three formulas can be seen in table 2. The results for F1 were stated to have the highest %EE value of 96.20%, which indicated the amount of kenikir leaf extract that was successfully adsorbed and protected by the polymer. The F1 produces a greater %EE compared to F3 and F3 because it uses a higher concentration of sodium tripolyphosphate. The concentration of sodium tripolyphosphate used can affect the amount of active substance encapsulated.

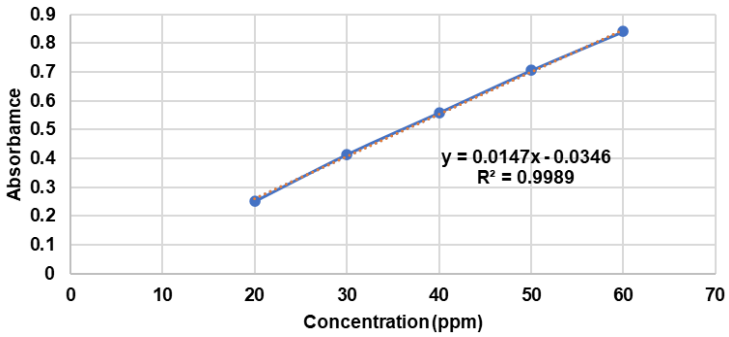

Fig. 1: Callibration curve

Particle size, PDI, and zeta potential

The all nanoparticle formulas of kenikir leaf ethanol extract were characterized in this study using a Particle Size Analyzer (PSA) to determine particle size, poly distribution index, and zeta potential. The results of the particle size, PDI, and zeta potential on the three formulas can be seen in table 2. The particle size produced in the three nanoparticle formulas shows particle sizes that can used for antidiabetic activity with results between 147-228 nm. Based on previous research, the particle size is 157,8 nm for antidiabetic testing, which was able to provide a better effect than extract without being made into nanosuspension preparations [19]. The particle size that is good for use in drug delivery systems is in the range<300 nm [20].

The results of measuring the polydispersity index in the nanoparticle system will indicate a uniform size dispersion value in the system. The requirements for the PDI (polydispersity index) value are in the range of 0 to 1. The closer the PDI values that exceed 0.5 are categorized as having many particles whose size is not uniform or the level of heterogeneity is high. The results of measuring the PDI values of the three formulas in this study showed that F3 have PDI>0.5. Meanwhile, F1 and F2 show PDI values<0.5, which indicates that the diameter size distribution is uniform. The results of the PSA test regarding zeta potential in this study from the three formulas obtained zeta potential values not towards more than±30 mV. Zeta potential test results on F1, F2, and F3 respectively+15.32±0.9550;+19.27±0.3950; and+18.56±0.7700 mV with this value have the potential to cause clumping and flocculation due to the smaller attractive forces compared to the relatively large repulsive forces between the bonded particles.

pH analysis

The pH requirements for preparations that are considered safe and have little chance of irritating the mucosa when administrated orally range from a pH of 4.0-6.5. The results of the pH on the three formulas can be seen in table 2. There is a difference in the pH value produced from each preparation formula. The factors that can affect the pH value of this nanosuspension preparation are the concentration of chitosan and the type of crosslinker used. The greater the concentration of chitosan solution, the lower the pH, and vice versa. The lower the pH value, the more protonated amine groups will be.

Measurement of the best formula

The results of the %EE normality test for the three formulas gave a significance result (sig)>0.05 which indicated that the data was normally distributed and homogeneous. The data then proceed to the one-way ANOVA test. The results of the one-way ANOVA test found differences from the three formulas with a significance value (sig)<0.05. Based on the test results, a significant difference was found in the data of the three formulas, namely F1, F2, and F3. This shows that variations in the concentration of sodium tripolyphosphate can affect the %EE of the nanosuspension of kenikir leaf extract. So further testing was carried out in the form of Duncan's post-hoc test to test and find out the specific differences of the three formulas. The results of Duncan's post-hoc test showed that the three formulas obtained three different column subsets. The results of the %EE test from F1, F2, and F3 with the formation of three subset columns show a significance value (sig) greater than alpha (p>0.05).

Based on the %EE analysis that was carried out in this study, the best formula was obtained, namely F1, which would be used in further *in vivo* test. F1 has the highest %EE which is an important parameter in this study because there are the most kenikir leaf extract that are successfully adsorbed in the chitosan-sodium tripolyphosphate F1 polymer. In addition, it is also seen that the best rsults of characterization of particle size, PDI, and zeta potential are owned by the F1 nanosuspension compared to F2 and F3.

Antidiabetic activity

Observation of blood glucose levels was carried out on the 30th day after high-fructose fat diet induction in each test group. Feeding a diet high in fat and fructose is expected to increase the content of cholesterol and free fatty acids in the blood and induce insulin resistance, resulting in type 2 diabetes mellitus. Test animals that have been induced by a high-fructose fat diet for 30 d are then measured for their blood glucose levels. The results can be seen in fig. 2.

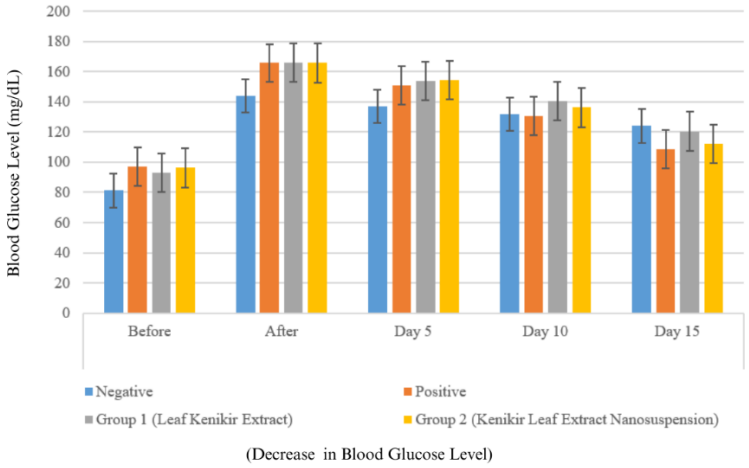

Fig. 2: Increase in rat blood glucose levels, there was significance among groups (p>0.05)

The results showed that the rat's blood glucose levels increased after high-fructose fat diet induction for 30 d. This indicated that the rats had diabetes mellitus and that the blood glucose levels of each group were>126 mg/dL. Test animals that were confirmed to have type 2 diabetes mellitus were then given further treatment in the form of administration of treatment in each test group, which had been divided for 15 d. This significance value (p<0.05) shows that there are differences in each group before and after induction. Therefore, it can also be seen that giving a diet high in fat and fructose for 30 d can cause type 2 diabetes mellitus in mice. Based on the result, fig 2 shows that there was significance among groups (p>0.05) in the data on reducing blood glucose levels after induced diet high fat and fructoce and treatment for 30 d. This showed that after treatment with kenikir leaf extract and nanoparticle-containing kenikir leaf extract obtaining decrease blood glucose levels.

There were various differences in each test group and on average, each group experienced a decrease in blood glucose levels. The explanation of the results of fasting blood glucose levels from before induction to the 15th day of treatment for the test rats of each is shown in fig. 3.

In this study, the effectiveness was measured between treatment I (kenikir leaf ethanol extract) and treatment II (nanoparticles containing kenikir leaf ethanol extract). The results of decreasing blood glucose levels between the two groups showed that treatment II had better ability than treatment I. Nanoparticles could reach a target and the ability of chitosan polymers to protect the active ingredient in kenikir leaf ethanol extract against environment degradation of compounds in the body and chitosan has mucho-adhesive properties. Therefore, it can also be seen that the administration of extract and preparations of nanoparticles of kenikir leaf ethanol extract can reduce blood glucose levels for 15 d of treatment. In addition, the negative group experienced a decrease, but not too significant.

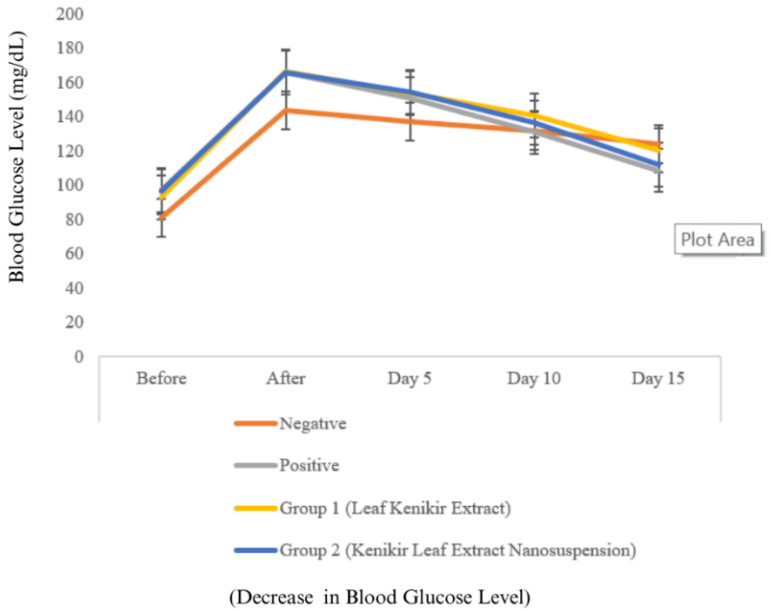

Fig. 3: Decrease of blood glucose levels. All data showed as mean±SD (n=3); where n is the number of observations

The results of this study indicate that the nanoparticles with chitosan and sodium tripolyphosphate as a carrier have better antidiabetic activity than the kenikir leaf ethanol extract. The nanoparticle system is used to improve the drug delivery system, especially the oral route used in this research. This nanoparticle system with antidiabetic activity is also able to increase glucose permeation and prolong the action of the active ingredients in the polymer nanoparticle. The encapsulation system in this nanoparticle is also able to increase bioavaibility and better pharmacological effectiveness [21].

The results of the data on decreasing blood glucose levels for each group were analyzed statistically using the SPSS and ANOVA. Based on the results it shows that there are significant differences between groups ($p<0.05$). Data obtained from the results of the one-way ANOVA test were continued with the LSD (Least Significant Differences) and Turkey post hoc tests because there were significant differences between the test groups. The test results showed that there was no significant difference in the data on reducing blood glucose levels belonging to the positive control group compared to each treatment group I and II with a significance value obtained ($p>0.05$). This shows that the kenikir leaf ethanol extract and the nanoparticle containing kenikir leaf ethanol extract work effectively in reducing blood glucose levels. Apart from that, there was a significant difference between the positive control group and the negative control group ($p<0.05$), then with group II there was no significant difference ($p>0.05$). This is because the concentration of blood glucose for treatment group II is almost close to the positive group where on the treatment.

CONCLUSION

Nanoparticle containing kenikir leaf extract has the potential to increase antidiabetic activity. The best formula obtained was of efficiency encapsulation of 96.20±0.0278%, a particle size of 144.6±7.8000 nm, a zeta potential of+15.32±0.9550 mV, and a PDI of 0.48±0.070. The decrease in blood glucose levels in the nanoparticles of kenikir leaf ethanol extract was not significant ($p>0.05$), different from the positive group (metformin) compared to kenikir leaf ethanol extract, which decreased not really significantly. Nanoparticle containing kenikir leaf ethanol extract have the potential to increase antidiabetic activity.

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AUTHORS CONTRIBUTIONS

Dina Permata Wijaya: The designed idea of the research, conceived of the original idea, contributed of the experiment, wrote the manuscript with support from Herlina and Raden Aulya' AH, Herlina: Editor the manuscript, contributed of the experiment, Raden Aulya' AH: contributed of an experiment, help to wrote the manuscript

CONFLICT INTERESTS

The authors declare no conflict of interest.

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