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Original Article

FORMULATION OF BINAHONG LEAVES (*ANREDERA CORDIFOLIA*) EXTRACT NANOPARTICLE AND EVALUATION OF *IN VIVO* ANTIDIABETIC ACTIVITY

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ABSTRACT

Objective: Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) is an herbal plant that is most commonly used to cure various types of diseases in Indonesia, including diabetes. The objective of this study was to conduct a comparative analysis of the antidiabetic activities between binahong leaves extract and binahong leaves extract nanoparticles.

Methods: Nanoparticle formulation was carried out using chitosan and sodium alginate polymers as crosslinkers. DDY strain mice with 70% ethanol extract of binahong leaves as test material Binahong leaf extract was tested on alloxan-induced diabetic mice at a dose of 200 mg/kgBW intraperitoneally. The dose of binahong extract used was 200 mg/kgBB and 200 mg/kgBB of chitosan-sodium alginate nanoparticles orally for 14 ds.

Results: Obtained extract nanoparticle met the specification, with average sizes of 192.7 nm, zeta potential of+25.3 mV. Extract nanoparticle with a dose equivalent to 200 mg/kgBB binahong leaf extract can reduce blood glucose levels in mice induced by alloxan was better than binahong leaf extract at a dose 200 mg/kgBW with respective percent reduction in blood glucose levels are 45.89% and 33.38%.

Conclusion: The formulated extract nanoparticle can enhance the antidiabetic activity of binahong leaves extract.

Keywords: *Anredera cordifolia,* Binahong leaves, Nanoparticle, Antidiabetic, Metabolic syndrome

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INTRODUCTION

The global prevalence might be estimated to be around one-quarter of the global population. To put it another way, Metabolic syndrome today affects over a billion people worldwide [1]. Metabolic syndrome comprises elevated glucose and blood pressure level. Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and metabolic disorders of carbohydrate, protein, and fats related to a deficiency in the insulin secretion by pancreas-β cells or a deficiency in the insulin action [2]. Diabetes mellitus remains a national health problem and ranks fourth in the priority of national research on degenerative diseases. It was estimated that 1.4 cases of diabetes mellitus occur in Indonesia annually [3, 4].

The utilization of herbal medicine in Indonesia has experienced a notable surge in recent years. As indicated by a national survey conducted in 2018, 44.2% of households in the country employ traditional healthcare methods, representing a significant increase from the fig. of 30.1% recorded in 2013. Within the group of traditional medicine users, approximately 70% reside in rural areas [5].

One of the Indonesian plants that can be used as a herbal medicine is the binahong leaves. Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) is an herbal plant that is most commonly used to cure various types of diseases in Indonesia. The compounds in this plant are often used as herbal medicine to treat diabetic, hypercholesterolemia, antiacne, and others. Binahong leaves have natural substances that can be extracted by maceration. It contains compounds such as flavonoids, phenols, and alkaloids.

Nanoparticles form dispersed particles on the nanoscale (10-1000 nm). The implementation of nanoparticles in the pharmaceutical field is an encapsulated drug in a certain nanometer-sized carrier system. The preparation of binahong leaves nanoparticles was based on the ionic gelation method [6]. The ionic gelation method is a method that involves cross-linking between polyelectrolytes in the presence of multivalent ion pairs [6, 7]. Chitosan and sodium alginate are two biopolymers that can entrap therapeutic agents which can maintain their structure and activity and protect them from metabolic or enzymatic degradation [8]. The aim of this study was to develop nanoparticulate systems based on ionic gelation between chitosan and sodium alginate for loading of binahong leaves extract and to compare binahong leaves extract and binahong leaves extract nanoparticle for their activities in term of antidiabetic. The properties obtained through the characterization of the nanoparticle comprise particle size, zeta potential, and particle morphology.

MATERIALS AND METHODS

Materials

Chitosan, sodium alginate, propylene glycol, tween 80, aquadest, glycerin, ethanol 96%, methanol, alloxan monohydrate (Sigma-Aldrich, USA); CMC Na (MedChemExpress); distilled water; oral antidiabetic drug: Glibenclamide (Indofarma), acetone, anhydrous acetic acid, NaCl, concentrated sulphuric acid, dichloromethane.

Preparation binahong leaves extraction

The preparation of binahong leaves extract nanoparticles was carried out by maceration technique using 70% ethanol solvent with the ratio of material and solvent 1 kg of dried powder with 6 l of 70% ethanol solvent for 2x24 h while stirring for the first 2-3 h. After the maceration process is complete, filter using filter paper to take the filtrate and separate the pulp. The results of the macerate were concentrated with a rotavapor at 40 °C for 6 h to obtain a crude extract, which was then weighed.

Preparation of extract binahong leaves nanoparticle

A total of 800 mg of binahong leaves extract was weighed carefully and then dissolved in tween 80, 96% ethanol, propylene glycol, and distilled water. Afterwards, 18 ml of chitosan was added and

gradually homogenized with a magnetic stirrer. 30 ml of sodium alginate was added dropwise and homogenized using a magnetic stirrer for 60 min at 300 rpm.

Table 1: The formula of extract binahong leaves nanoparticles

Materials	Formula
Binahong leaves extract (mg)	800
Sodium Alginate 0.1% (ml)	30
Chitosan 0.5% (ml)	18
Propylene glycol (ml)	5
Ethanol 96% (ml)	15
Tween 80 (ml)	6
Aquadest (ml)	Ad 100

Determination of antidiabetic activity

Through letter number KET-121/UN2. F1/ETIK/PPM.00.02/2023, the Faculty of Medicine, Universitas Indonesia Health Research Ethics Committee has granted permission (ethical approval) for this study. All mice were adapted for one week. On day 0, all mice used in the study fasted for 16 h, then the initial blood sample (normal mice) was taken by taking blood from the lateral vein of the tail of the mice and measuring blood glucose levels at the start of the experiment. 5 mice were randomly selected as the normal group. All mice, except the normal group, were made hyperglycemic by induction of alloxan monohydrate (dose 200 mg/kg BW) intraperitoneally for 3 consecutive days. On the 9th day, blood glucose levels were measured to determine hyperglycemic conditions (mice were fasted for 16 h). Random groups of mice were carried out; each group consisted of 5 mice, which were taken randomly and given the following treatment:

- Group I served as a control without any treatment
- Group II as a negative control by giving distilled water.
- Group III as a positive control with glibenclamide administration as a comparison.
- Group IV was given binahong leaves extract at a dose of 200 mg/kg BW
- Group V was given chitosan alginate nanoparticles with a dose equivalent to 200 mg/kg BW of binahong leaves extract.

Blood samples were taken on the 9th and 16th d after treatment from the lateral tail vein and blood glucose was measured using a glucometer. On day 23, all mice had neck dislocations. Blood glucose data were then analyzed using a one-way ANOVA test. Kruskal Wallis and Mann – Whitney tests were used for non parametric data. Data were assessed for normality with one-sample Kolmogorov-Smirnov and homogeneity of variances was tested with levene tests. Significant differences were accepted at $P<0.05$.

RESULTS AND DISCUSSION

Particle size distribution

The particle size range of nanoparticles was 10-1000 nm. The results of particle measurements on binahong leaves extract nanoparticles using the Malvern Zeta Sizer showed that the formed nanoparticles had an average size of 192.7 nm. The measurement output showed the nanoparticles met the specification of nanoparticle size.

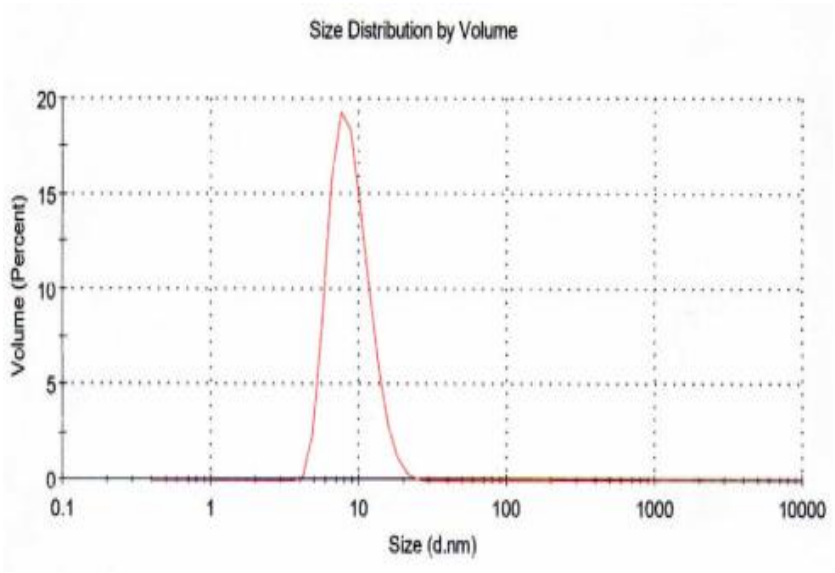

Fig. 1: Particle size of binahong leaves extract nanoparticles

Zeta potential

Zeta potential is one of the parameters indicating the stability of a colloidal nanoparticle solution. Zeta potential represents the force of repulsion between particles. A favorable zeta potential value is one that passes or approaches (+/-) 30 mV or moves away from zero. If the zeta potential value approaches zero (either positively or negatively), it can lead to aggregation, resulting in an unstable nanosuspension. Nanoparticles with zeta potential values between-10 and+10 mV are considered neutral, whereas nanoparticles with zeta potential values exceeding +30 mV or falling below -30 mV are classified as highly cationic and highly anionic, respectively. Therefore, higher charges result in more stable particles due to increased resistance between particles. Nanoparticles with positively charged zeta potential values can rapidly penetrate the mucous layer because they can electrostatically interact with negatively charged mucin [9].

The result of the zeta potential value was+25.3 mV. The charge possessed by the nanoparticles is far from the value of 0 and is almost close to+/-30 mV so that nanoparticles are obtained which are stable and do not easily aggregate. Also, from these results, it could be assessed that the obtained zeta potential values were positive because the nanosuspension was predominantly composed of positively charged chitosan, resulting in a positive potential difference between the electrical double layer and the medium [10].

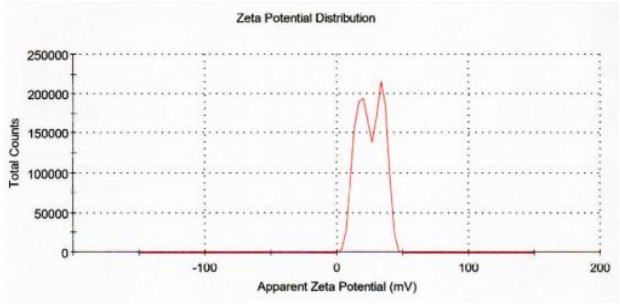

Fig. 2: Zeta potential of binahong leaves extract nanoparticle

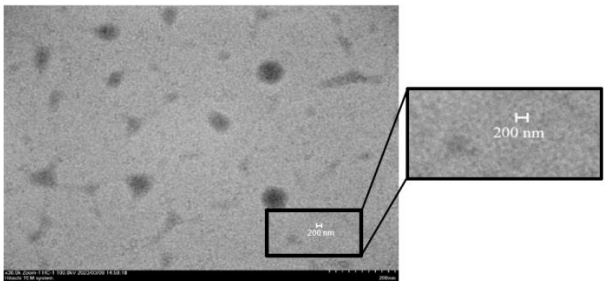

Fig. 3: Observation results of extract nanoparticle morphology using TEM (30000x magnification)

Table 2: Blood glucose levels of mice

Blood glucose levels	Day 0 (mg/dl)	Day 9 (mg/dl)	Day 16 (mg/dl)	Day 23 (mg/dl)
Group I (normal control)	121.2±9.9	108±20.2	111±16.7	121.8±16.1[b]
Group II (negative control)	94.2±8.4	369.4±42.7[a]	374±37.4	385.4±38.8[ac]
Group III (positive control)	104.6±10.11	349±13.1[a]	148±12.23	115±12[b]
Group IV (extract)	99±11.4	466.8±34.9[a]	150±13.9	111.6±54.2[abc]
Group V (nanoparticle)	109±8.3	361.8±23.3[a]	157.8±8.0	155.2±9.3[b]

The data was presented in mean±SD, n=5, [a]p<0.05: significant difference from normal control, [b]p<0.05: significant difference from negative control, [c]p<0.05: significant difference from positive control

Particle morphology

Based on TEM observations of the nanoparticles with 30000x magnification, it is known that the nanoparticles are spherical in shape. Surface morphology affects the ability of nanoparticles to penetrate the target cell membrane. The spherical surface of the nanoparticles can enter cells more easily [11].

Mice blood glucose levels

Blood glucose levels of mice were shown in table 2. Table 2 showed that the blood glucose levels of mice on day 0 were 86-135 mg/dl.

Blood glucose levels on day 0 meet the requirements for normal fasting blood glucose levels, namely 80-135 mg/dl [12]. Based on the results of statistical analysis, there were significant differences in the blood glucose levels of mice in initial conditions for groups I, I, III, IV, and V ($p<0.05$). The blood glucose levels of mice in groups II, III, IV, and V became hyperglycemic due to the administration of alloxan monohydrate. This proves that alloxan has been proven to cause a hyperglycemic condition, which is characterized by an increase in blood glucose levels in mice through the formation of ROS (Reactive Oxygen Species) and through increased concentration of calcium ions in pancreatic cells. In these conditions, the insulin concentration will increase rapidly and will significantly result in impaired insulin secretion in a short time. Hyperglycemia occurs due to a decrease in insulin production, which causes blood glucose not to be absorbed into cells.

From the results of statistical tests on the blood glucose levels of mice on day 23 (14 d after treatment), the results obtained were that the blood glucose results of the chitosan-sodium alginate nanoparticle group were significantly different from the blood glucose of the negative group and binahong leaf extract and were not significantly different from the normal and normal groups. Positive indicating a difference in blood glucose reduction ($p<0.05$).

The blood glucose values obtained are then used to calculate the Area Under Curve (AUC). Area Under Curve (AUC) is used to see the effect of reducing blood glucose in test animals. The smaller the AUC value, the better the effect of lowering blood glucose. The results of calculating the AUC of blood glucose in mice can be seen in table 3.

$$AUC = \left(\frac{a+b}{2} \text{ x day difference}\right) + \left(\frac{b+c}{2} \text{ x day difference}\right)$$

a = 9th d blood glucose levels

b = 16th d blood glucose levels

c = 23th d blood glucose levels

Table 3: AUC after treatment

Group	AUC (mg/dl x day)
Group I	1600±131.9
Group II	5260±357.6
Group III	2666±137.6
Group IV	3504±130
Group V	2846±57.6

The data was presented in mean±SD, n=5

From the results of the average AUC value in table 3, it was found that the average AUC value of the negative control group was the highest compared to all groups. The results of this data show that alloxan as a diabetogenic agent, is able to provide hyperglycemic conditions. The AUC data also shows that chitosan-sodium alginate nanoparticles are able to lower blood glucose better than binahong

leaf extract, this is shown from the average AUC value of chitosan-sodium alginate nanoparticles smaller than binahong leaf extract. The AUC results of the sodium alginate chitosan nanoparticle group were significantly different from the AUC of the normal, negative, and binahong leaf extract groups ($p<0.05$).

The percentage of decreased blood glucose levels of mice

The percentage of decreased blood glucose levels of mice was shown in table 4. The data in table 4 shows that chitosan-sodium alginate nanoparticles have stronger antihyperglycemic activity than binahong leaf extract in lowering blood glucose levels.

Table 4: The percentage of decreased blood glucose levels of mice

Test preparations	Percentage of decreased blood glucose levels in mice (%)
Glibenclamide	49.31%
Binahong leaves extract	33.38%
Chitosan-sodium alginate nanoparticles	45.89%

The percentage of decreased blood glucose levels of mice obtained from the value =

$$P = \frac{AUC\ control - AUC\ sample}{AUC\ control}\ x\ 100\%$$

The stronger antihyperglycemic activity could be because chitosan-sodium alginate nanoparticles would increase the poor bioavailability of binahong leaf extract so that the pharmacological effect could increase. Binahong leaves extract nanoparticles using Chitosan could improve drug penetration in the cell.

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AUTHORS CONTRIBUTIONS

Deni Rahmat: conceived and planned the experiments supported the financial research; Azarine Mediory Saka Waluyo and Teddy Himawan: carried out the experiments and analyzed the data; Yati Sumiyati, Sarah Zaidan, Ni Made Dwi Sandhiutami: analyzed the data and contributed to the interpretation of the results; Safira Nafisa and Yesi Desmiaty: administration of the research, supervised and verified the result; All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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