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Parijoto Fruit Extract Nanoparticles As Glucose-Lowering Agent In Vitro

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Abstract

Parijoto fruit is an herbal that is the potential to be used for the treatment of lowering blood glucose. The active compound in parijoto fruit can decrease glucose levels is flavonoids. The use of natural materials is limit because of their low solubility. It often failed in the clinical phase. The solution to this problem is that the parijoto fruit extract is formulated in the form of nanoparticles using chitosan and STPP as encapsulate materials. This study aims to determine the activity of nano chitosan parijoto fruit extract (NPFE) as a glucose-lowering agent by applying nanoparticle technology. PFE was made by ionic gelation reaction by mixing extract chitosan solution of 0.2% concentration, and NaTPP concentration of 0.1%. The characteristic of NPFE was particle size, PdI, and % transmittance. The effect of a glucose-lowering agent of NPFE tested by the Nelson Somogyi method. The particle size of the NPFE result is 269.3 nm, PdI value is 0.372, and the percentage of transmittance is 99.397%. The glucose-lowering effect of NPFE was more significant (EC50 <2 ppm) than parijoto fruit extract (EC50 48,750 ppm) and quercetin (EC50 6,831 ppm). Nano chitosan parijoto fruit extract affects a glucose-lowering agent.

Keywords: Parijoto Fruit; Extract; Glucose

INTRODUCTION

Basic Health Research in Indonesia (2018) showed that the prevalence of diabetes mellitus in Indonesia increased from 6.9% to 8.5%. Indonesia is estimated to be ranked 4th in the number of people with diabetes mellitus. The prevalence of diabetes mellitus in Indonesia in 2030 expects to increase to 21.3 million. They are increasing the number of suffers each year due to changes in people's lifestyles that consume foods high in carbohydrates and fast food. Epidemiological transitions in Indonesia also cause changes in disease patterns that cause an increase in degenerative diseases, one of which is diabetes mellitus (Frankilawati, 2014), (Yuliani *et al.*, 2014).

If not appropriately managed, Diabetes Militus can cause complications such as microvascular, macrovascular, and neuropathic complications (Febrilian and Pujiastuti, 2017; Villarruel- Ló pez *et al* ., 2018).

Diabetes management, in addition to pharmacological therapy, can also be used as a complementary herbal treatment (Leonita and Muliani, 2015). Herbal medicine is intended for degenerative diseases because the treatment is long term, and the side effects are relatively small. This Herbal medicine is related to the working of herbal medicine, which generally has a slower effect than chemical drugs (Sari, 2012).

Parijoto fruit (*Medinilla speciosa* Reinw. Ex Blume) is a typical flora of the Colo region, Muria Mountains, Kudus. The fruit used as an ulcer drug, anti-inflammatory, and antibacterial empirically. According to research Amin (2015) and Tussanti and

Johan (2014), parijoto has been investigated adequately as a cholesterol-lowering and cytotoxic agent. Wijayanti and Lestari (2018) have researched that ethanol extract of parijoto fruit at a dose of 250 mg/kg bb and 500 mg/kg bb was able to reduce blood glucose levels in Wistar male models of diabetes mellitus. The content of active compounds in parijoto fruit that are supposed to reduce glucose levels is flavonoids (Febrilian and Pujiastuti, 2017). The result of total flavonoid levels in the n-hexane fraction was 1.11 mg QE / g, the ethyl acetate fraction was 46.83 mg QE / g, and the ethanol fraction was 66.07 mg QE / g. This result showed that ethanol is the best solvent to take flavonoid compounds from parijoto fruit (Vifta and Advistasari, 2018).

The use of natural materials is limited because it often fails in clinical testing. This is due to the low bioavailability of natural materials (Alam *et al* ., 2012). The low solubility of natural compounds causes low bioavailability, and the lack of permeability of compounds to penetrate the absorption *barrier* (Ramadon and Mun'im, 2016). Increasing the ability of the affinity of active substances to the target can be done with nanoparticle systems.

The purpose of nanoparticle technology is to protect the active compound from degradation and deliver it directly to the target. The method of making nanoparticles carried out using ionic gelation. The ionic gelation process used to prepare chitosan nanoparticles because this method is straightforward. This method does not require heating, so the possibility of damage to active compounds can be avoided (Rismana *et al.*, 2014). Chitosan is a *carrier* polysaccharide developed in various pharmaceutical dosage forms that increases the bioavailability of biomolecules because it has good diffusion and penetration capabilities into the mucus. Nanoparticles with chitosan *carriers* can be made by the ionic gelation method, which is the method that attracts the most researchers because of its simple process. Sodium tripolyphosphate is used as a crosslinking agent because it is non-toxic and it has a multivalent nature.

The effect of reducing glucose levels tested using the in vitro method, which is non-enzymatic Nelson Somogyi method. The principle of the test is to oxidize the glucose complex with the Nelson reagent, then form a greenish-blue molybdenum complex after the addition of the arsenmolybdate reagent, so that its absorbance can measure by Uv-Vis spectrophotometry. This method is selected because of the reagent and straightforward testing process (Vifta and Advistasari, 2018a).

Based on this background, researchers will conduct further studies on the manufacture of nanoparticles on the activity of reducing glucose levels in parijoto fruit (*Medinilla speciosa* Reinw. Ex Blume) by *in vitro* testing. It is expected that from this research, the application of nanoparticle technology in parijoto fruit extracts can increase the ability of functional activity and bioavailability of parijoto phytochemical compounds.

METHOD

1. Tools and Materials

This study used many tools such as: standard glass equipment, *rotary evaporator* (RE 100-Pro), *water bath* (Memmert), moisture balance (OHAUS), *magnetic stirrer* (Thermo Scientific Cimarec), UV-Vis spectrophotometer (Shimadzu UV-1800), *Particle Size Analyzer* (*Particle Size Analyzer*) Malvern), micropipette 10-100 µL (Nesco) and 100-1000 µL (Socorex), heaters, a set of *centrifuges*, analytical scales (OHAUS).

Materials used were Parijoto, quercetin, ethanol 96% (Brataco), ethanol pa (Merck), chitosan powder, NaTPP (Brataco), glacial acetic acid pa (Merck),

aquadest, glucose anhydrous, Nelson reagent, arsenicolagen reagent, chloroform, potassium dichromate, sulfuric acid.

2. Procedure

Parijoto Fruit Extraction

The ratio of dried powder and solvent (ethanol 96%) used was 1:10. The dried powder weigh is 200 g, macerated with 1.5 L of ethanol solvent for two days, after two days the filtrate separated, and the residue remunerated for one day with 500 ml of 96% ethanol. The obtained liquid filtrate, separated with the solvent using a *rotary evaporator* at 80ºC until it becomes a thick extract.

Parijoto Fruit Nanoparticles (NPFE)

The formulation NPFE did by weighing 100 mg of parijoto fruit extract. The extract dissolved into *Beaker* glass containing 35 mL pa ethanol and 15 mL aquabidest. The liquid extract took 10 mL and then added with a solution of 0.2% chitosan as much as 50 ml. Gradually, 10 mL of NaTPP solution added as much as 10 mL using a dropper drop into the solvent mixture. The mixing process carried out with a *magnetic* stirrer (for 20 minutes, with a speed of 400 rpm) (Kurniasari and Atun, 2017; Choiri *et al.*, 2016).

The result of the mixture formed a nano chitosan suspension of ethanol extract of Parijoto fruit. Then, the nano chitosan extract of parijoto ethanol separated between the supernatant and the colloidal precipitated by centrifugation (for 15 minutes, 3000 rpm).The obtained supernatant (upper layer) used for characterization, including percent transmittance, particle size, and distribution.

Characterization Test of Parijoto Fruit Nanoparticles (NPFE)

1. Percent of Transmittance

A total of 1 mL of nano chitosan parijoto fruit extract added to the final volume of 50 ml Aquadest. Homogenization was carried out by a *magnetic stirrer* for 30 seconds. Then, the transmittance value formula NPFE was measured using a spectrophotometer at a wavelength of 650 nm (Huda and Wahyuningsih, 2016).

2. Particle Size and Distribution

The determination of particle size and particle distribution (polydispersion index) carried out using a *Particle Size Analyzer* (PSA) (Choiri *et al* ., 2016).

Glucose LoweringTest

1. Maximum Wavelength of Glucose Test

40 ppm standard glucose solution took 1 mL, 1 mL Nelson reagent is added, then covered with cotton. The solution heated over boiling water ± 10 minutes. The solution cooled for 5 minutes. The solution took into a 10 mL volumetric flask, and 1 mL of arsenomolybdate reagent added. Aquadest was added to the limit and homogenized, allowed to stand for incubation time. The absorbance read at a wavelength of 700-780 nm.

2. Operating Time Test

40 ppm standard glucose solution takes 1 mL, 1 mL Nelson reagent is added, then covered with cotton. The solution is heated over boiling water ± 10 minutes. The solution cooled for 5 minutes. The solution took into a 10 mL volumetric flask, and 1 mL of arsenomolybdate reagent added. Aquadest was added to the limit and homogenized, allowed to stand for incubation time. Absorbance was measured for 40 minutes at maximum duration at intervals of 1 minute. The optimum time is obtained based on a stable absorbance value at each time interval. (Vifta and Advistasari, 2018a) .

3. Standard Curve Test

Glucose standard series 8, 16, 24, 32 and 40 ppm from 1000 ppm solution. Total of 80, 160, 240,

320 ,400 dan 1000 ppm glucose solution pipetted into a 10 mL volumetric flask, then diluted to the limit, then pipette 1 mL of the solution was put into a test tube then added a 1 mL Nelson reagent, then covered with cotton. The solution is heated over boiling water ± 10 minutes. Sampels is stand for 5 minutes. The solution is taken into a 10 mL volumetric flask and 1 mL of arsenomolybdate reagent is added. Aquadest was added to the limit and homogenized, allowed to stand for incubation time . Absorbance is read at maximal lamda.

4. Glucose Lowering Test in Quercetin

Quersetin solution was made in series of concentrations of 2, 4, 6, 8, and 10 ppm by piping 20, 40, 60, 80, and 100 µL 1000 ppm quersetin solution into a 10 mL volumetric flask and then diluted to the limit. Each series of quersetin solution of 2 mL was taken plus 2 mL of standard solution of glucose concentration of 40 ppm into a test tube. As much as 1 mL of the solution mixture is put into a test tube, 1 mL Nelson reagent is added, then covered with cotton. The solution is heated over boiling water ± 10 minutes. The solution is cooled for 5 minutes. The solution is taken into a 10 mL volumetric flask and 1 mL of arsenomolybdate reagent is added. Aquadest was added to the limit, and homogenized, allowed to stand for incubation time . Absorbance is read at maximal lamda.

5. Glucose Lowering Test in Parijoto Extract and Nano Parijoto Fruit Extract

Nano extracts and parijoto fruit extracts were made in series of concentrations 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 ppm by piping 20, 40, 60, 80, 100, 200, 300, 400, 500 and 600 µL of nano extract and 1000 ppm extract were put into a 10 mL volumetric flask and then diluted to the limit.

Each series of nano extract and 2 mL extract were added plus 2 mL of standard glucose solution with a concentration of 40 ppm into a test tube. As much as 1 mL of the solution mixture is put into a test tube, 1 mL Nelson reagent is added, then covered with cotton. The solution is heated over boiling water ± 10 minutes. The solution is cooled for 5 minutes. The solution is taken into a 10 mL volumetric flask and 1 mL of arsenomolybdate reagent is added. Aquadest was added to the limit and homogenized, allowed to stand for incubation time. Absorbance is read at maximal wavelength.

RESULT AND DISCUSSION

Table 1. Characteristic of Parijoto Fruit Extract

Powder weight	Extract weight	Rendemen	Characteristics		
			Shape	Color	Smell
200 gram	35,113 gram	17,56%	Thick	Chocolate	Characteristic odor

Table 2. The Characteristics of Dried Powder and Parijoto Fruit Extract

Sample	Characterization		
	Water content (%)	Water soluble (%)	Ethanol soluble (%)
Dried powder	1,06	16,3	20,2
EEBP	0,38	51,4	75,7

The Result of Water Content

The results of the water content of dried powder and extract was respectively 1.06% and 0.38%. These results were accordance with the applicable parameters ie not more than 10% (Puspitasari & Prayogo, 2017). The water content is too high will cause microbial growth. It will affect the quality of the ingredients (Wardatun *et al* ., 2016.

The Result of Water Soluble and Ethanol Soluble

The result of ethanol soluble for dried powder was 20.2%, and extract was 75.7%. The results of water soluble for dried powder was 16.3% and extract was 51.4%. The purpose was to find out the amount of compounds that are solved by polar (water) or non-polar (ethanol) solvents. (Handayani *et al* ., 2017). The percentage of ethanol dissolved content was greater than water soluble content. This showed the most of the parijoto fruit bioactive compounds tend to be non-polar.

The Formulation of Nano Chitosan Parijoto Fruit Extract

Nanoparticle formulation based on ionic gelation method. The method is mixing parijoto extract, chitosan 0.2% and NaTPP 0.1%. The process of mixing chitosan solution with NaTPP causes the positively charged amine group (NH_3) in chitosan to interact with the positively charged tripolyphosphate group ($H_3P_3O10^{-2}$). Ionic interactions will form crosslink bonds (Rahayu and Khabibi, 2016).

The Characterization of Nano Chitosan Parijoto Fruit Extract (NPFE)

The nanoparticle that have been made was tested for characterization, the results of which can be seen in Table 3.

Table 3. The Characterization of NPFE

Chitosan 0,2% : NaTPP 0,1%	Extract	Particle Size	Polydispersity Index	The Average Transmittance Value
5 : 1	0,2%	269,3 nm	0,372	99,379 %

The percentage of transmittance produced by nanoparticle was 99.379%, the results were close to 100%. It showed that the parijoto nanoparticles made were well-formed. It is known that the higher the percentage value of nanoparticles, the smaller the size of the nanoparticle.

The results of particle size in the formula of NPFE were 269.3 nm and the polydispersion index was 0.372. Particle size results were by the requirements, where the nanoparticle size is in the range of 10-1000 nm (Nagavarma, Yadav, Ayaz, Vasudha, and Shivakumar, 2012). If the polydispersity index is in the range 0.3, the formed nanoparticles have a range of proper particle size distribution or similar level of uniformity (Mardliyati *et al* ., 2012). Non-uniform particle size caused by particles agglomerated to form a large aggregate of particles. Factors that can affect particle size and polydispersion index are chitosan and crosslinker concentration, volume, and mass ratio between chitosan solution and crosslinker, stirring speed and stirring time.

The Results of Glucose-Lowering Activity

The activity of reducing glucose levels used non-enzymatic methods, namely Nelson Somogyi (in vitro). The principle of the Nelson Somogyi method is to oxidize glucose with the Nelson reagent, which will convert glucose to gluconic acid so that its absorption will read with a Uv-Vis spectrophotometer.

Glucose is a reducing sugar that converts Nelson's pereagen copper (II) ions to copper (I). The reaction accelerated by the heating of the sample solution, marked by the red deposits of copper (I) oxide. After a cold solution, Arsenomolybdate reagent is added, which functions as the oxidation of copper (I) ions to copper (II), marked greenish-blue formed (molybdenum complex). Then, the absorbance value of the sample is measured (Aprizayansyah et al., 2015).

Determination of the Maximum Wavelength of Glucose

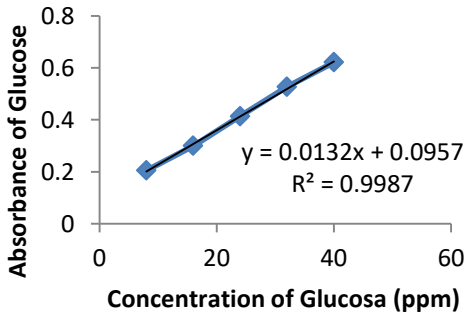
The first step is to know the maximum wavelength. The purpose is to find out the maximum absorption of the analyzed sample solution. Wavelength measured in the range of 700-780 nm, which is the absorption area of nelson Somogyi reagents. Wavelength obtained by 757.4 nm; this result is not much different from the study conducted by 760.5 nm (Vifta and Advistasari, 2018).

Determination of Optimum Glucose Incubation Time

The second step after determining the maximum wavelength doing by determining the operating time. Operating time is carried out for 40 minutes, and an optimum time obtained in the 17th minute. Determination of operating time carried out to determine the time needed for a substance to react optimally and stable (Wardatun *et al* ., 2016).

Glucose Standard Curve

The aim of making the standard curve is to know a linearity equation between absorbance and concentration of glucose. Preparation of standard curve done by making a series of concentrations of a glucose solution at 8, 16, 24, 32, and 40 ppm, so the linear regression equation y = 0,0132x + 0.0957 with relation coefficient (R2) = 0.9987. There was a correlation between absorbance and concentration. The glucose standard curve equation is in Figure 4.



Picture 1. The Curve of Glucose Standard

4. Glucose- Lowering Test

Glucose level testing carried out to determine the activity of NPFE to decrease glucose levels. The calculation based on the value of a linear equation of glucose standard. The percentage decreasing in glucose level and EC 50 values in table 4 and 5.

EC50 quercetin, NPFE, and parijoto fruit extracts respectively showed that quercetin could reduce 50% glucose levels, 6,831 ppm, NPFE <2 ppm, and parijoto fruit extract 48,750 ppm. NPFE was more effective in reducing 50% glucose 24x higher than the extract, and NPFE has effectiveness 8x higher than the comparative control of quercetin.

Research of Kesharwani *et al.,(*2018) stated that the development of nanocarriers (nanoparticles, liposomes, dendrimers, niosomes, and micelles) can increase hypoglycemic activity. In addition, the presence of nanocarriers as encapsulates can provide hypoglycemic agent protection so as to avoid degradation and its effect is more optimal as a glucose-lowering agent. Encapsulation of insulin nanoparticles at a dose of 100 IU / kgBB can reduce preprandial and postprandial glucose levels in Wistar female rats by 104, 32 % ± 9.74%, and 72.20 ± 5.53%. Insulin formulated into nanoparticles has 2x fold activity compared to insulin without formulation (Hamindar & Nugroho, 2016) Nano chitosan extract *Echinacea purpurea* dose 465 mg / kgBB can reduce the AUC value of blood glucose in male Sprague-Dawley strain (Mao *et al*, 2018).

Its secondary metabolite, flavonoids influenced the activity of decreasing glucose levels in parijoto fruit. Secondary metabolites in parijoto fruit have low solubility so that the ability to penetrate target cells is also low (small bioavailability). The encapsulated extract of the ionic gelation method produced better activity than the extract. Encapsulation of bioactive compounds with chitosan (cross-linking) will increase the activity and strengthen the mechanical strength of the particles formed so that it can maintain the stability of secondary metabolites (Abdassah, 2015). The nanoparticle system can increase the number of bonds between the active substance and the target due to the increased contact surface area (Martien *et al* ., 2012).

Glucose-lowering activity caused by glucose solutions that react with flavonoids to form glucose complexes. The -OH group at C-3 in flavonoids can bind glucose which makes the glucose levels in the standard solution will be reduced (Wardatun *et al* ., 2016). This according to research from Sarian *et al* ., (2017) that C-2, C-3 double bonds, ketone groups on C-4 in the flavonoid structure can affect the increase in xanthine oxidase activity, α-glucosidase, and inhibitory activity of DPP-4. The hydroxyl group plays an essential role in regulating the flavonoid bioactivity.

Table 5. The Activity of Glucosa-Lowering Extract, NPFE, and Quercetin

Sample	Concentration	Average	%	EC_{50}

	(ppm) (x)	Absorbance±SD	Decreased Glucose Level (y)	(ppm)
Extract	2	0,439±0,0030	34,646	48,750
	4	0,435±0,0010	35,408	
	6	0,430±0,0025	36,296	
	8	0,424±0,0012	37,438	
	10	0,418±0,0025	38,707	
	20	0,405±0,0015	41,055	
	30	0,386±0,0026	44,736	
	40	0,373±0,0030	47,211	
	50	0,355±0,0017	50,637	
	60	0,343±0,0030	52,922	
NPFE	2	0,334±0,0025	54,571	< 2
	4	0,327±0,0021	55,904	
	6	0,323±0,0006	56,792	
	8	0,319±0,0025	57,554	
	10	0,314±0,0035	58,379	
	20	0,293±0,0035	62,377	
	30	0,274±0,0015	66,121	
	40	0,247±0,0026	71,197	
	50	0,230±0,0026	74,433	
	60	0,206±0,0021	79,066	
Quercetin	2	0,512±0,0015	20,813	6,831
	4	0,449±0,0020	32,742	
	6	0,391±0,0042	43,847	
	8	0,319±0,0025	57,554	
	10	0,255±0,0035	69,611	

CONCLUSION

The characteristics of nano chitosan parijoto fruit extract (*Medinilla speciosa* Reinw. Ex B.) with chitosan 0.2% and NaTPP 0.1% (volume ratio = 5: 1) included the value of particle size was 269.3 nm, particle distribution was 0.372, and percent of transmittance was 99.379%. EC50 of nano chitosan parijoto fruit extract has <2 ppm. The researcher used a non-enzymatic method (*in vitro),* namely Nelson-Somogyi, based on changes in the color of the reaction. The research conducted has not been able to produce conclusions that occur on cellular effects on living subjects. It needs to emphasized the antidiabetic effectiveness of nanoparijoto using the in vivo method. So we can find out the effective dose as a glucose-lowering herbal medicine.

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