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RESEARCH PAPER

The Effect of Stirring Speeds to the Entrapment Efficiency in a Nanoparticles Formulation of Java Plum's seed Ethanol Extract *(Syzygium cumini)*

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Abbreviations:

Abstract: Java Plum's *(Syzygium cumini)* seed contains flavonoids in the form of quercetin. Quercetin plays an essential role in stimulating insulin production from pancreatic beta cells. However, it could be easily degraded by gastric acid or the digestive system. Thus, in this research, a good delivery system for quercetin will be established, namely nanoparticles. The study objectives are determining the entrapment efficiency's percentage of nanoparticle preparations from ethanol extracts of Java Plum's seeds and observing the effect of stirring speed on the percentage of entrapment efficiency. Java Plum's seed extract was obtained by maceration method using ethanol 70% with the ratio between the simplicial powder and solvent is 1:4 respectively. Meanwhile, the solvent evaporation process was undergone by using a water bath with a temperature not exceeding 70°C. The nanoparticle formulation of Java Plum's seed extract was made by adding polymers in chitosan and sodium tripolyphosphate cross-linker with three variations of stirring speed (500, 1000, and 1500 rpm) using a magnetic stirrer. The percentage of entrapment efficiency was obtained by subtracting the flavonoids levels of Java Plum's seeds extract, and flavonoids levels of Java Plum's seeds extract in nanoparticles. Then, the value was divided by flavonoids levels of Java Plum's seeds extract and multiplied by 100%. Quercetin levels obtained by absorbance readings using spectrophotometry UV-Visible, then absorbance value-added into variable X on equation quercetin's standard curve y = 0,0229x + 0,0644. The results show that the percentage of entrapment efficiency at speed variations of 500, 1000, and 1500 rpm are 48.8459%, 49.1064%, and 56.0413%. According to the t-test two independent samples statistical test, the data has a 0.961 probability value (500 rpm vs. 1000 rpm), 0.324 probability value (1000 rpm vs. 1500 rpm), 0.25 (500 rpm vs. 1500 rpm). Probability value > 0.05, which means the stirring speed does not significantly influence the percentage of entrapment efficiency.

Keywords: Java Plum's Seed, Flavonoids, Nanoparticle, Stirring Speed, Entrapment Efficiency

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INTRODUCTION

Java Plum (*Syzygium cumini*), also known as Syzygium jambolanum or Eugenia cumini is a native plant in India, Bangladesh, Myanmar, Nepal, Pakistan, Srilanka, and Southeast Asia, such as Indonesia [1,2]. Java Plum's has been used by the public as a traditional medicine to treat diabetes, anti-inflammatory, ulcers, and diarrhea [3]. Generally, people only consume the fresh Java Plum fruit directly and leave the seeds behind. Meanwhile, the fresh Java Plum seed is very effective

as an antidiabetic agent. Thus, Fresh Java Plum seed can reduce the sugar level in the urine quickly [4].

The extracts from Java Plum's seeds are also liable to lower blood sugar since it contains flavonoids in quercetin. Quercetin works by stimulating insulin production from pancreatic beta cells [5]. Even whole Java Plum's seeds are likely to have a similar effect to glibenclamide, a well-known commercial drug for diabetes mellitus [6]. Therefore, currently, Java Plum seed has the potential as raw material for natural medicine.

Many plants extracts are formulated in various dosage forms, but sometimes it is unreliable because the usage is only based on empirical evidence [7]. Furthermore, active compounds based on natural ingredients also have bioavailability problems. Quercetin, for example, is easily degraded by gastric acid or the digestive system. Therefore, the delivery system in the form of nanoparticles can be used as a quercetin carrier. One of the advantages of nanoparticles is that they can protect the compounds from extreme pH [8]. Nanoparticles are defined as dispersed particles or solid particles with sizes from 1-1000 nm [9]. Nanoparticles are designed as a drug delivery system (active substance) that can regulate the release rate of active substances, increase solubility and drug absorption in the body [10].

In this study, nanoparticles from Java Plum's seed extract with a carrier solution in the form of chitosan and sodium tripolyphosphate (Na-TPP) cross-linker were prepared using the ionic gelation method. The ionic gelation method is a method to form the nanoparticles with constant stirring at room temperature. The stirring process is an essential factor in the manufacture of nanoparticles. Thus, the stirring speed variation was carried out to determine a good stirring speed in the manufacture of Java Plum's seed extract nanoparticles. The appropriate stirring speed was observed and linked to the amount of flavonoids absorbed in each sample.

In this research, aside from active compounds based on natural ingredients, chitosan, a nanoparticle carrier system, is also a natural material. Chitosan is obtained from the hydrolysis of chitin polymer, which is obtained from the shells of crustaceans such as shrimp, lobster, and crab [11]. Therefore, these ingredients are commonly consumed, so they are unlikely to cause toxicity at therapeutic doses [12]. It is by one of the primary factors for consideration of pharmaceutical therapy, namely reducing the harmful effects of the system [13].

MATERIALS AND METHODS

Materials

The ripe Java Plum's fruits with blackish purple color were used as the main samples. As the reagents, polymer chitosan, cross-linker sodium tripolyphosphate, ethanol 70%, glacial acetic acid, aquadest, aluminum chloride, hydrochloric acid, sodium acetate, quercetin standards, ethanol pro analysis, and paper filter were utilized in this experiment.

Sample Preparation and Extraction

Java Plum fruit was washed 3 times and separated from the seeds. The Java Plum's seeds are then washed again until there is no pulp attached to the seeds. After being washed, it was dried until the sample weight was constant. The dried Java Plum's seeds were then mashed and sieved using sieve mesh number 10. In the next step, the Java Plum's seed powder was extracted using the maceration method with 70% ethanol as the solvent. The ratio between Java Plum's seed powder and ethanol solvent was 1: 4. The maceration process was carried out for 3 x 24 hours. The results were then filtered to separate the Java Plum's seed ethanol extract from the precipitate. Furthermore, the extract was concentrated using a water bath at 70 ° C so that the thick extract of the Java Plum's seeds was obtained.

Determination of Maximum Wavelength, Operating Time, and Standard Quercetin Curve

The 1000 ppm quercetin main solution was prepared by dissolving 10 mg of standard quercetin with 10 mL ethanol pro analysis. It was then diluted to form several concentration series, namely 10, 15, 20, 25, 30, and 35 ppm. The determination of the maximum absorbance wavelength was observed in the range 400-450 nm using a UV-Visible spectrophotometer [14]. After the maximum wavelength was obtained, the operating time can be determined by reading the absorbance every 1 minute until a stable absorbance was occurred [15]. Finally, the determination of the standard quercetin curve is obtained by reading the absorbance of each series of quercetin solution concentrations. 1 ml solution was taken from each concentration and mixed with 10% AlCl3 reagent, 1M sodium acetate, 1M HCl, and 0.5 mL of distilled water each [16]. Then, the absorbance is read at the maximum wavelength and the operating time previously obtained.

The Formulation of Nanoparticles Ethanol Extract of Java Plum's Seed

The nanoparticles ethanol extract of Java Plum's seed can be obtained by adding the chitosan carrier and cross-linker in the form of sodium tripolyphosphate (Na-

TPP). Hence, the formula can be seen in the following table:

Table 1. The Formula of Nanoparticles Ethanol Extract of Java Plum's seed

Reagents	Concentration (% b/v in gram/mL)	Volume (mL)
Chitosan	0,55	2
sodium tripolyphosphate	0,06	2
Ethanol Extract of Java Plum's Seed	1,55	2

The first step was to make 0.6% chitosan main liquor, 0.5% Na-TPP main liquor, and 2% extract ethanol Java Plum's seed main liquor. A 0.55% chitosan solution was made from those main liquors by dissolving 9.167 mL of main chitosan liquor with 1% acetic acid ad 10 mL. Then, 0.06% Na-TPP solution was obtained from 1.2 mL of Na-TPP ad 10 mL aquadest. Finally, the 1.55% extract solution was obtained by dissolving 7.75 mL of the mother liquor extract with 70% ethanol ad 10 mL.

The preparation of the nanoparticles was then carried out by mixing 2 mL of 1.55% ethanol extract of Java Plum's seed and 2 mL of 0.55% chitosan solution on a magnetic stirrer at a speed of 500 rpm. While stirring, 2 mL of 0.06% Na-TPP solution was wisely dropped into the solution. For repetition, the formula was replicated into 3 replications. The whole process was then repeated with different speeds, 1000 rpm, and 1500 rpm.

Percentage of Transmission

To determine the percentage of transmission, 3 mL of the nanoparticles that have been made were taken using a cuvette. Then, the value was measured using a UV-Visible spectrophotometer with a blank in the form of aquadest at a maximum wavelength of 650 nm [17].

Entrapment Efficiency

To evaluate the entrapment efficiency, 1 mL of the nanoparticles that have been produced were added with 10% $AlCl_3$ reagent, aquadest, 1M HCl, and 1M Na-acetate 0.5 mL each. The solution was then incubated regarding the operating time. The absorbance was read at the maximum wavelength obtained previously. The same procedure was done for the ethanol extract of Java Plum's seed. Flavonoids levels are obtained by entering the absorbance value into the standard curve equation. Based on this value, the entrapment efficiency percentage can be obtained through the following equation [18].

$$\%\ EE = \frac{Ca - Cb}{Ca}\ x\ 100\%$$

where:

% EE = entrapment efficiency

Ca = flavonoids content of the ethanol extract of Java Plum's seed

Cb = flavonoids content of the nanoparticles ethanol extract of Java Plum's seed

RESULTS AND DISCUSSION

The Java Plum's samples were secured from Lendang Batu Village, Kayangan District, North Lombok Regency with latitude coordinates of 8 ° 16'18.749 "and east longitude 116 ° 14'26.474". Juwet parts such as stems, bark, leaves, fruit and roots were also collected for determination. Determination was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University. Based on the determination results, the sample used in this study was the Java Plum with the scientific name Syzygium cumini. The Java Plum's seed sample was then prepared to obtain simplicia powder. The percentage of simplicia yield was 44.2049%. The simplicia powder was then extracted, and the yield value of the extract was 11.4234%.

The extraction technique used in this research was the maceration technique. It was a cold extraction technique (without heating) that was able to avoid damage to thermolabile compounds such as flavonoids in the samples. The solvent used for extraction was 70% ethanol. The ethanol was used because flavonoids are polar compounds. Thus they will dissolve well in polar solvents such as ethanol. However, flavonoids are bound in glycosides, so the solvent mixture with water is a good solvent for flavonoids [19]. In this study, it is expected that flavonoids in quercetin can be absorbed in the nanoparticles.

In this study, the nanoparticles used were the nanocarrier, it is a cross-linked nanoparticle with the ionic gelation method. Nanocarrier has several advantages, including increasing the solubility of active drug substances, protecting the recombinant proteins and genes, circulating in blood vessels for a long time without being noticed by macrophages (so that they are controlled release in the targeted area) [20]. It is expected that this nanoparticle preparation can work as a controlled release that targeting the location of the pancreas to produce insulin, thereby reducing blood sugar levels (antidiabetic).

The nanoparticles were produced through the ionic gelation method with a carrier system in chitosan and a cross-linker in the form of a polyanion, namely sodium tripolyphosphate (Na-TPP). Nanoparticles are formed

due to the ionic interaction between free ammonium groups (positive ionized groups) on chitosan with the negative charge in polyanions. The ionic gelation method can form nanoparticles spontaneously with the constant stirring speed at room temperature [21]. According to this, the stirring speed is an essential factor in nanoparticles manufacturing. Slow stirring might form the large droplets, which means the reaction is not complete and nanoparticles are not fully formed. Stirring too fast could cause the particles that are formed to be too small so that they are unable to absorb the active compounds in the matrix of each particle [22]. The appropriate speed of stirring can be seen from the amount of active compound absorbed in each sample. This variable is known as entrapment efficiency. So, in this study, the stirring speed is an independent variable that might affect the entrapment efficiency percentage.

Entrapment efficiency is a variable that can be used to determine the ability of chitosan (polymer) to protect the active substance that forming nanoparticles [23]. In other words, entrapment efficiency shows the ability of chitosan (polymer) to carry active substances into the body [24]. The higher values show the more significant the ability of chitosan to protect the active substance from external destructive influences. Furthermore, the high percentage of entrapment efficiency means the higher the bioavailability of the active substance and vice versa [23].

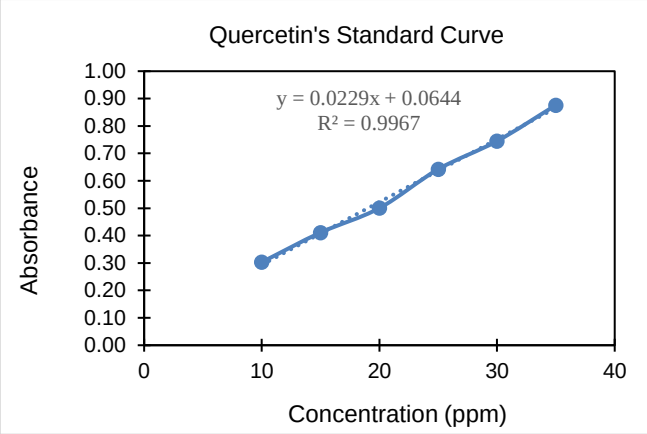

Figure 1. Quercetin's Standard Curve

The obtained nanoparticles were centrifuged at a speed of 15000 rpm for 30 minutes to separate solids from colloidal nanoparticles. Supernatant then mixed with 10% $AlCl_3$ reagent, aquadest, 1M HCl and 1M Na-acetate 0.5 mL each. The solution was then incubated for 20 minutes, and the absorbance was read at a maximum wavelength of 417 nm. The same procedure was carried out for the ethanol extract of Java Plum's seed. Flavonoid levels are obtained by entering the absorbance value into the standard curve equation (y = 0.0229x + 0.0644) (Figure 1) for the x variable, where y is a variable representing the flavonoids level.

The entrapment efficiency value can then be calculated through the gap between the extract flavonoids content and the nanoparticles. The value is then divided by the extract flavonoids content and multiplied by 100%. The entrapment efficiency value can be seen in Table 4. The entrapment efficiency test results are then statistically tested using t-test two independent samples. In the t-test, if the Sig. value or p> 0.05, it can be concluded that there is no significant difference between groups. On the other hand, if p<0.05, there is a significant difference between groups [25]. Based on According to the t-test two independent samples statistical test, the data has a 0.961 probability value (500 rpm vs. 1000 rpm), 0.324 probability value (1000 rpm vs. 1500 rpm), 0.25 (500 rpm vs. 1500 rpm). Probability value > 0.05, so there was no significant difference between groups. Thus, it can be concluded that the increase in stirring speed does not cause a significant difference in the percentage of entrapment efficiency.

Effective absorption is acquired when the percentage of entrapment efficiency is close to 100% [26]. In addition, the good percentage of entrapment efficiency has to be more than 60% [18]. The nanoparticle system is said to be successful if it has high entrapment efficiency since it can reduce matrix components. It means that the drug can give pharmacological effects at smaller or more efficient doses. However, based on the research results, it is obtained that the low average value of the entrapment efficiency at each speed because the value is below 60%.

The short stirring duration can cause the low entrapment efficiency percent value for only 1 hour (probably not the optimal time), while the percent entrapment efficiency value is not only influenced by the stirring speed but also by the stirring time. The short stirring might cause the reaction is not complete (the nanoparticles are not yet fully formed) so that the active substance has not been fully absorbed. Stirring too long can also cause the particle size to be too small so that the ability to absorb active substances is low [22]. Therefore, it is expected that in further research, the effect of stirring time on the formulation of the ethanol extract of Java Plum seed nanoparticles needs to be carried out.

Apart from this, it is possible that the low entrapment efficiency percentage value can also be caused by the absence of a pH setting (acidity). It could cause the dissociation rate between polymer (chitosan), cross-

linker (Na-TPP), and active substance (Java Plum seed ethanol extract) is not regulated. The unregulated level of dissociation might result from a low ionic group formation among the three so that the percent entrapment efficiency obtained is also low.

Table 2. Absorbance and flavonoids level of the ethanol extract of Java Plum's seed

Stirring speed (rpm)	Replications	Absorbance	Flavonoids Levels
500 rpm	1	0,6035	23,5415
	2	0,6103	23,8384
	3	0,5755	22,3188
1000 rpm	1	0,7199	28,6244
	2	0,6269	24,5633
	3	0,6569	25,8734
1500 rpm	1	0,6686	26,3843
	2	0,6698	26,4367
	3	0,6938	27,4847

Table 3. Absorbance and flavonoids level of the nanoparticles ethanol extract of Java Plum's seed

Stirring speed (rpm)	Replications	Absorbance	Flavonoids Levels
500	1	0,3108	10,7598
	2	0,3649	13,1223
	3	0,3338	11,7642
1000	1	0,3451	12,2576
	2	0,3834	13,9301
	3	0,3793	13,7511
1500	1	0,2918	9,9301
	2	0,3153	10,9563
	3	0,3967	14,5109

Table 4. *Entrapment Efficiency* of the nanoparticles ethanol extract of Java Plum's seed

Stirring Speed (rpm)	Replication	*EE* (%)	Mean
500	1	54,2943	48,8459
	2	44,9531	
	3	47,2902	
1000	1	57,1778	49,1064
	2	43,2890	
	3	46,8524	
1500	1	62,3636	56,0413
	2	58,5565	
	3	47,2037	

CONCLUSION

Based on the research that has been done, it can be concluded that the average entrapment efficiency at the stirring speed of 500 rpm, 1000 rpm, 1500 rpm is 48.8459%, 49.1064%, 56.0413%, respectively. This entrapment efficiency value shows chitosan (polymer) ability to protect the active substance in the form of flavonoids. The most significant percentage of entrapment efficiency is at a speed of 1500 rpm. The greater the entrapment efficiency value, the greater the ability of chitosan to protect the active substance from external destructive influences. So that the bioavailability of the active substance also increases. However, based on the t-test two independent samples static test, the probability value is > 0.05, which indicates that the stirring speed has no significant effect on the entrapment efficiency.

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