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Characterization of Nano Chitosan-Cucumber Suri (*Cucumis melo* L.) Seeds with Sodium Tripolyphosphate as Crosslinker

*Rissa Laila Vifta¹, Fania Putri Luhurningtyas², Febi Indah Fajarwati³

^{1,2} Department of Pharmacy, Faculty of Health Science, Universitas Ngudi Waluyo, Indonesia

³ Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Islam Indonesia, Indonesia

*rissa_laila@yahoo.co.id. (Corresponding author 1)

*febi.indah@uii.ac.id. (Corresponding author 2)

Abstract. Cucumber suri (*Cucumis melo* L.) is one of natural materials which has many bioactivities, such as antioxidant, antifungal, antibacterial, anti-cholesterol, antidiabetic, and several other activities. Eventough, Cucumber suri (*Cucumis melo* L.) has a fairly low flavonoid content. In addition, bioactive compounds in cucumber suri seeds tend to be difficult to dissolve in water, unstable to pH, and even have a low bioavailability. Nanoparticles can be used to increase the activity of natural materials by using chitosan and sodium tripolyphosphate as crosslinking agents. This study aims to determine the characteristics of nano chitosan-cucumber suri seeds (*Cucumis melo* L.). First, Cucumber Suri seeds was previously extracted using maceration method and identified their bioactive compounds. And then, Cucumber Suri seed extract was encapsulated with Chitosan: STPP (5: 1). Characterization test was carried out using UV-Vis Spectrophotometry, Infrared Spectrophotometry, and Particle Size Analyzer (PSA). The encapsulation process of Cucumber Suri seed extract produces a clear, homogeneous and stable supernatant. The values of characterization with UV-Vis Spectrophotometry in 0 to 60 minutes was >90%, that indicated nanosized particles was successfully formed. The IR characterization values indicate a shift in the nano chitosan-cucumber suri seeds wavelength compare with chitosan. The shift of -OH stretching vibration at wavelength 3403 cm⁻¹ and stretching vibration of C=O in 1642 cm⁻¹ showed there was an interaction between chitosan and Cucumber Suri seed extract. Analysis using Particle Size Analyzer (PSA) showed that chitosan-cucumber suri seeds had a normal polydispersion index (PI) of 0.677 and average particle diameter size was 360 nm.

1. Introduction

Cucumber suri (*Cucumis melo* L.) is an annual plant that produces fruit, speradly growth, and has yellow and bell-shaped flowers [1]. Cucumber suri contains secondary metabolites which generally have a low bioavailability and they are very sensitive to some processing factors. Because it is difficult to dissolve in water, it becomes difficult to absorb. In addition, many compound of the active ingredients has a bitter taste that limits their use in oral form [2]. To resolve the insolubility in water and also low bioavailaibility, another technology needs to be applied [3]. One of many technologies that can be applied is nanotechnology. The synthesis of nanoparticles was designed as a delivery system to control the particle size, the character of the surface and drug releasing system to achieve specific drug action to optimize of therapeutic effects [4]. The application of nanotechnology can be a solution to the main



problem of active compounds that have low bioavailability, because nano-sized particles have a very large surface area that allows it to be more effective and easy to pass through the intestinal wall [5].

Chitosan is kind of polymers that often used as encapsulant. The advantages of chitosan as encapsulant has been widely used to improve the drug release and other substances [6,7]. The use of chitosan as a nanopolymer material needs the addition of Sodium tripolyphosphate (STTP) as a crosslinker to stabilize the nanoparticles polymers [8]. This can be occur because of the positive charge of chitosan will bind to the negative charge of STPP (Sodium tripolyphosphate) [9]. Tripolyphosphate (TPP) its self is a polyanion which can be used as crosslinker in nanoparticles formation [10].

In the previous study, chitosan as an encapsulant had been used to develop the mechanical and physicochemical properties of *Yerba mate* extract [7]. In another research group, chitosan and tripolyphosphate as crosslinkers was found to be useful to increase the antiulcerogenic activities of *Arrabidaea chica* extract [11]. In another hand, polymers such as chitosan can be used to deliver the active compounds contained in natural product. El-Aziz et al., 2018 reported that combination of Chitosan and *Mentha longifolia* extract showed the highest effect in antifungal activities between bulk chitosan and chitosan nanoparticles. Chitosan was temporary used in deliver the active compounds because of its abilities to provide protection and stability [12].

In this study will be carried out the synthesis and characterization of the combination between nanochitosan-*Cucumis melo* L. with Sodium Tripolyphosphate (STPP) as the crosslinker. The result is expected to provide a preliminary study of the formation of nanoparticles between chitosan and *Cucumis melo* L. This research can be used as an alternative way in phytopharmaca, especially about the functionalization and optimization of natural product as an alternative for complementary treatment through the nanoparticles formation.

2. Method

2.1. Plant material

Cucumber suri (*Cucumis melo* L.) seeds was obtained from Salatiga, Central Java and previously determined in Laboratory of Ecology and Biosystematics, Universitas Diponegoro.

2.2. Testing material

Testing materials were Ferri-chloride 1%, dragendorff reagent, Mayer reagent, sitroborate reagent, HCl pro analysis (Merck®), Chloride acid 2N, H₂SO₄ pro analysis, n-hexane, ethyl acetate, ethanol 96%, Chitosan, Sodium Tripolyphosphate (STPP) from PT. Brataco, and Aquades from CV. Bratachem.

2.3. Extraction procedure

Making Cucumber Suri (*Cucumis melo* L.) seed extract was made by maceration method using 1500 grams of cucumber suri seed powder that added by ethanol 96% (1:10), and then macerated for 24 hours. The maserate was collected and the pulp was remecerated for 24 hours until the maserate was obtained. The collected maserate was evaporated using a rotary evaporator at 60°C until the thick extracts was obtained.

2.4. Phytochemical screening

Phytochemical screening using the thin layer chromatography (TLC) method was carried out to determine the secondary metabolites contained in the extract. The mobile phase used was n-hexan and ethyl acetate with a ratio of 8: 2. Identification of the compounds was carried out using several spray reagents, namely identification of the flavonoids (using H₂SO₄), identification of alkaloids (using Dragendorff reagent), and identification of tannins (using FeCl₃ 1%).

2.5. Synthesis of nano chitosan-cucumber suri

One milligram of Cucumber Suri extract was diluted in 10 ml of ethanol pro-analysis and stirred for 20 minutes. After that, the sediment and the solution were separated. The solution was collected and ready to be encapsulated. 0.069 grams of chitosan was diluted in 11 ml of glacial acetic acid 2.5% (pH 4). The chitosan solution that was obtained then added with the Cucumber Suri seed extract. The next step, the solution was stirred at 400 rpm for 20 minutes. Then, the STPP solution with a concentration of 0.25% as much as 300 μ l was added and stirred at 400 rpm for 20 minutes. The result of the formulations that was obtained, was centrifuged for 15 minutes.

2.6. Characterization of nano chitosan-cucumber suri

The Characterization of Chitosan-Cucumber Suri Seed nanoparticles was carried out using UV-Vis Spectrophotometer (Shimadzu, UVmini-1240), IR Spectrophotometer (Perkin Elmer, Frontier FT-IR 96772), and Particle Size Analyzer (PSA) (Malvern, Zetasizer Nano-S90). The initial characterization was done by observing the transmittance at the maximum wavelength. The scanning of wavelengths was carried in the range of 190-900 nm according to the research conducted by Yasin *et al.*, 2013. Identification of specific groups of nano chitosan-cucumber suri seed was carried out using Infrared Spectrophotometer at the wave number 450-4000 cm^{-1} . The particle size and polydispersion index tests were carried out using Particle Size Analyzer.

3. Result and Discussion

The synthesis of nano chitosan-cucumber suri seeds was began by determining the material. The determination was aimed to find out the truth of the plants and avoid errors in the material collection [1]. The result of the determination of cucumber suri seeds conducted at the Laboratory of Ecology and Biosystematics, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang showed that the sample that used in this research was Cucumber Suri seeds (*Cucumis melo* L.) which included in the family of Cucurbitaceae.

The extraction of cucumber suri seed was done using maceration method by ethanol 96% as the solvent. The maceration method was chosen because the process is easy and does not use high temperatures which might damage the secondary metabolites contained in the Cucumber Suri seeds. The extraction was carried out using two processes, maceration and remaseration for five days in total it produced of 135.335 grams extract with a percentage of the rendement obtained 9.022% b/b. The rendement of the extract was obtained based on comparison between constant weight of the thick extract and the initial weight of the powder used.

Table 1. The analysis of rendement cucumber suri seed extract

Powder mass (gr)	Constant mass (gr)	Rendement (% b/b)	Characteristic of the Extract		
			Form	Colour	Odor
1500	135.335	9.022	Thick	Brown	Typical

Phytochemical screening was carried out using Thin Layer Chromatography (TLC) that used n-hexane: ethyl acetate (8: 2) as mobile phases and silica GF₂₅₄ as the stationary phase. The solvent had been choosen related with the properties of the compound and the stationary phase used. The solvents should not be carcinogenic and also does not react with samples during the test. The stationary phase that was used is silica GF₂₅₄ (Merck®) because its can be separated the phenolic compounds, alcaloids, fatty acids, sterols, and terpenoids widely. The result was shown in Table 2 which indicated that the active compounds contained in Cucumber Suri seeds were flavonoids, alkaloids, and tannins.

Table 2. Thin layer chromatography of cucumber suri seed extract

Secondary metabolites compounds	Reagent for Identification	Visualization	UV ₂₅₄ nm	UV ₃₆₆ nm	Result
Alcaloids	Dragendorff	Yellowish-White	Yellowish-Brown	Yellow	+
Flavonoids	Sitroborate	Yellow	Brown	Green	+
Tannins	Ferri-chloride	Yellowish-White	Blackish-Green	Blackish-Green	+

Based on the TLC result of the Cucumber Suri seed extract reacted with dragendorff reagent, yellowish brown spot was seen on the UV₂₅₄ nm and yellow spot on the UV₃₆₆ nm were showed the alcaloid content. The test using sitroborate reagents was showed brown spots on the UV₂₅₄ nm and green spots on the UV₃₆₆ nm which indicated the flavonoid content. The test using ferri-chloride reagent showed in blackish-green spots on the UV₂₅₄ nm and blackish-green on the UV₃₆₆ nm which indicated the tannin content.

The synthesis of nanochitosan-cucumber suri was carried using ionic gelation method by adding chitosan and STPP to form the nanoparticles. After that, the characterization of particle had been seen using the spectrophotometry to know the nanoparticles formed. The principles of using UV-Vis Spectrophotometry method was to measure the transmittance values from nanoparticles. Based on the result shown in Table 3, the transmittance was >90%, which indicated that the nanoparticles has been formed quite well.

Table 3. The Formulation of nanochitosan-cucumber suri seeds result

Chitosan: STPP ratio	Result	
	Visual	Transmittance (%)
5:1	Clear-Transparant	99.5

Chitosan was chosen as the encapsulant material with several advantages, such as chitosan is a polymer that has bioactive properties, biocompatible and biodegradable, but chitosan has several disadvantages which are, it can absorb water quickly, so it is easy to be swollen which will decrease the ability in delivering and releasing system of the drug. Therefore, it is necessary to add a crosslinking material, such as Sodium Tripolyphosphate (STPP) which can reduce the swelling degrees and improve the biocompatibility. The use of STTP as a low-dose crosslinker was intended to lower the bond between the polionic TPP and the amine group on chitosan [9].

The characterization of nano chitosan-cucumber suri using UV-Vis spectrophotometer was carried out by observing the transmittance for 60 minutes with 5 minutes time interval. The purpose of this is to determine the stability of nanoparticles formation. The result showed that Nanochitosan-cucumber suri had a good stability which was identified by looking transmittance values that not exceeding the initial interval (>90%). The transmittance >90% means that the smaller particle size and larger surface area are make it easier to read the absorbance. The small size of particle resulted in the faster brown motion, prevent the sedimentation process and cause the supernatant becomes clearer [13].

Table 4. The analysis of transmittance measurement in nanochitosan-cucumber suri seeds

Time (minutes)	Visual	%T (average)	K*%T
0-60	Clear	99.50±0.066	99.640

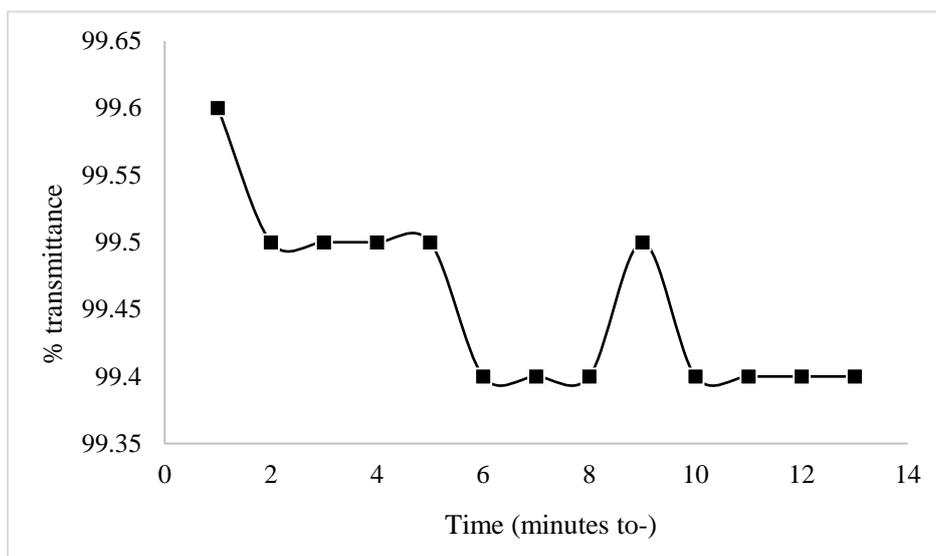


Figure 1. The correlation between time and transmittance percentage of Nanochitosan-Cucumber Suri Seeds.

The nanoparticles formation was confirmed by looking at result from the values of the PSA with a value of Polydispersity Index (PI) of 0.677 and an average diameter of 360 nm which shown in the Table 5. Polydispersity Index (PI) is used to estimate the particle size distribution range and determine whether there is an aggregation formation in nanoparticles samples or not. The particle size distribution is expressed uniformly if the PI value is in the range 0.01-0.7 [14]. Polidispersity index (PI) measured was around 0.677 which indicated a good level of particle distribution uniformity. The large size of the particle can be caused by the small number of active substances that bonded with the chitosan, so that the remaining active substances formed larger size particle aggregations [15].

Table 5. Particle size analysis of nanochitosan-cucumber seeds result

Chitosan:STPP ratio	Characterization Result (PSA)	
	PI	Average diameter (nm)
5:1	0.677	360.00

The characterization of nano chitosan-cucumber suri seeds using infrared spectrophotometry which shown in Figure 1 was carried out to identify the effect of encapsulation between chitosan and cucumber suri seed extract which was identified by the shifting of the absorption wave number. Chitosan has a typical absorption of its -OH stretch vibration in the 3400an cm^{-1} region, -CH stretching vibration in 2800 cm^{-1} , C=O stretching vibration in 1651 cm^{-1} , -NH bending vibration at 1597 cm^{-1} , and -CO stretching vibration in 1080 cm^{-1} [16]. The presence of -OH stretching vibration shift at 3403 cm^{-1} , C-H stretching vibrations at 2928 cm^{-1} , and C=O stretching vibrations at 1642 cm^{-1} showed that there was interaction between encapsulated chitosan and flavonoids contents in the cucumber suri seed extract.

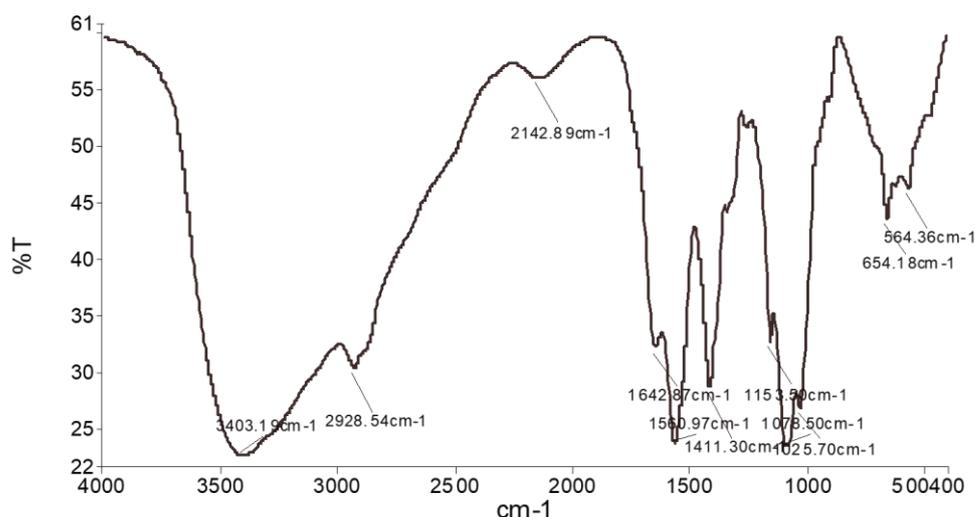


Figure 2. The analysis result using infrared spectroscopy of nanochitosan-cucumber seed.

4. Conclusion

Nanochitosan-Cucumber Suri Seeds had been successfully synthesized using ionic gelation method and Sodium Tripolyphosphate (STPP) as the crosslinker. The characterization result using UV-Vis spectrophotometry showed the presence of nanoparticles formation by looking at the transmittance that was more than 90% and confirmed using Particle size Analyzer with polydispersity index (PI) at 0.677 and the average particle diameter was 360 nm. The analysis using infrared spectrophotometry showed that shift in wavenumber of chitosan which indicated that was interaction between chitosan and cucumber suri seed extract.

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