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Silver Nanoparticles Synthesis from Dragon Fruit (*Hylocereus polyrhizus*) Peel Extract and Its Potential as Antiseptic Mouthwash

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ABSTRACT

This study examines the potential compounds dragon fruit peel extract (Hylocereus polyrhizus) which are synthesized into silver nanoparticles, then used as the antiseptic mouthwash. The synthesis process is carried out using the Green Synthesis Nanoparticle method, which utilizes natural materials as metal bioreductants due to it is more herbal and does not have a poisonous effect on health. Secondary metabolite compounds contained in dragon fruit peel extract (H. polyrhizus), namely flavonoid compounds functioned by donating electrons to Ag+ ions to produce silver nanoparticles that can be used as mouthwash because they are antimicrobial. Based on the results of the UV-Vis spectrum, the best nanoparticle synthesis results were obtained at the extract concentration of 0.25% for 30 minutes duration with a synthesis temperature of 70°C, which the absorbance peak of silver nanoparticles was obtained at wavelength of 444.5 nm, and visually changes the color of the solution to brown. Furthermore, based on the test results obtained from incubation for 24 hours, it was found that the mouthwash solution showed an inhibition zone on the two test bacteria. Escherichia coli bacteria produced an inhibition zone of 28 mm, while Staphylococcus aureus bacteria produced an inhibition zone of 25.5 mm.

Keywords: *Silver Nanoparticle, H. ployrhizus, Mouthwash, Escherichia coli, Staphylococcus aureus*

1. INTRODUCTION

Nanoparticles are nano-sized particles around 1-100 nm. Nanoparticles based on their composition can be divided into two types, namely organic and inorganic. Organic nanoparticles are carbon-based nanoparticles, while inorganic nanomaterials are composed of inorganic materials such as metals, magnetic materials and semiconductors [1,2]. One method of synthesizing nanoparticles that uses natural materials sourced from living organisms, such as plants, animals, and microorganisms that live on land or in the sea, as metal bio-redactors is the Green Synthesis Nanoparticle method. Living organisms produce metabolites, both primary and secondary which can reduce metals, such as alkaloids, amines, amides, proteins, carbonyl groups, and pigments [3,4]. Secondary metabolite compounds contained in plants work by donating electrons to Ag^+ ions to produce silver nanoparticles [5].

Silver nitrate ($AgNO_3$) is an organic compound that is a versatile precursor for other silver compounds, because this compound is relatively stable to light, so it can dissolve in many solvents, including water [6]. Ag is formed through the oxidation reaction of Ag^+ ions present in solution and Ag^+ ions contained in certain plant compounds, such as enzymes and reductases derived from plant parts. Functional groups in secondary metabolites work by donating electrons to Ag^+ ions to form Ag nanoparticles. The reduction process until the formation of silver nanoparticles cannot be separated from the role of secondary metabolite compounds, one of which is the flavonoid compounds contained in dragon fruit peel extract [7].

Indonesia is one country in Asian continent with many natural plants/materials, one of them is dragon fruit. Dragon fruit (*H. polyrhizus*) is currently cultivated by the community because it has high nutritional value

and efficacy. Part of the dragon fruit 30-35% is the skin of the fruit which is very beneficial for health but in reality it is only thrown away and considered as waste, even though 100 g of dragon fruit peel (*H. polyrhizus*) has many ingredients such as 60 kcal calories, 0.53g protein, carbohydrates 11.5g, fiber 0.71g, calcium 134.5mg, phosphorus 87mg, iron 0.65mg, vitamin C 9.4mg, anthocyanin, antioxidants, phenols, flavonoids, protein, fat, water, carbohydrates, ash, pentacyclic triyepene taraxast 20ene 3aol and taraxast 12,20(30) dien 3aol, and the water is 90% [8,9].

One alternative to improve dental and oral health is to use mouthwash by utilizing dragon fruit peel extract (*H. polyrhizus*) which contains secondary metabolites such as flavonoids which have the potential as natural antimicrobial ingredients. Flavonoids work by donating electrons to Ag^+ ions to produce silver nanoparticles that can be used as mouthwash because they are antimicrobial [8,5]. Based on the description of the background, this research is needed to obtain silver nanoparticles ($AgNO_3$) through green synthesis using dragon fruit peel extract (*H. polyrhizus*), to determine the effect of $AgNO_3$ concentration on nanoparticle size and its effect on bacterial growth and the benefits of dragon fruit peel extract (*H. polyrhizus*) as an antiseptic mouthwash.

2. MATERIALS AND METHODS

2.1. Sample preparation

The peel of the dragon fruit (*H. polyrhizus*) was washed, dried and then mashed into powder, then sieved using a 20 mesh sieve. The sieve was called simplicia powder. Then dragon fruit simplicia powder (*H. polyrhizus*) was weighed 10.0 grams, added 80 ml of aquabidest. Straining the extract with a buchner funnel, add 20 ml of aquabidest and then filter it into the same erlenmeyer, then added 100.0 ml of aquabidest. This solution was referred as the main extract. The main extract was diluted at an equivalent concentration of 0.125%; 0.25% and 0.5%, the results of these dilutions were then referred to as tested extracts.

2.2. Preparation of AgNO3 solution

A total of 0.425 grams of $AgNO_3$ powder was dissolved into aquabidest to a volume of 250 mL and then homogenized. Furthermore, as much as 60 mL was pipetted from 1 mM $AgNO_3$ solution into 1000 mL erlenmeyer and added aquabidest to 600 mL.

2.3. Composition Optimazion

1 mM $AgNO_3$ solution was pipetted as much as 20 mL for each concentration of dragon fruit peel extract (*H. polyrhizus*) which had been diluted equally (0.125%; 0.25% and 0.5%) and each solution was put into a glass bottle, Then 10 mL of dragon fruit peel extract (*H. polyrhizus*) was added in a ratio (1:2). The mixture was stirred until homogeneous.

2.4. Silver Naoparticle Shynthesis Process

The solution was heated by stirred with a synthesis temperature of 70°C for 30 minutes. The absorbance of this solution was observed every 15 minutes (t0; t15; t30) with a spectrophotometer, to monitor the formation of silver nanoparticles. After 60 minutes, 0.2 M NaOH was added dropwise while observing the color change and continuously stirring without heat. The results of the synthesis of silver nanoparticles were then used for the characterization and antibacterial activity testing.

2.5. Silver Naoparticle Characterization

The compound obtained from the synthesis of silver nanoparticles using water extract of dragon fruit peel (*H. polyrhizus*) was then characterized on several parameters, namely organoleptic, the yield was observed visually for its color characteristics. The absorbance of the synthesis solution was observed over time to confirm the formation of silver nanoparticles using a Uv-VIS Spectrophotometer instrument.

2.6. Determination of Antibacterial Activity by the Paper Disk Method

Bacterial suspension was made into 5 mL of sterile distilled water. It was shake until homogeneous and immediately rubbed evenly on the NA medium to make a layer of bacteria. Then sterile paper disks were taken and immersed in each of the nanoparticle synthesis sample solutions, dragon fruit peel extract (*H. polyrhizus*), $AgNO_3$, positive control (gentamicin) and negative control (aquabidest), then the paper disk was placed on the surface of the medium in petri dish. Petri dishes were allowed to stand for 30 minutes, then wrapped without turned and then incubated at 37°C for 48 hours. Then the inhibition zone formed around the paper disk was measured for its vertical diameter and horizontal diameter in millimeters (mm).

2.7. Making Mouthwash and Observation UV-Vis Spectrophotometer

The water-soluble ingredients were prepared, namely 50 mL aquabidest, 0.2 gram sodium benzoate, 1 gram saccharin sodium, 3.75 gram tween 80, 1.25 gram glycerin and 3.12 gram dragon fruit peel extract (*H. polyrhizus*), water insoluble material was pipermint oil 0.5 grams. Then mixed together while stirring until dissolved then filtered and put into a clear glass bottle. Furthermore, the re-test was carried out using the Uv-Vis Spectrophotometer instrument to determine the

formation of silver nanoparticles in the mouthwash solution.

2.8. Antibacterial Activity Test

Cotton swab sterile was dipped in bacterial suspension until wet and then rubbed on NA media to make an even layer of bacteria. After that, the paper disks were immersed in each mouthwash solution, positive control (gentamicin), negative control (aquabidest), AgNO3 and dragon fruit peel extract solution (*H. polyrhizus*), then the paper disk was placed on the surface of the agar medium in a petri dish. Petri dishes were allowed to stand for 30 minutes, then wrapped without turning and then incubated at 37°C for 1 x 24 hours. The inhibition zone formed around the paper disk was measured for its vertical diameter and horizontal diameter in millimeters (mm).

3. RESULT AND DISCUSSION

3.1. Isolation of silver nanoparticles ($AgNO_3$) through green synthesis using dragon fruit peel extract (H. polyrhizus)

Figure 1. Dragon Fruit Peel Extract (*H. polyrhizus*).

In this study, the isolation of silver nanoparticles was carried out by utilizing dragon fruit peel extract (*H. polyrhizus*) as a bioreductant through a maceration process using aquabidest solvent, because aquabidest was a good solvent for the extraction of compounds with low molecular weight such as flavonoids [1]. The skin of the dragon fruit (*H. polyrhizus*) used was the skin that fresh, not dry or rotten, the skin was still shiny, and the scales were still reddish pink. In this study 1 mM $AgNO_3$ was used as a source of nitrate which was reacted with dragon fruit peel extract (*H. polyrhizus*). The greater the concentration of silver nitrate, the faster the reduction process, this was due to more Ag ions in solution and natural reducing agents were not difficult to interact with or bind to Ag ions [1].

The results of the extraction of dragon fruit skin (*H. polyrhizus*) obtained show a purplish red color, where the purplish red color on the skin of dragon fruit (*H. polyrhizus*) contained active compounds, namely flavonoids which could react with AgNO3 to form silver nanoparticles [7]. Flavonoids has effective properties in inhibiting the growth of viruses, bacteria and fungi [10].

The way plantas work in reducing silver nanoparticles is by utilizing the performance of chemicals contained in plants so that they can reduce Ag^+ to Ag^0. Positive charge contained in the Ag element will be reduced to uncharged Ag nanoparticles, namely by utilizing plant axtracts as reducing agents [1]. Where flavonoid compounds in plants could react with Ag^+ which is hydroxyl group that interacts with the carbon atom of the aromatic ring which could reduce silver ions into silver nanoparticles and provided stability to agglomeration [11].

3.2. Absorbance value of silver nanoparticles on dragon fruit peel extract (H. polyrhizus) using UV-Vis Spectrophotometer

The initial parameter that became the benchmark for the formation of silver nanoparticles with increasing synthesis time can be seen from the results of the UV-Vis spectrum and the change in the color of the solution. UV-Vis spectrum characterization was carried out by measuring the maximum wavelength of the dragon fruit peel extract solution (*H. polyrhizus*) synthesized in the wavelength range of 200-800 nm. While the change in the color of the solution which indicated the formation of silver nanoparticles was from pale yellow to brownish yellow then to reddish brown. The color change of the solution in the synthesis of silver nanoparticles occurred due to surface plasmon resonance and reduction of silver ions [12].

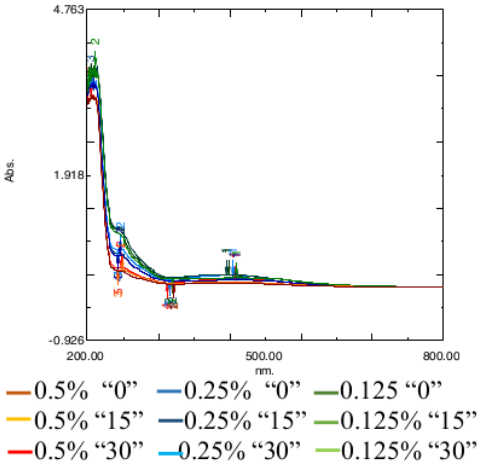

Figure 2. The absorbance of the solution with increasing time of the synthesis process of silver nanoparticles of dragon fruit peel extract (*H. polyrhizus*) at a temperature of 70°C.

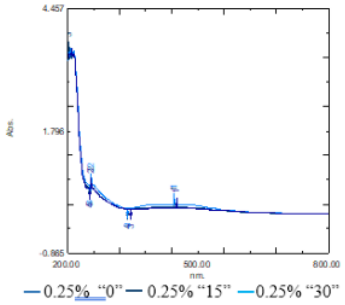

Figure 3. The absorbance of the solution with increasing time of the synthesis process of silver nanoparticles of dragon fruit peel extract (*H. polyrhizus*) with a concentration of 0.25% at a temperature of 70°C.

Parameters that could confirm the formation of silver nanoparticles can be seen from the UV-Vis spectrum, in Figure 2. the absorbance peak of silver nanoparticles in this experiment was at a wavelength of 444.5 nm, this indicates that modification of silver nanoparticles has occurred between dragon fruit peel extract and $AgNO_3$ solution. silver. Colloidal solutions can be absorbed maximally at a wavelength of 400-500 nm, this is a feature of the plasma surface of silver nanoparticles [13].

Based on Figure 3. show the best nanoparticle synthesis results were produced at 0.25% extract concentration with a duration of 30 minutes at a synthesis temperature of 70°C compared to the synthesis results at 0.125% and 0.5% extract concentrations, because the highest absorbance value was 444, 5 nm with the most contrasting color change. High temperatures were needed to help the reduction process [14]. The nucleation and formation of silver nanoparticles depended on the reaction temperature used [15].

In this study, the absorbance of the solution increases with the increase in synthesis time, this show that the rate reduction was directly proportional to the increase in synthesis time. However, 30 minutes the change in the absorbance value and the change in the color of the solution were no longer significant. This indicated that at a certain time the reduction rate was constant, where the formation of silver nanoparticles no longer occured because the silver salt had been completely reduced [16]. The large amount of Ag nanoparticles formed can be seen from the absorbance value, the higher the absorbance indicated the increasing number of silver nanoparticles [17].

Based on the Figure above, visual characterization show that the resulting colloid had formed silver nanoparticles which were characterized by a change in the color of the solution from light yellow to reddish brown with an increasingly concentrated intensity as the $AgNO_3$ composition increased and after additional NaOH. The intensity of the color of the solution that was getting more concentrated indicated a correlation with the number of nanoparticles formed [18]. The result stable silver nanoparticles were characterized by the formation of yellow colloidal silver nanoparticles [19].

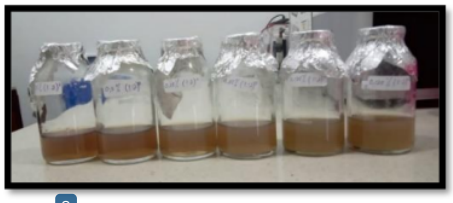

Figure 4. Synthesis of silver nanoparticles at 70^{0}C with the addition of NaOH. Concentration (0.5%; 0.25%; 0.125%) duration 0 minutes.

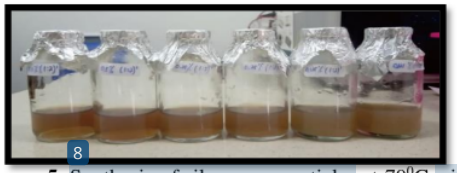

Figure 5. Synthesis of silver nanoparticles at 70^{0}C with the addition of NaOH. Concentration (0.5%; 0.25%; 0.125%) duration 15 minutes.

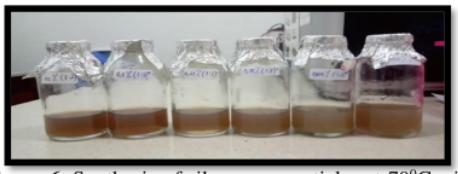

Figure 6. Synthesis of silver nanoparticles at 70^{0}C with the addition of NaOH. Concentration (0.5%; 0.25%; 0.125%) duration 30 minutes.

3.3. The effectiveness of antiseptic nanoparticles of red dragon fruit peel extract (H. polyrhizus) against pathogenic microbes

In this study, to see the antibacterial activity of dragon fruit peel extract was carried out by the paper disc diffusion method against pathogenic bacteria, namely *E. coli* and *S. aureus*. Antibacterial testing was carried out to show that silver nanoparticles had good antibacterial ability. The inhibition zone on the paper disc became a parameter for determining antibacterial activity. Inhibitory activity against bacterial growth has several categories, namely, if the value of the inhibition zone is less than 5 mm then it is categorized as weak, 5-10 mm is categorized as moderate, 10-20 mm is categorized as strong and more than 20 mm is categorized as very strong [20].

Based on table 4, showed the results of the inhibitory test obtained at incubation for 24 hours and 48 hours, each concentration of nanoparticle solution indicated the presence of an inhibitory zone in the two test bacteria.

However, the resulting inhibition zone was smaller than the inhibition zone produced in $AgNO_3$ and control (+) solutions. The best results were obtained at a concentration of 0.25% solution at 30 minutes on *E. coli* bacteria of 14.5 mm while *S. aureus* bacteria of 18.5 mm. This show the ability of dragon fruit peel extract silver nanoparticles (*H. polyrhizus*) in inhibiting bacteria categorized into strong inhibitory and antimicrobial properties.

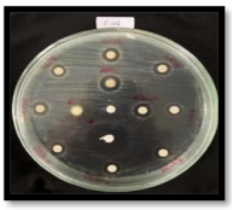

Figure 7. Testing the antibacterial activity of silver nanoparticles from dragon fruit peel extract (*H. polirhyzus*) on *E. coli* bacteria (24 hours).

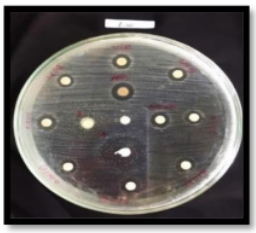

Figure 8. Testing the antibacterial activity of silver nanoparticles from dragon fruit peel extract (*H. polirhyzus*) on *E. coli* bacteria (48 hours).

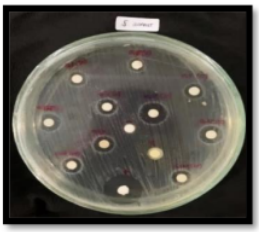

Figure 9. Testing the antibacterial activity of silver nanoparticles from dragon fruit peel extract (*H. polirhyzus*) on *S. aureus* bacteria (24 hours).

The synthesized sample of silver nanoparticles used in the manufacture of mouthwash was synthesized at a concentration of 0.25% for 30 minutes at a synthesis temperature of 70°C, because the absorbance value was the highest at 444.5 nm with the most contrasting color change. The addition of $AgNO_3$ in the manufacture of mouthwash from dragon fruit peel extract nanoparticles (*H. polyrhizus*) because the nanoparticles have a size of 1-100 nm, meaning that the particle structure of the nanoparticles was smaller when compared to the direct structure of the dragon fruit peel extract (*H. polyrhizus*) which was not added. $AgNO_3$. So that the smaller the size of a nanoparticle, it was absorbed more quickly into the bacterial cell wall layer to lyse the peptidoglycan layer in bacteria.

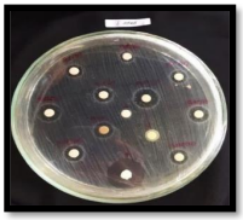

Figure 10. Testing the antibacterial activity of silver nanoparticles from dragon fruit peel extract (*H. polirhyzus*) on *S. aureus* bacteria (24 hours).

Table 1. Testing the inhibition zone of variations in the concentration of nanoparticles from dragon fruit peel extract (*H. polirhyzus*) (24 hours and 48 hours).

Test Sampel	Inhibition Zone (mm)			
	E. coli		*S. aureus*	
	24 h	48 h	24 h	48 h
Control (+)	28,5	29,5	25	25
Control (-)	-	-	-	-
$AgNO_3$	15	15	13,5	13,5
H. polyrhizus	9	9	9,5	10
0,125% (0)	14	14	15,5	15,5
0,25% (0)	12,5	13,5	12	13
0,5% (0)	14	14	10	10,5
0,125% (15)	13	13	11,5	12,5
0,25% (15)	13	13,5	14	15,5
0,5% (15)	11	11,5	11,5	13
0,125% (30)	13,5	14	12	13
0,25% (30)	14,5	14,5	18,5	18,5
0,5% (30)	13	13	11,5	12

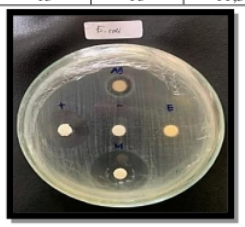

Figure 11. Testing the antibacterial activity of mouthwash on *E. coli* bacteria (24 hours).

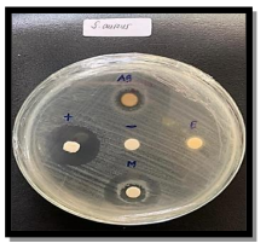

Figure 12. Testing the antibacterial activity of mouthwash on *S. aureus* bacteria (24 hours).

Table 2. Testing the inhibition zone of Mouthwash from dragon fruit peel extract (*H. polirhyzus*) (24 hours).

Test Sample	Inhibition Zone (mm)	
	E. coli	*S. aureus*
Control (+)	23,5	24,5
Control (-)	-	-
$AgNO_3$	12	13
H. polyrhizus	7,5	9
Mouthwash	28	25,5

Table 2. showed the results of the inhibition test obtained from the mouthwash solution, on *E. coli* bacteria by 28 mm, while in *S. aureus* bacteria by 25.5 mm. This show ability mouthwash in inhibiting bacteria was categorized into a very strong inhibitory and antimicrobial.

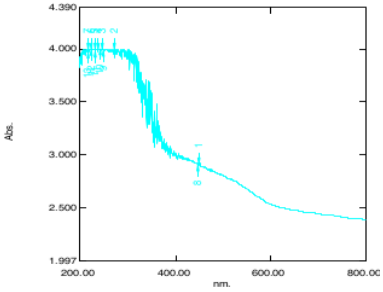

Figure 13. Results of UV-Vis Spectrophotometer Mouthwash Solution.

From the value of the inhibition of the two types of bacteria, it was related to the mechanism of silver nanoparticles in penetrating the outer layer of each type of bacteria. Gram-negative bacteria had an effective permeability barrier, namely a thin layer of lipopolysaccharide on the outer membrane of the peptidoglycan layer which could limit the penetration of the silver nanoparticle solution. While gram-positive bacteria had a cell wall consisting of a thick layer of peptidoglycan. Thicker peptidoglycan layer was thought to be the cause of silver nanoparticles providing lower antibacterial activity. This made it difficult for silver nanoparticles to penetrate the outside of the bacteria so they could not enter the internal bacterial organelles [21].

Based on Figure 13. the results of UV-Vis spectrophotometer from mouthwash solution of dragon fruit peel extract (*H. polyrhizus*) showed the presence of silver nanoparticles formed with absorbance peaks at a wavelength of 448.5 nm which indicated that the silver nanoparticles obtained had undergone a reduction process between the synthesis results. silver nanoparticles at a concentration of 0.25% for 30 minutes with a mouthwash formulation of dragon fruit peel extract (*H. polyrhizus*).

4. CONCLUSION

In this study, the isolation of silver nanoparticles was carried out by utilizing dragon fruit peel extract (*H. polyrhizus*) as a bioreductant because it contained bioactive compounds, namely flavonoids, which could be used in the process of isolating silver nanoparticles. Based on the results of the UV-Vis spectrum, the best nanoparticle synthesis results were obtained at an extract concentration of 0.25% for 30 minutes with a synthesis temperature of 70°C, where the absorbance peak of silver nanoparticles obtained was at a wavelength of 444.5 nm, while visually the isolation color changed into brown. Based on the results of the inhibition test on incubation for 24 hours, it was found that the mouthwash solution showed the presence of an inhibitory zone on the two test bacteria. E. coli bacteria produced an inhibition zone of 28 mm, while S. aureus bacteria produced an inhibition zone of 25 mm, it showed the mouthwash solution made from dragon fruit peel extract nanoparticles (*H. polyrhizus*) has the potential as antiseptic.

AUTHORS' CONTRIBUTIONS

LISA AINAYAL FATIHA AS A RESEARCHER AND AUTHOR, ZARASWARI DWYANA AND EVA JOHANNES AS A SUPERVISOR

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