

Eco-friendly silver nanoparticles (AgNPs) fabricated by green synthesis using *Actinidia deliciosa* extract: biosynthesis, characterization, and antibacterial activity

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Abstract—Biosynthesis of silver nanoparticles (AgNPs) have many benefits, one of which is in the health sector. AgNPs also have received a great attention in nanotechnology because of antimicrobial and biomedical activity. Green synthesis of AgNPs have a cost- effective and enviromentally alternative. In this research, *Actinidia deliciosa* were used to reduce the size of Ag to become AgNPs. Biosynthesis, characterisation, and antibacterial activity of AgNPs were studied in this research. The synthesized of AgNPs were characterized by UV- Visible spectroscopy (UV-Vis), Fourier- transform Infrared Spectroscopy (FTIR), and Particle Size Analyzer (PSA). The formation of AgNPs was confirmed by optical performance using UV-VIS spectroscopy and showing a peak of AgNPs at 415 nm. C-H, C-N, and C=O groups in *Actinidia deliciosa* were confirmed by FTIR. The size of AgNPs was studied using PSA and obtained a size of 65,01 nm. This extract was used to synthesized AgNPs exhibited good in antibacterial activity.

Keywords— *Actinidia deliciosa*, Green synthesis, AgNPs, antibacterial activity.

INTRODUCTION

Nanotechnology is an important field in the field of modern research. The nanotechnology approach is concerned with the design, synthesis, and manipulation carried out to obtain particle structures with sizes ranging from 1-100 nm [1,2]. The use of large nanoparticles in the biomedical field creates its own challenges in the synthesis method. The nanoparticle synthesis method is based on three process approaches, namely chemistry, physics, and biology [1]. In this study, the synthesis of AgNPs was carried out in an environmentally friendly manner using a green synthesis technique. Nowadays, biological nanoparticle synthesis is preferred over physicochemical methods, because it is considered to have many advantages, including: more eco-friendly [2], non-toxic, more reproducible, easier to scale up, and better morphology. Green synthesis of silver nanoparticles is suggested as an environmentally friendly (without environmental damage method) and low cost process. Several reports are available on the biosynthesis of silver metal nanoparticles using plant extract [3]. Sources of biological synthesis of nanoparticles are microorganisms and plants. The use of plants for the synthesis of nanoparticles is preferred over microorganisms for various reasons, including: a simpler, faster, more cost-effective, more biocompatible step so that it is more applicable for use in the medical field. Almost all components in plants such as proteins, amino acids, organic acids, vitamins, and secondary metabolites such as flavonoids, alkaloids, polyphenols, terpenoids, heterocyclic components, and polysaccharides, have significant functions in reducing metal salts, acting as capping and stabilizing agents in synthesis. nanoparticles [4]. The biological activity of AgNPs depends on factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type, and the type of reducing agents used for the synthesis of AgNPs are a crucial factor for the determination of cytotoxicity. Basically, the reduction of silver salts involves two stages (1) nucleation; and (2) subsequent growth. In general, silver nanomaterials can be obtained by two methods, classified as “top-down” and “bottom-up”[5]. This study used kiwi fruit extract as a silver metal reducing agent. The initial process of synthesizing silver nanoparticles in this study was started by optimizing the synthesis parameters using uv-vis, FTIR, and psa instruments. the use of uv-vis is used to ensure the formation of silver nanoparticles which are characterized by the wavelength formed and a change in color. ftir was used to determine the presence of active groups C=O and O-H which functioned as reducing agents in the synthesis of silver nanoparticles. PSA is used to

determine the average size distribution of silver nanoparticles in the synthesized solution. In this study also studied the antibacterial activity of AgNPs that have been synthesized using kiwi fruit extract.

METHODS

2.1 Materials

Silver nitrate (AgNO_3) from Sigma- Aldrich (Darmstadt), kiwi fruit, Ultra High Pure Water (UHP), All culture media for bacterial and fungal growth. The glassware and dishes were immersed in sulfuric acid and then washed by using deionized water. All the prepared media were then sterilized through autoclaving at (121°C) for 20 min. Bacterial strains, *Escherichia coli* (E.coli) and *Staphylococcus aureus* (S. aureus). The pure bacterial cultures were grown on Mueller Hinton Agar (MHA) medium. After that, each bacterial culture was kept through regular subculturing on the same medium and kept at 4°C prior further researches.

2.2 Preparation of Plant Extract

254,4 grams of kiwi fruit was crushed, then added 100 mL of UHP. The solution is kept for about 1 hour. The next step is to stir and let stand at room temperature for 1 hour. The mixture was filtered to obtain a clear yellowish green solution. The solution is stored in a dark glass bottle at room temperature.

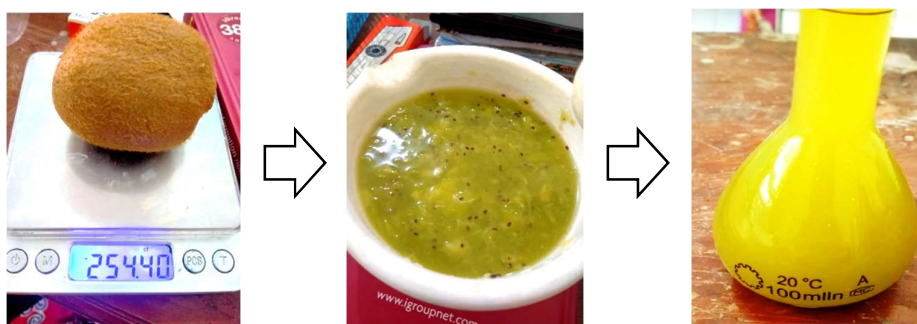


Figure 1. preparation of plant extract

2.3 Synthesis of AgNPs

Silver nanoparticles were synthesized by mixing 10 ml of aqueous extract of kiwi fruit with 50 mL of 1mM AgNO_3 . the solution was stirred for 30 minutes. After that 10 mL plant extract which was left for incubation over 24 hours, 30°C . The solution was stored in a dark glass bottle and a dark room to prevent photooxidation. The color change indicates that silver nanoparticles have been formed. The synthesized AgNPs were characterized using uv-vis, FTIR, and PSA.

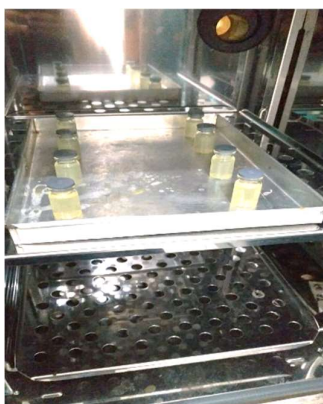


Figure 2. 10 mL plant extract which was left for incubation over 24 hours, 30°C

2.4 Characterization Techniques

Silver nanoparticles were characterized using UV-Visible Spectrophotometer Simadzu (UV- 1800, Japan) at 300-700 nm. The probable functional groups that present within plant extract were identified using FTIR analysis. The size of AgNPs was determined using Particle Size Analyzer (PSA), Malvern.

RESULTS AND DISCUSSIONS

3.1 Colour change of solution

The synthesis of silver nanoparticles using plant extracts is of great interest to researchers. The use of plant extracts is considered easy, inexpensive, and environmentally friendly, and provides satisfactory nanoparticle results. Kiwi plant extract can be used as a reducing agent because it has reducing compounds that can change the size of AgNO_3 into AgNPs. A colour change from light yellow to brown of silver nitrate solution indicates that AgNPs have been formed. Figure 3 show us the colour changes that occurred in the 1 mM of silver nitrate solution from light yellow to brown by the addition of 10 mL kiwifruit extract which was left for incubation over 24 hours, 30°C . This color change indicates that AgNPs have been formed due to Surface Plasmon Resonance (SPR). The synthesized AgNPs were later subjected to various characterization methods. Secondary metabolites that contained in kiwifruit extract will stabilized the size of AgNPs and lead the reduction of $\text{Ag} \rightarrow \text{Ag}^0$. The secondary metabolites can reduce the size of Ag to become silver nanoparticles [5].

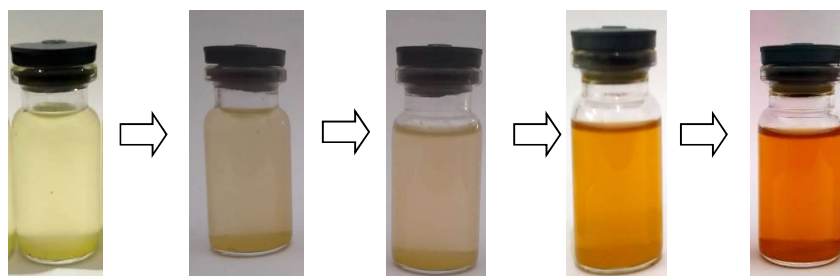


Figure 3. Colour change of solution

3.2 UV- Visible Analysis

UV-vis spectroscopy is a significant technique to authenticate the formation and stability of AgNPs in aqueous solution. It is renowned that AgNPs exhibit dark brown colours, depending on the intensity and the size of nanoparticles; the colours arise due to the excitation of surface plasmon resonance (SPR) of the AgNPs [5]. Analysis using UV-VIS spectrophotometer was used to confirm and characterize the formation of AgNPs through the resulting color changes. Observations were made at a wavelength of 300-700 nm. Reaction time had significantly effect on shape and size of AgNPs and the UV- Vis spectrum. Based on Figure 4, there are differences in the UV-Vis analysis on the kiwi fruit extract solution + Ag and AgNO_3 solution. AgNO_3 used as a comparison did not show any peaks when analyzed using UV-Vis. We can observe that there is a peak at the wavelength of 415 nm. This is in accordance with research that has been done previously, that silver nanoparticles are formed in the wavelength range of 410 and 450 nm [6]. The sized of silver nanoparticles that have been formed were confirmed by the wavelength of 415 nm.

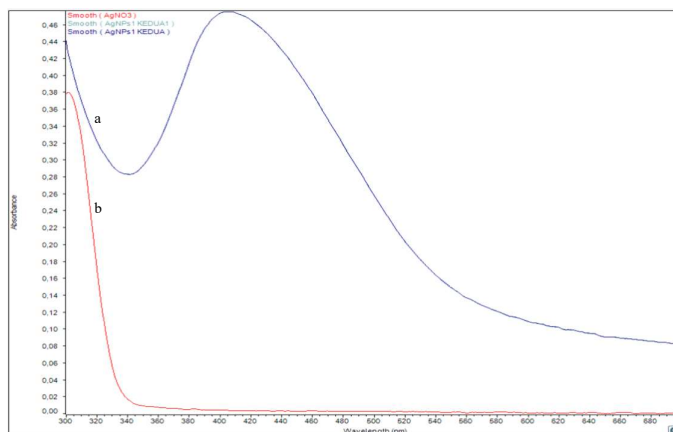


Figure 4. (a) UV- Vis analysis of kiwifruit extract + Ag (b) UV- Vis analysis of AgNO_3 1 mM

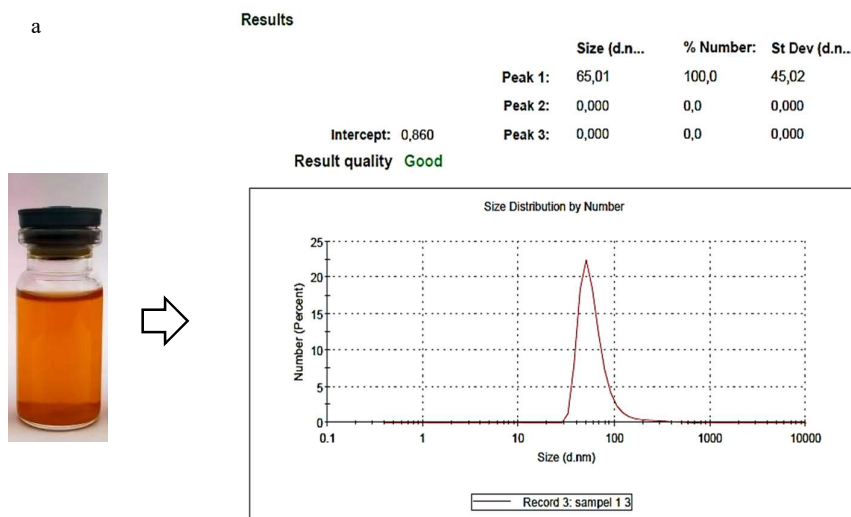
Based on the observation, the formed silver nanoparticles were stable for 14 days. After more than 14 days, the silver nanoparticles agglomerated. This is indicated by a change in the color of the solution to blackish gray (figure 5), from this colour we can indicated that Ag^0 has aggregated into a bulk form, not in the form of silver nanoparticles anymore.



Figure 5. Blackish gray solution, Ag not stable after 14 days

3.3 Particle Size Analyzer (PSA) Analysis

Synthesis of nanoparticles using reducing agents is the most common way of synthesizing AgNPs using organic and inorganic reducing agents. continues through a process to produce a silver colored solution, this is because the metal surface has free electrons in the conduction band and a positively charged nucleus. To reduce AgNO_3 solution to form nanoparticles, reducing agents such as Sodium borohydride (NaBH_4), elemental hydrogen, polyol process, N,N-dimethylformamide (DMF), Ascorbic acid, poly(ethylene glycol)-block copolymers, hydrazine, and ammonium formate are applied for reduction of silver ions (Ag^+) in the aqueous or nonaqueous solution. This study used kiwi fruit extract as a reducing agent which aims to reduce the size of the nanoparticles and stabilize the size of the silver nanoparticles. To characterize the size distribution of nanoparticles, PSA was used. Analysis using PSA is intended to determine the particle size distribution of AgNPs in solution. Based on the results of characterization using the PSA instrument, the size of the distribution of AgNPs was around 65 nm. The nanotechnology approach is concerned with the design, synthesis, and manipulation carried out to obtain particle structures with sizes ranging from 1-100 nm [1,2]. However, when the solution changed color to blackish gray and analyzed using PSA, the data on the size distribution of Ag was around 111 nm. (Figure 6). Based on these data, it can be concluded that the presence of a color change is a physical marker of the instability of the AgNPs formed. If the solution remains brown, it indicates that Ag is still in the form of nanoparticles, whereas if the solution changes color to blackish gray, it indicates that Ag has agglomerated into a bulk form and is no longer in the form of silver nanoparticles.



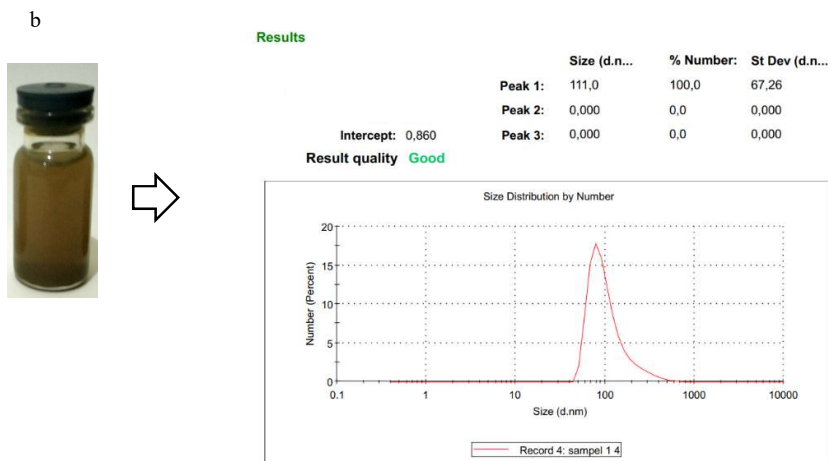


Figure 6. Comparison analysis using PSA between kiwi fruit extract + Ag solution which is brown (a) and blackish grey (b).

3.3 FTIR Analysis

The FTIR test was carried out to determine the presence of functional groups contained in the extract which would stabilize the presence of AgNPs [6]. Based on the FTIR data, several functional groups were found at varying wavelengths including 618, 1104, 1234, 1383, 1617, 1637, 1745, 2925, and 3414 cm^{-1} . peak at wavelength 2925 cm^{-1} indicates C-H stretch, peak at wavelength 1745 cm^{-1} indicates C=O stretch, and peak at wavelength 1383 cm^{-1} indicates amaida bonds C=N stretch and peak at wavelength 618 cm^{-1} indicates the reduction of Ag to Ag^0 [7]. The presence of C=N, C-H, and C=O groups were reduced the sized of Ag $\rightarrow$ Ag^0 and stabilized the sized of Ag^0 .

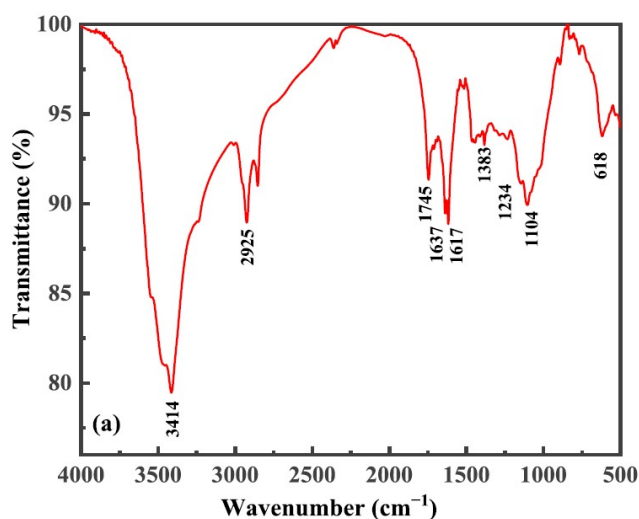


Figure 7. FTIR Analysis of kiwifruit extract + Ag

3.4 Antibacterial activity of the AgNPs

Infections caused by bacteria are very dangerous, so antibacterial agents are needed to prevent them. Plants and organic ions have been used as antibacterial agents to fight infections in various industries. The development of antibacterial agents is increasingly widespread, one of which uses the concept of green chemistry [8]. This study used kiwi fruit extract to synthesize Ag into AgNPs and stabilize the formed AgNPs. The most important thing in doing green synthesis using plant extracts is the selection of the right solvent, as well as consideration of the content of metabolites present in the plant extract. If the ha is met, then AgNPs will be formed which are stable and can be used to kill pathogenic bacteria. The antibacterial test in this study used gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*). Based on the research conducted, the inhibition data obtained were 16.71 mm and

19.41 mm, respectively. The bacterial test method used in this study is the disc diffusion method using gram-positive and gram-negative bacteria. Bacterial strain activation was carried out by loopful inoculation of the strain in 30 ml nutrient broth to maintain McFarland standard turbidity 6 (10cells/ml) of bacterial strains. The next step is to incubate for 6 hours. 0.1 ml of inoculums of various bacterial strains were inoculated into the molten Muller Hinton agar and spreaded uniformly over Petri plates with a sterile glass spreader. The solution derived from AgNPs synthesis was prepared in UHP and 40 L of the sample was poured into a 6 mm disc and kept dry. The standard drug used in this study was streptomycin. the seeded agar plates were impregnated with the disc. These plates were kept for 1 hour for pre diffusion of the biosynthesized nanoparticles and incubated at 37°C for 24 H. The diameter of the zone of inhibition was measured in mm around each paper disc.

Table 1. Antibacterial activity of AgNPs

S No.	Bacteria	Diameter of zone of inhibition (mm)			Standard drug
		25	50	75	
1	<i>S. aureus</i>	11	14	15	18
2	<i>E. coli</i>	12	14	13	20

CONCLUSIONS

AgNPs synthesis using kiwi fruit extract has been successfully developed. This method is an environmentally friendly method, low cost, and does not use harmful chemicals. AgNPs were formed, indicated by a change in the color of the solution from light yellow to brown. AgNPs were characterized using UV-Vis, PSA, and FTIR. Based on the characterization results, AgNPs were formed and were stable for 2 weeks. The size of the AgNPs distribution is 65 nm. Anti-bacterial potential calculated by disc diffusion method shows more inhibition zone in gram-negative bacteria (*Escherichia coli*) as compared to gram-positive (*Staphylococcus aureus*).

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