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Article

Green Synthesis Silver Nanoparticles (AgNPs) Utilizing Ari (Pithecellobium jiringa) Skin Waster Extract as a Bioreductor

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Abstract. In this study, silver nanoparticles (AgNPs) are widely applied as antibacterials and can be composited with polymers for automotive components. Pithecellobium jiringa epidermis waste extract contains several secondary metabolite compounds that can act as bioreductors to produce AgNPs. This research aims to synthesize silver nanoparticles (AgNPs) using P. jiringa epidermis waste extract as a bioreductant. The volume ratio of P. jiringa epidermis extract and 1 mM silver nitrate ($AgNO_3$) solution is 3:7 at 25 ℃. Characterization of AgNPs using U-Visible (UV-Vis) Spectrophotometer, Particle Size Analyzer (PSA), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), and X-Ray Diffraction (XRD) The results of UV-Vis, PSA, and SEM-EDX analyses, respectively, obtained AgNPs with an adsorbance of 2.121 at a wavelength of 430 nm at a reaction time of 96 hours, an average size distribution of 46.6 nm with a round shape, the element content of AgNPs, namely Ag, C, and O. wave number 2867.74 cm^{-1}, and the C=C functional group at wave number 1613.18 cm^{-1}.

Keywords: Bioreductor, green synthesis, pithecellobium jiringa, silver nanoparticles.

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1. Introduction

Green synthesis is an environmentally friendly method in the field of nanotechnology. Nanotechnology is the development of materials in the nanometer size range (10-9 m) and structures whose dimensions range from 1-100 nm. Particles at the nanoscale (nanoparticles) have high reactivity and surface energy, as well as a large surface area [1]. Nanoparticles are widely applied in various fields, including the automotive industry, biomedical industry, food packaging industry, and others [2,3]. Pithecellobium jiringa epidermis waste extract is known as jengkol and is consumed as food in Indonesia. Jengkol plants can be cultivated in almost all regions of Indonesia. Jengkol epidermis contains several secondary metabolite compounds (biomolecules), including flavonoids, saponins, tannins, and alkaloids [4]. These compounds are combined with hydroxyl (O-H) and carbonyl (C=O) functional groups [5,6]. Organic compounds such as flavonoids, alkaloids, terpenoids, tannins, saponins, etc. have an important role in producing silver nanoparticles (AgNPs) [7-9].

Silver (Ag) has antimicrobial properties with high biocompatibility compared to other metals such as titanium dioxide, gold, copper, and others. Silver also has long-term antibacterial efficiency against different bacteria. Silver nanoparticles (AgNPs) have good biological activity, high conductivity, chemical stability, and catalytic properties. AgNPs have the potential to be composited with polymers as antimicrobials, one of the car interior components that has the most direct contact with humans, such as door panels, dashboards, and seat belts [10]. Green synthesis is a method that uses plants as reducing agents and has advantages including being environmentally friendly, faster synthesis, requiring low energy, abundant and renewable materials, and reducing waste, thus producing products with low levels of environmental pollution. Apart from this, plant extracts also have phytochemicals that show greater reduction and stabilization for the synthesis of AgNPs [11].

Green synthesis is a method that utilizes plants as reducing and capping agents [12], has advantages including faster synthesis, does not require high energy, is cost-effective, easy, environmentally friendly, and can reduce waste, thereby producing products with high levels of low environmental pollution. The plant sources used to synthesize nanoparticles are plentiful, affordable, renewable, and easy to mass produce. Plant extracts also have phytochemicals that make AgNP synthesis less likely to happen and keep it stable [13]. No one has studied the epidermis extract of P. jiringa for AgNP synthesis, and several previous studies synthesized AgNPs using extracts of fruit peels, vegetables, and other plant leaves at temperatures above room temperature. According to Nazir et. al., [14], the use of high temperatures may incur greater costs. In this research, we synthesized AgNPs using P. jiringa epidermis extract at room temperature. No one has carried out research on the synthesis of AgNPs using P. jiringa epidermis extract at room temperature. Researchers have made AgNPs both at room temperature and above room temperature in the past. These studies used stem and leaf extracts from Swertia chirata, green tea leaves, Enicostema axillare leaves, Saliva Sclarea leaves, Allium cepa bark, Citrus sinensis bark, and Carica papaya peel. AgNPs with size and synthesis temperature, respectively, 80–110 nm (15–25 ^{o}C), 50 nm (room temperature), 70–80 nm (room temperature), 5–30 nm (room temperature), 12.5 nm (T = 90 ^{o}C), 16 nm (T = 60 ^{o}C), and 25–27 nm (T = 80 ^{o}C) [15-18].

Several previous studies used extracts of onion peel (Allium cepa L.), and papaya peel (Carica papaya) to produce AgNPs with sizes of 12.5 nm (t = 48, T = 90 ℃), 16 nm (t = 3.5 hours, T = 60 ℃), and 25 - 27 nm (t = 1 hour, T = 80 ℃) [5, 11]. The study aimed to optimize factors such as temperature, time, pH, ratio of plant extract, and concentration.

Another paper was determined the reduction of nitro-compounds into corresponding amines, where AgNPs^{-2} was found to be an to be an efficient reductive catalyst [14]. Some of these studies synthesized AgNPs using fruit and vegetable peels at temperatures above room temperature (25-35 ℃) using sodium dodecyl sulphate (SDS) and sunlight, so in this study, we synthesized AgNPs using P. jiringa epidermis extract at room temperature to reduce energy use [19]. The developed nanoparticles were the potential low-cost and effective photocatalyst in the treatment of wastewater and also act as potential antibacterial and antioxidants [20].

2. Materials and Method

2.1. Materials Preparation

This study utilized mature jengkol epidermis sourced from the Pondok Gede Traditional Market in Bekasi, West Java, Indonesia. Silver nitrate ($AgNO_3$), distilled water, and analytical-type NaOH were supplied by Harum Kimia, Jakarta. $FeCl_3$ produced by Merck KGaA, Pudak brand K_2CrO_4, and Pudak brand K_2CrO_4 (purity ~98.5%) analytical types were purchased at Phy Edumedia, analytical type C_3H_6O was purchased at Bumi Agung Kimia, and H_2SO_4 ~90% purity level and analytical type Dragendorff solution were purchased at the Pharmapreneur Store. All reagents used were analytical grade. All glassware was rinsed with distilled water before being used for AgNPs synthesis. This final mixture appeared as a white gel. It was allowed to stand for 1 h to achieve a smooth and uniform gel [21, 22].

The Ari (P. jiringa) extract is washed clean, dried, crushed by 1 gram, and then heated with 100 mL of distilled water at a temperature of 85℃ for 30 minutes. After cooling system, the dark red extract was filtered using Whatman No. 1 paper and stored at 4℃ for further use. All the chemicals were used as received with further purification.

2.2. Biosynthesis of Nanoparticles

A 1 millimolar (mM) solution of $AgNO_3$ was prepared by dissolving 0.0849 grams of $AgNO_3$ in aquadest to a final volume of 500 mL. Subsequently, the solution was agitated until it had a uniform composition. The manufacture of AgNPs utilized a 1 mM solution of silver nitrate ($AgNO_3$) (purity ~99.5%) as the source of Ag^+ ions.

The synthesis of AgNPs was conducted by combining a solution of $AgNO_3$ with an Ari (P. jiringa) extract at a volume ratio of 3:7 at room temperature in a dark and enclosed environment. Measurements of solution color, pH, and UV-Vis spectrum were conducted at different time intervals. The solution underwent a chromatic transition from transparent to a pale brown hue in a time span of 6 hours.

3. Phytochemical and Characterization of Ari Jengkol Extract

3.1. Flavonoid Analysis

The saponin test involved quickly mixing 0.2 mL of Ari (P. jiringa) extract with 5 mL of aquadest, then heating the mixture to its boiling point. Foam formation is indicative of the existence of saponin [19].

3.2. Alkaloid Analysis

Alkaloid testing was identified by adding 5 mL of Ari (P. jiringa) extract and 1 mL of Dragendorff's solution into a test tube. The presence of alkaloids in the extract can be determined by observing a color change in the solution, which will turn either orange or red color [20].

3.3. Saponin Analysis

The saponin test was identified by shaking 0.2 mL of Ari (P. jiringa) extract with 5 mL of aquadest and then heating it until it boiled. The appearance of foam indicates the presence of saponin [19].

3.4. Tannin Analysis

The tannin test was identified by mixing 0.5 mL of Ari (P. jiringa) extract solution of with 1 mL of hot water in a test tube, then dripping with 2-3 drops of 1% $FeCl_3$ solution. Then the extract will be indicated to contain tannin if it is dark blue or greenish black [23, 24].

The results of the experiments are outlined in this section, which are broken down into various subheadings, including the characteristics of AgNPs, composition of Ag, NPs in the catalysts, surface areas and particle size of the AgNPs (i.e., UV-Vis, FTIR, SEM, EDX, XRD, and PSA analyzer.

4. Results and Discussion

4.1. Phytochemical Characterization of P. jiringa Extract

A phytochemical analysis was carried out to identify the secondary metabolite chemicals present in the extract of P. jiringa epidermis. Visual observation of phytochemical testing was conducted following the mixture of the extract with several chemical solutions. The study results indicate that the epidermal extract of P. jiringa contains a variety of biomolecules, such as flavonoids, alkaloids, saponins, and tannins (Table 1). The extract's color changed from clear to yellow during the flavonoid test, indicating the presence of flavonoids. The change in color from clear to orange and greenish black during the alkaloid and tannin tests, respectively, also supported the presence of alkaloids and tannins. The presence of foam and changing color in the extract indicates the presence of tannins. The findings align with prior research [25]. Figure 1 presents the data regarding the outcomes of the phytochemical test.

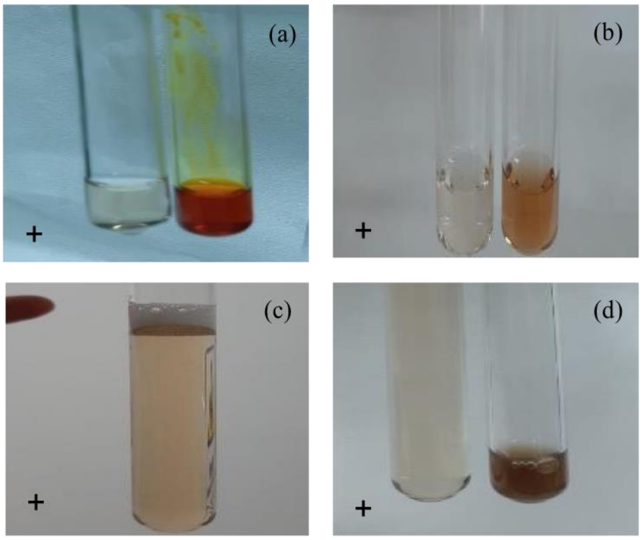

Fig. 1. Phytochemical analysis conducted on the epidermal extract of P. jiringa.

Table 1. Phytochemical analysis of P.Jiringa.

Secondary metabolites	Color change	Results
Flavonoid	White to yellow	-
Alkaloid	White to orange	-
Saponin	brown	Foam forms
Tannin	green	Foam forms

4.2. UV-Vis Spectrophotometer Analysis

An ultraviolet-visible (UV-Vis) spectrophotometer was utilized to detect the presence of silver nanoparticles (AgNPs) within the wavelength range of 400-550 nm. The presence of produced AgNPs in solution can be

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confirmed by analyzing the absorbance spectrum. Figure 2 displays the outcomes of UV-Vis analysis, indicating the peak absorption of AgNPs at different time intervals: 6 hours with a value of 0.329 (λ=380 nm), 12 hours with a value of 0.778 (λ=420 nm), 24 hours with a value of 0.964 (λ=420 nm), and 96 hours with a value of 2,121 (λ=430 nm). The manufactured silver nanoparticles exhibited characteristic wavelengths ranging from 400 to 550 nm after 12, 24, and 96 hours. The Surface Plasmon Resonance (SPR) band of AgNPs produced with Ari (P. jiringa) extract falls within the range of AgNPs synthesized in previous studies [21]. The highest absorbance and corresponding wavelength were recorded at 2.121 and 430 nm, respectively, after 96 hours at room temperature. The results indicate that AgNPs can be obtained during a synthesis period of 12 hours. The growth of AgNPs can be analyzed qualitatively by examining the particular surface plasmon resonance (SPR) features of the nanoparticles. RPS refers to the process of exciting electrons from the valence band to the conduction band. When resonance occurs, a prominent absorbance band arises from the surface plasmon [26]. At high concentrations of AgNPs, smaller average sizes were linked to maximum wavelength (λ_{max}) values with both high and low absorbance. The presence of broad and narrow peaks at high and short wavelengths, respectively, suggests an augmentation and reduction in the dimensions of the AgNPs generated. Small nanoparticle sizes are indicated by narrow and low wavelength absorption peaks, but big sizes or aggregation are indicated by broad peaks at high wavelengths [27].

The occurrence of surface plasmon resonance (SPR) leads to a transition in hue from transparent to deep brown, signifying the generation of silver nanoparticles (AgNPs) due to the SPR phenomenon [22]. The observed color shift is indicative of the decrease and formation of nanoparticles, which is associated with the surface plasmon resonance (SPR) bands of silver nanoparticles (AgNPs) [28]. Visual observations indicated that color changes commenced after 6 hours and progressively intensified over time. The solution's color turned into a dark brown shade after 96 hours, which served as confirmation of the production of AgNPs as indicated by the UV-Vis examination. The shift in color to brown or dark brown aligns with prior studies [29-32]. The alteration in color is believed to be caused by the conversion of Ag^+ ions to Ag^0 through the action of secondary metabolites present in the oxidized Ari (P. jiringa) epidermis extract. This finding aligns with the findings reported [33].

The presence of SPR results in a color change from clear to dark brown, which indicates the formation of AgNPs as a result of the SPR phenomenon. This change in color shows that nanoparticles are being reduced and made, which is linked to the SPR bands of AgNPs. The results of visual observations showed that color changes began to occur at 6 hours and became more intense as time increased. The color of the solution became dark brown at 96 hours, which confirmed the AgNPs produced from UV-Vis analysis. The color change to brown or dark brown is in accordance with previous research [31]. The color change thought to be the result of the reduction of Ag^+ ions to Ag^0 by secondary metabolites in the oxidized Ari (P. jiringa) epidermis extract; this is in accordance with what was reported [33].

The pH measurement results in Fig. 3. and Fig. 4. shows the relationship between absorbance and reaction time, and after synthesis were 4 and 8, respectively. The growth of AgNPs was favorable at alkaline pH settings. Variations in pH within the sample exert an impact on the morphology and dimensions of AgNPs. This phenomenon is believed to arise from alterations in the ion charge of secondary metabolites, which therefore modulate the absorption of Ag^+ ions. Under alkaline conditions, the oxidation of materials leads to a more limited range of sizes for AgNPs, as opposed to acidic conditions. AgNPs exhibit enhanced stability under high pH conditions due to reduced aggregation [2, 18].

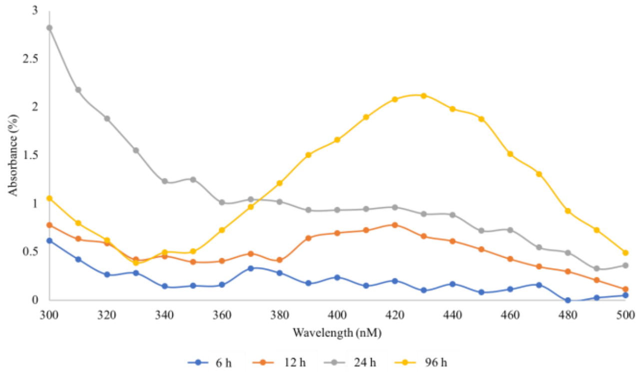

Fig. 2. UV-Vis spectrophotometer analysis at 6, 12, 24, and 96 hours.

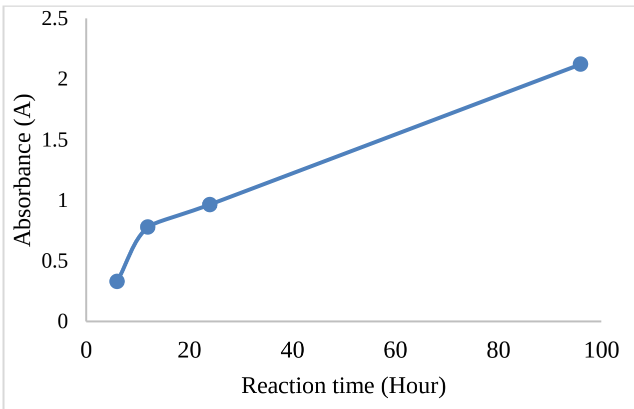

Fig. 3. The relationship between absorbance and reaction time.

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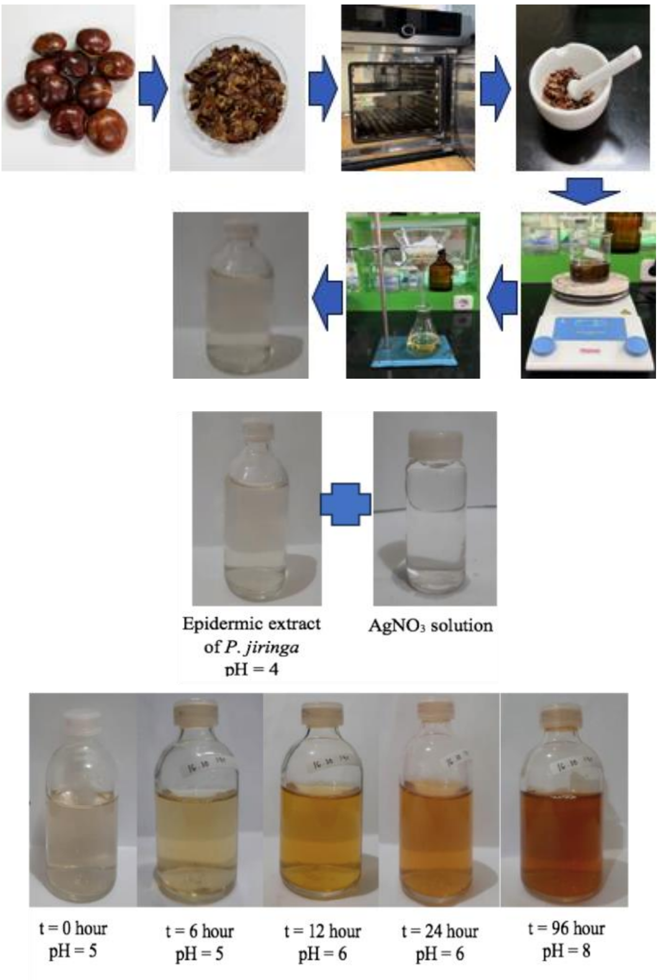

Fig. 4. Synthesis of AgNPs.

4.3. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

To determine the biomolecular functional groups present in the epidermal extract of P. jiringa used of FTIR analysis. These functional groups are responsible for converting Ag^+ to Ag^0. Additionally, the molecular functional groups present in AgNPs colloids were also identified. The FTIR spectrum illustrates the variations in absorption bands between the compounds found in the extract (Fig. 6a) and the produced AgNPs (Fig. 6b). The extract contains O-H functional groups that are ideal for alcohol and phenolic compounds. These functional groups are present at several wave numbers, specifically 3250.47, 3287.62, 3395.79, and 3454.94 cm^{-1}. The peak observed in the wave number range of 3200 to 3600 cm^{-1} indicates the existence of phenolic monomer properties commonly found in flavonoids, tannins, saponins, alkaloids, and polyphenols. The C-H functional group is detected at the wave number of 2980.81 cm^{-1}, which is indicative of alkane (hydrocarbon) molecules. Additionally, there is a C=O functional group present at the specific wavenumber of 1613.18 cm^{-1}. The wave number 1053.27 cm^{-1} signifies the presence of the C-O functional group [34].

P. jiringa epidermis extract as a bioreductor at the same time caused AgNPs to be made by breaking down $AgNO_3$. This process caused a noticeable shift in the peak of the FTIR spectrum of AgNPs, as depicted in Fig. 4. The O-H functional groups present in the alcohol and phenolic extract underwent shifts in the AgNPs colloid. These shifts were observed at specific wavenumbers, such as 3250.47 cm^{-1} to 3252.32 cm^{-1}, 3287.62 cm^{-1} to 3330.10 cm^{-1}, 3395.79 cm^{-1} to 3400.54 cm^{-1}, and 3454.94 cm^{-1} to 3540.85 cm^{-1}. These shifts resulted in the stretching of the NH band, which is associated with the amino group or OH hydroxyl group. The C-H functional group of alkanes (hydrocarbons) underwent a transition from 2980.81 cm^{-1} to 2867.74 $cm^{-1.}$ A transition takes place at the wavenumber 1607.37 cm^{-1} to 1613.18 cm^{-1}, indicating a change in the C=O functional group. The breakdown of hydrogen bonds and the interaction of the alcohol extract and phenolic groups with the $AgNO_3$ solution are what result in the shift in the O-H functional group [35]. This interaction is conduct for the reduction of Ag^+ to Ag^0 (AgNPs) [36]. The C=O functional groups absorption moved to a higher wave number, which suggests that secondary metabolite compounds from the extract and silver might interact (Fig. 4). As was already known, flavonoid and tannin compounds help keep AgNPs stable. This change in the C=O functional groups backs this up [30, 37]. The remaining peaks in the FTIR spectra of the extract and AgNPs had similar patterns. This suggests that the biomolecules in the extract help the AgNPs by both reducing them and covering them.

The biomolecules in the P. jiringa epidermis extract help to lower, cap, and stabilize the production of AgNPs [38]. A capping agent is a chemical that prevents the clumping together of silver nanoparticles during their creation. This results in the AgNPs maintaining stability, with only little fluctuations in peak absorbance values and maximum absorption [17]. According to this analysis, it can be inferred that the reaction mechanism aligns with previous studies [2, 39]. Biomolecules interact with metal ions using the O-H/C=O functional groups that have been extracted. These interactions are significant in molecular imprinting to create selective binding sites in polymers for biomolecule recognition. Additionally, weak interactions like hydrogen bonding and aromatic ring stacking are essential for molecular recognition in biological systems, highlighting the importance of noncovalent interactions in metal complex formation and structural organization [40]. Metal complexes can be specifically designed to interact with biomolecules, altering biological processes and offering diverse properties for protein modulation and imaging in living cellsThis causes reactive hydrogen to be released. This hydrogen then converts enol biomolecules into their keto form, leading to the formation of Ag^0. An illustration of the formation and redox reactions of AgNPS colloids can be described in Fig. 5. An approximate reaction mechanism is proposed for the interaction between metal ions (Ag^+) and reducing agents of tannin biomolecules during the formation of AgNPs.

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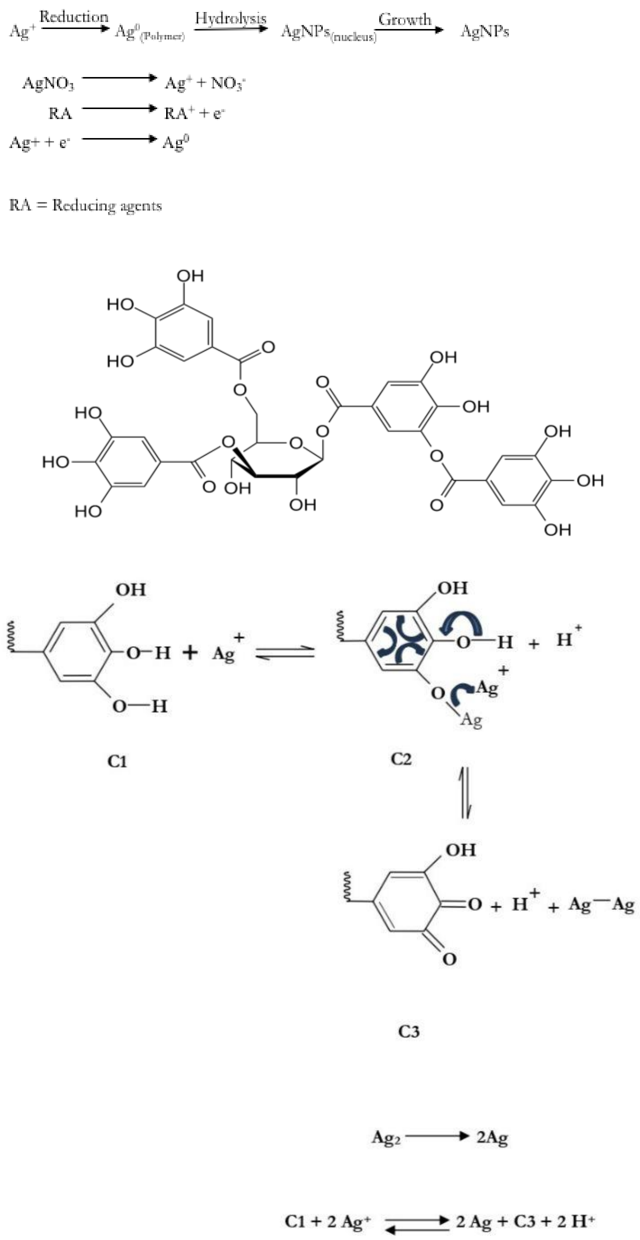

Fig. 5. Mechanism interaction between metal ions (Ag^{+}) and reducing agents of tannin formation of AgNPs.

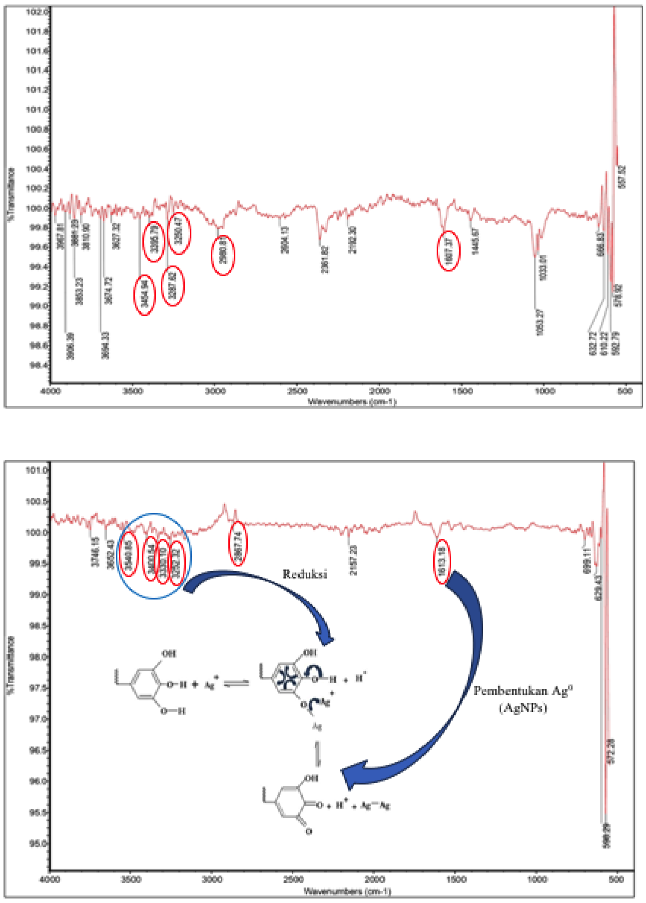

Fig. 6. Spectrum FTIR analysis of (a) P. jiringa epidermis extract, (b) AgNPs.

4.4. Scanning Electron Microscopy (SEM) Analysis

SEM analysis was carried out to determine the morphology, shape, and size of AgNPs, which were synthesized using P. jiringa epidermis extract. SEM analysis results in Fig. 7 show that the size of AgNPs is in the range of 28-34 nm with a round shape [2, 41]. The varying sizes are thought to be due to aggregation during sample preparation; something similar has been reported previously [42]. Poly dispersion of nanoparticles is also visible in SEM images. The AgNPs produced are thought to be due to the presence of several reducing phytochemicals in the P. jiringa extract. Similar observations have previously been reported [43, 44].

4.5. Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

EDX analysis was used to find out what elements were in the sample and to confirm that the AgNPs were made by mixing an $AgNO_3$ solution with secondary metabolite compounds from a P. jiringa (PJ) epidermis extract. EDX analysis of PJ-AgNPs synthesized from P. jiringa epidermis in Fig. 8 shows the highest weight percentage of silver 76.9, followed by carbon (13.0) and

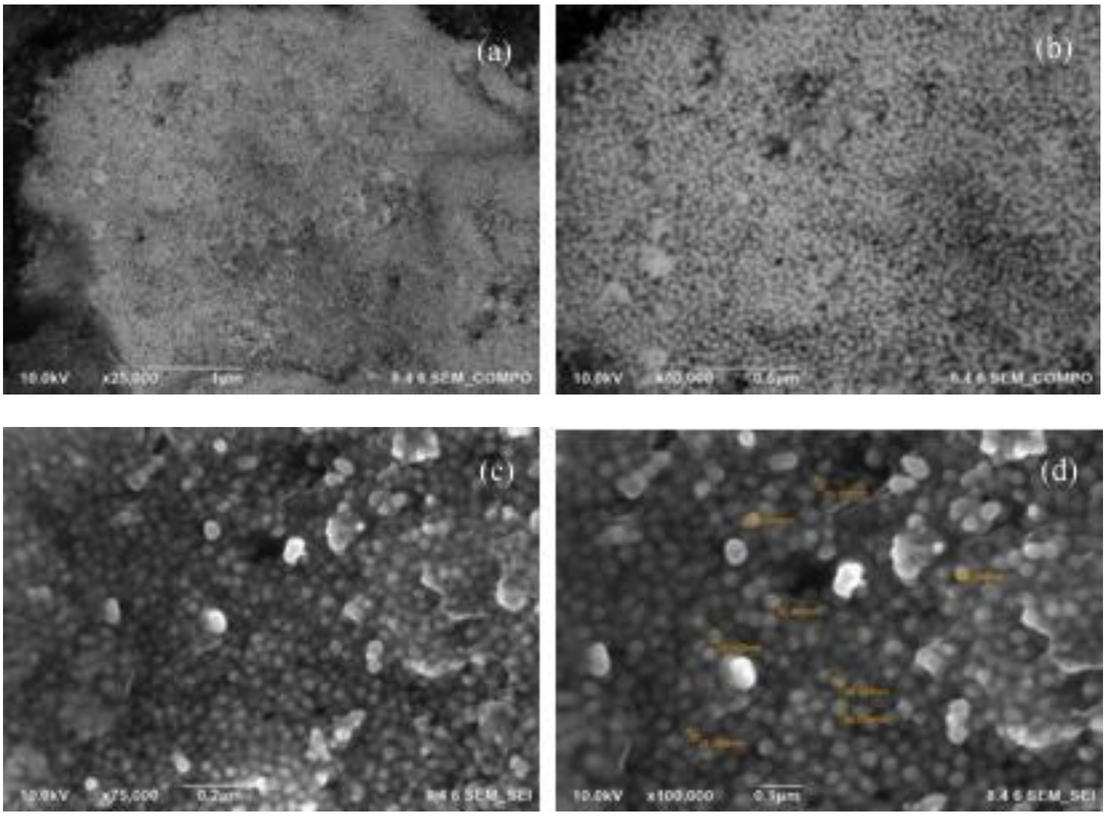

Fig. 7. SEM analysis of AgNPs with different magnification (a) 1 µm; (b) 0.5 µm; (c) 0.2 µm; and (d) 0.1 µm.

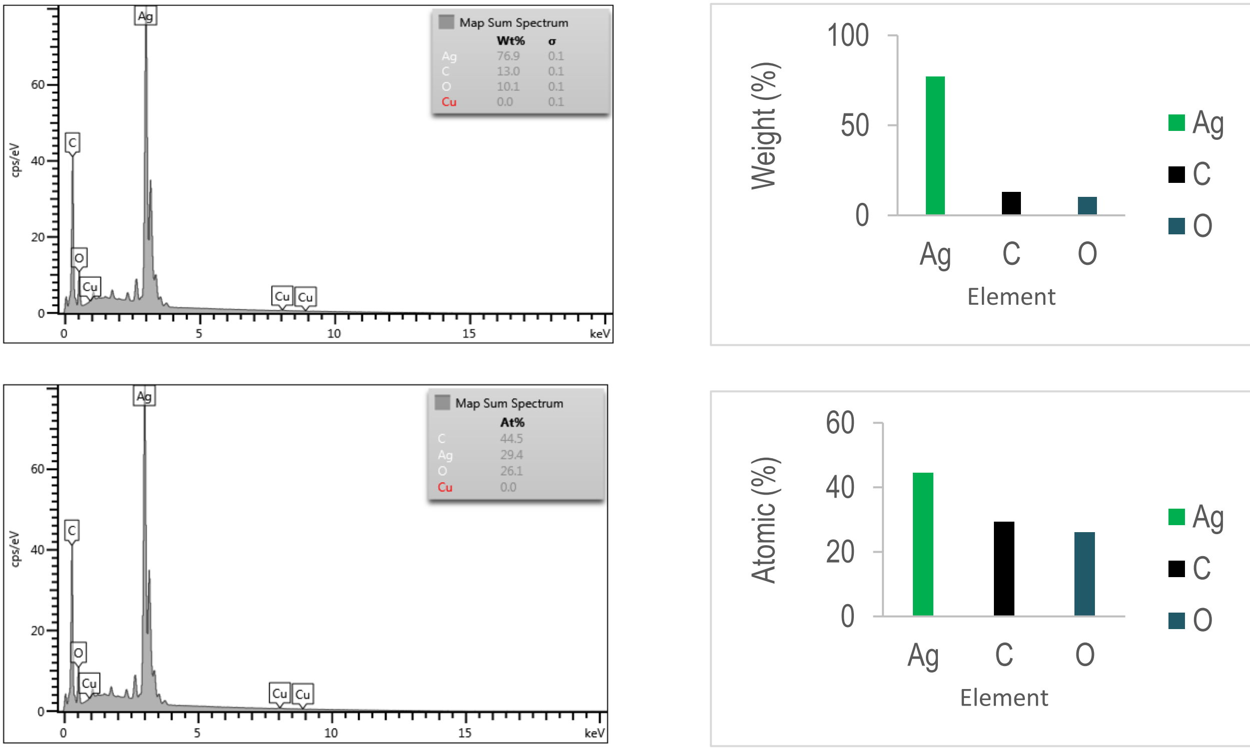

Fig. 8. Qualitative analysis of EDX elements (a) weight; (b) empirical atomic.

oxygen (10.1). The highest silver level indicates the formation of AgNPs in the sample. EDX analysis confirmed the presence of silver, while oxygen showed that extracellular organic material was thought to be adsorbed on the surface of PJ-AgNPs; something similar has been reported previously [39, 45]. As for the percentage of the number of empirical atoms, the highest is C at 44.5, followed by Ag at 29.4 and O at 26.1.

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These results are also in accordance with research conducted by Saygi et al, [24] Quantitative analysis of the elements in Fig. 8 shows that the sample shows (PJ)-AgNPs bonds, mainly containing Ag, C, and O. C and O atoms. This confirms the content of organic compounds, which are responsible for reducing and stabilizing AgNPs. Generally, AgNPs show specific optical absorption peaks in the range of 2.5-3.5 keV, which is characteristic of the absorption of silver metal due to SPR with silver, carbon, and oxygen [46, 47]. The first peak shows the alignment of silver and carbon and the presence of the bond between the two elements, followed by the oxygen peak (Fig. 7a).

Table 3. EDX analysis from PJ-AgNPs.

Element	Weight (%)	Atomic (%)
Ag	76.9	44.5
C	13.0	29.4
O	10.1	26.1
Total	100	100

4.6. X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was carried out to identify the crystal structure of particle. At the atomic level, XRD is a method frequently used to identify the crystalline state of substances. X-ray diffraction spectroscopy was used to determine the phase angle and crystal size of the resulting AgNPs [48]. There are four diffraction rings referred to as (111), (200), (220), and (311) Bragg reflection lattice planes that correspond to elemental silver, thus confirming the crystallite nature of AgNPs [26]. Figure 9 shows that the pattern obtained is characteristic of AgNPs peaks in accordance with previous literature [11, 49]. The sample confirmed the presence of AgNP crystals synthesized using P. jiringa epidermis extract, and the (311) plane Bragg reflection lattice confirmed that AgNPs have face-centered cubic (FCC) crystals. These results are in accordance with those reported previously [50].

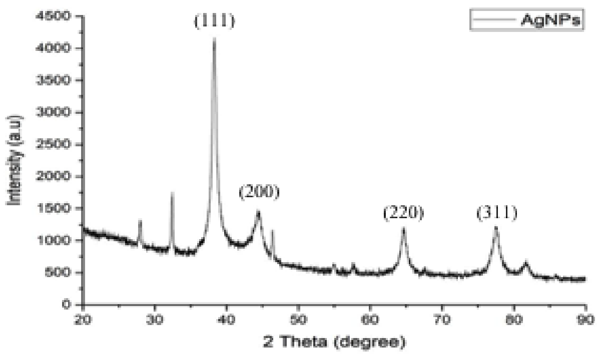
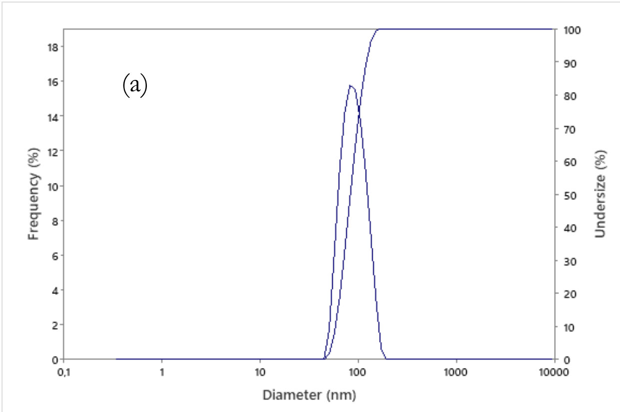
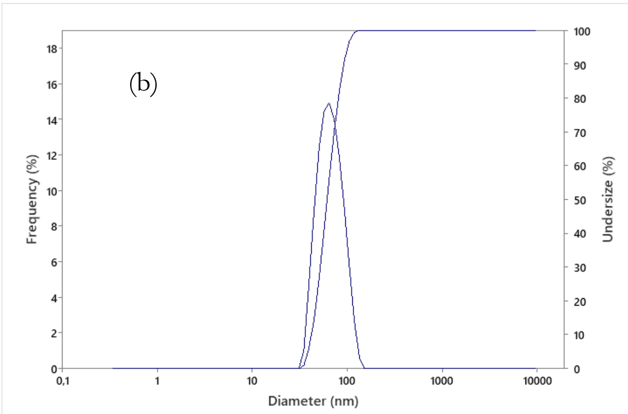


Fig. 9. XRD pattern analysis of P-jiringa-AgNPs.

4.7. Particle Size Analyzer (PSA) Analysis

Analysis using PSA was carried out to identify the average size distribution of the AgNPs produced. PSA testing was carried out at reaction times of 12, 24, and 96 hours, which had wavelengths in the typical range of AgNPs resulting from UV-Vis analysis. The PSA analysis results in Fig. 10 show the average size distribution of AgNPs at each time, namely 76.3, 64.2, and 46.6 nm. The size of the AgNPs produced strengthens the results of UV-Vis analysis, according to the literature and previous research, namely in the range 1-100 nm [51, 52]. The average size distribution of AgNPs at the 96-hour reaction time was smaller than the 12-hour and 24-hour reaction times; likewise, for the 24-hour reaction time, it was smaller than the 12-hour reaction time. The difference in size is thought to be due to the difference in reaction time; a long reaction time causes a longer collision between the biomolecules of P. jiringa epidermis extract and the $AgNO_3$ solution, thus allowing the formation of more and smaller AgNPs [53]. In this study, the best reaction time to form AgNPs was 96 hours, with the smallest size being 46.6 nm. This is supported by the results of UV-Vis analysis at the 96th hour reaction time, which had the highest absorption peak, namely 2.121 with a wavelength of 430 nm, while at the 24 and 12 reaction times, the absorption peaks were 0.964 and 0.778, respectively. with a wavelength of 420 nm each [54].





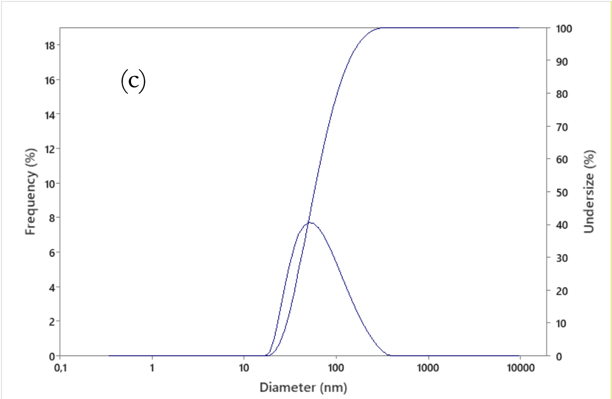


Fig. 10. PSA synthesis time (a) 12-hours; (b) 24-hours; and (c) 96-hours.

Table 2. Analysis of PSA results.

Concentration of $AgNO_3$	Average Size Distribution of AgNPs (nm)	Synthesis Time (hour)
1	76.3	12
1	64.2	24
1	46.6	96

5. Conclusions

This study examines an analysis of epidermis waste extract of P. jiringa contains flavonoid, alkaloid, saponin, and tannin biomolecules. P. jiringa epidermis waste extract can be used as a bioreductant to produce AgNPs. Synthesis at room temperature can produce AgNPs starting at 12, 24, and 96 hours with an average size distribution of 76.3, 64.2, and 46.6 nm, respectively. The nanoparticles obtained were round silver particles with a face-centered cubic (FCC) structure. Therefore, P. jiringa epidermis waste is very promising as a bioreductant source for producing AgNPs via a green synthesis method that is cost and energy-efficient and easy to carry out.

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