Rosella Petal Extract (*Hibiscus sabdariffa* Linn.) as a Reducing Agent in Silver Nanoparticle Synthesis and Its Antibacterial Activity

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Article Info

Abstract

Silver nanoparticles have superior properties that attract much attention because they can increase their effectiveness and efficiency compared to their macroscopic size. Silver nanoparticles have several benefits, one of which is as an antibacterial agent. Synthesis of silver nanoparticles was carried out by reducing silver ions (Ag^+) to uncharged silver (Ag^0) using a reducing agent from *Hibiscus sabdariffa* Linn. petal extract. This research aims to synthesize silver nanoparticles by studying several parameters that influence their formation, such as the pH of *Hibiscus sabdariffa* Linn. petal extract, reaction time, silver nitrate concentration, and *Hibiscus sabdariffa* Linn. petal extract concentration. The silver nanoparticles formed were then tested for their antibacterial activity. The experimental results showed that the best synthesis was performed using *Hibiscus sabdariffa* Linn. petal extract with pH 10, reaction time of 45 minutes, silver nitrate concentration of 1×10^{-4} M, and *Hibiscus sabdariffa* Linn. petal extract concentration of 0.004%. The synthesized silver nanoparticles, examined through a transmission electron microscope, exhibited a size distribution ranging from 2 to 26 nm with an average size of 12 ± 3 nm, which was stable with a storage time of 3 months. Silver nanoparticles showed antibacterial activity against *Staphylococcus aureus*. *Hibiscus sabdariffa* Linn. petal extract is a promising reducing agent for producing antibacterial silver nanoparticles, showcasing its potential in this application.

1. Introduction

Silver nanoparticles are particles of silver metal with a size range of 1-100 nanometers [1]. The different characteristics between nanoscale and macroscopic materials brought a new revolution in industrial technology in the early 2000s. Increasing effectiveness and efficiency in nanometer-sized materials has attracted much attention from researchers [2]. The favorable attributes of silver nanoparticles have yielded a positive impact, leading to their widespread application across various areas of life. Several noteworthy applications of silver nanoparticles encompass serving as an antibacterial agent within the health sector [3], functioning as a metal ion sensor in chemical measurements [4], and acting as a degrader of indigo blue dye [5].

In general, nanoparticles can be produced through two primary methods: physical (top-down) and chemical (bottom-up) approaches. The physical method involves reducing larger-sized materials into smaller, nano-sized particles. Conversely, the chemical method entails the formation of nanoparticles by combining basic atomic materials, which then aggregate to form nanostructures [6].

Both chemical and physical methods for synthesizing silver nanoparticles come with inherent disadvantages. Physical methods suffer from drawbacks such as the complexity of equipment utilized and the substantial associated costs. On the other hand, the chemical synthesis of silver nanoparticles involves using hazardous materials, and the resulting waste is environmentally unfriendly. Consequently, there is a pressing need for alternative approaches to silver nanoparticle synthesis that address these drawbacks [7].

Silver nanoparticles can be synthesized through chemical reduction methods, a process involving reduction and oxidation (redox) reactions that necessitate the use of a reducing agent. Several publications have highlighted that certain reducing agents also serve as stabilizing agents, playing a dual role [8]. In this context, the reducing agent is responsible for reducing Ag^+ to Ag^o, while the stabilizing agent is a preventive measure to inhibit the agglomeration of formed silver nanoparticles, ensuring their stability [9]. However, the consistent reliance on reducing and stabilizing agents derived from chemical sources in significant quantities poses environmental limitations, as these agents may not be environmentally friendly. To address this concern, there is a growing interest in exploring alternative reducing agents sourced from natural materials, such as plants. This shift aims to minimize the generation of toxic waste or hazardous by-products associated with traditional chemical agents, promoting a more environmentally sustainable approach to silver nanoparticle synthesis [10].

The abundant natural resources in Indonesia provide an opportunity to leverage the secondary metabolite compounds found in plants as reducing agents for synthesizing silver nanoparticles. Plants are preferred for synthesis due to their inherent advantages, such as not requiring specialized treatments like isolation or tissue culture, resulting in a faster, more economical, and simpler process [11]. *Hibiscus sabdariffa* Linn. petals, known for their high antioxidant content [12], and *Hibiscus sabdariffa* Linn. petal extract, reported to contain various secondary metabolite compounds such as flavonoids, alkaloids, phenols, and glycosides [13], serve as promising candidates. These secondary metabolite compounds extracted from plants play dual roles as reducing and stabilizing agents in the synthesis process, contributing properties for both reduction and stabilization [14]. Functional groups present in these secondary metabolite compounds, such as hydroxyl groups, amines, carboxylates, and amides, are electron-rich, enabling them to effectively reduce Ag^+ to Ag^o [15]. Furthermore, nanoparticles synthesized using reducing agents derived from plant extracts retain functional groups on the surface of silver nanoparticles. This retention of functional groups enhances the nanoparticles' ability to prevent aggregation, contributing to the overall stability of the synthesized silver nanoparticles [16].

The remarkable properties exhibited by silver nanoparticles significantly enhance their antibacterial activity. The ability of silver nanoparticles as an antibacterial is associated with their smaller size than microorganisms, enabling them to penetrate bacterial cell walls. Once inside, silver nanoparticles attack the bacterial DNA, dephosphorylate proteins (deactivating the bacteria), and inhibit enzyme activity, leading to bacterial cell death [17]. Employing silver nanoparticles as an antibacterial agent offers a distinctive advantage—acting as a reservoir for Ag^o. This reservoir undergoes oxidation to Ag^+, gradually interacting with the bacterial cell wall. The controlled release of Ag^+ in minimal amounts exerts a toxic effect on bacteria, endowing silver nanoparticles with the capability to deliver potent antibacterial activity over an extended duration and at low concentrations [18]. This study examines the capacity of *Hibiscus sabdariffa* Linn. petal extract as both a reducing and stabilizing agent in synthesizing silver nanoparticles, showcasing its effectiveness by evaluating antibacterial activity.

2. Experimental

2.1. Tools and Materials

The tools used in this research include a UV-Vis spectrophotometer (UV-1800 Shimadzu), FTIR spectrophotometer (IR Prestige-21 Shimadzu) and transmission electron microscope (1400-Jeol Jem), centrifuge (TOMY MX307), water heater, analytical balance, oven, pH meter (Hanna-HI110) micropipette, rotary evaporator and thermometer. The materials used were distilled water, *Hibiscus sabdariffa* Linn. petals obtained from one of the plantations in the Kubu Raya area of West Kalimantan, silver nitrate (Merck), sodium hydroxide (Merck), methanol, nutrient agar (NA) (Merck), nutrient broth (NB) (Merck), two types of bacteria namely *Staphylococcus aureus* ATCC 12600 and *Escherichia coli* ATCC 11775.

2.2. Extraction of *Hibiscus sabdariffa* Linn. Petals

The *Hibiscus sabdariffa* Linn. petals were first separated from the seeds and subsequently cut into smaller pieces until their flower petals were obtained. The obtained flower petals were then thoroughly washed with water and air-dried. The dried petals were ground and sieved using a 40-mesh sieve. A quantity of 500 g of *Hibiscus sabdariffa* Linn. petal powder was soaked in a methanol solvent and stirred. Following this, maceration was conducted by immersing the powder for 5×24 hours at room temperature. The obtained filtrate was concentrated using a rotary evaporator at a temperature of approximately 40°C, resulting in a thick extract of *Hibiscus sabdariffa* Linn. petals. This resulting product was intended for use in the synthesis of silver nanoparticles.

2.3. Qualitative Analysis for Phytochemical Screening

A qualitative analysis was undertaken to identify the chemical constituents present in the extract [19]. The concentrated extract derived from *Hibiscus sabdariffa* Linn. petal was dissolved thoroughly in a methanol solvent. Subsequently, a phytochemical test was performed to assess the presence of alkaloids, flavonoids, phenolics, saponins, terpenoids, and steroids.

2.4. Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by mixing an $AgNO_3$ solution and a *Hibiscus sabdariffa* Linn. petal extract solution, with the addition of sodium hydroxide. The mixture was then heated in boiling water until a noticeable color change to yellow occurred. This color change signified the completion of the reduction process from Ag^+ to Ag^o. The assessment of this reduction process, performed using a UV-Vis spectrophotometer, revealed a maximum absorption peak in the wavelength range of 400-450 nm [20]. The formation of silver nanoparticles

was influenced by several parameters, including the pH of the extract, reaction time, silver nitrate concentration, and *Hibiscus sabdariffa* Linn. petal extract concentration.

2.4.1. Determination of the pH of *Hibiscus sabdariffa* Petals Extract Linn.

A 5 mL of *Hibiscus sabdariffa* Linn. petal extract solution was mixed with a 0.01% sodium hydroxide solution until the pH reached values of 10, 11, and 12. Subsequently, for each pH adjustment, 5 mL of a 1.0×10^{-4} M silver nitrate solution was introduced. The resulting mixture in each test tube was then heated at the temperature of boiling water for an hour, followed by a cooling process using running water. The silver nanoparticles produced from each pH variant of the extract solution were quantified using a UV-Vis spectrophotometer, utilizing a wavelength range from 300 to 700 nm.

2.4.2. Determination of Reaction Time

In the experimental procedure, 5 mL of a 1.0×10^{-4} M silver nitrate solution was introduced to 5 mL of a 0.01% extract solution with a pH adjusted to 10, within a test tube. The resulting mixture was heated in a water bath at boiling water temperature for varying durations: 5, 10, 15, 30, 45, and 60 minutes. Subsequently, the mixture was cooled using running water. The silver nanoparticles generated at each distinct heating time were then quantified utilizing a UV-Vis spectrophotometer, with a wavelength range from 300 to 700 nm.

2.4.3. Determination of Silver Nitrate Concentration

Initially, the initial concentration of the silver nitrate solution was varied from 0.5×10^{-4} M, 1.0×10^{-4} M, 1.5×10^{-4} M, 2.0×10^{-4} M, 2.5×10^{-4} M, 3.0×10^{-4} M, and 4.0×10^{-4} M. For each concentration, 5 mL of the silver nitrate solution was combined with 5 mL of *Hibiscus sabdariffa* Linn. petal extract solution under pH 10 conditions with a 0.01% sodium hydroxide solution. Each mixture was heated for 45 minutes in a water heater and then cooled using running water. Silver nanoparticles produced from each concentration of silver nitrate were measured using a UV-Vis spectrophotometer with a wavelength range of 300–700 nm.

2.4.4. Determination of *Hibiscus sabdariffa* Linn. Petals Extract Concentration

In this experimental series, 5 mL aliquots of *Hibiscus sabdariffa* Linn. petal extract, each possessing concentrations of 0.002%, 0.004%, 0.006%, 0.008%, 0.01%, and 0.02% at a pH of 10, were individually combined with 5 mL of a silver nitrate solution at a concentration of 1.5×10^{-4} M. Each mixture was heated for 45 minutes in a water heater and then cooled in running water. The resulting silver nanoparticles were measured using a UV-Vis spectrophotometer in the wavelength range of 300–700 nm.

2.5. Stability Test for Silver Nanoparticles

Stability was determined on nanoparticles produced from synthesis under optimum reaction conditions. Nanoparticles were synthesized by reacting with a 0.004% solution of *Hibiscus sabdariffa Linn.* petal extract under pH 10 conditions with 1.5×10^{-4} M silver nitrate solution and heated in boiling water for 45 minutes. The stability of the resulting silver nanoparticles was observed for 3 months of storage, focusing on changes in the maximum wavelength position, absorbance intensity, and full width at half maximum (FWHM).

2.6. Characterization of Silver Nanoparticles

The synthesized nanoparticles were then characterized using FTIR and TEM instruments. The sample for FTIR analysis consisted of *Hibiscus sabdariffa* Linn. petal extract and synthesized silver nanoparticles. The silver nanoparticle sample was centrifuged at 15,000 rpm for 20 minutes. Centrifugation of silver nanoparticles at high speed causes the silver nanoparticle colloid to settle at the bottom of the centrifuge tube. After the silver nanoparticle colloid was separated from the solvent, it was dried in an oven at a temperature of 45°C. FTIR analysis was carried out using the KBr plate method and measured in the range 400–4000 cm⁻¹. Meanwhile, 5 mL samples for TEM analysis were prepared to determine the morphology and size distribution of silver nanoparticles.

2.7. Antibacterial Activity of Silver Nanoparticles

2.7.1. Rejuvenation of Test Bacteria

The test bacteria were rejuvenated by inoculating them onto nutrient agar media and subsequently incubating them at a temperature of 28°C for 12 hours. The specific test bacteria employed for evaluating antibacterial activity were *Staphylococcus aureus* ATCC 12600 and *Escherichia coli* ATCC 11775.

2.7.2. Preparation of Bacterial Culture

The rejuvenated test bacteria were then used for culturing the test bacteria. A total of 1 ose of test bacteria was inoculated in liquid nutrient broth (NB) media and incubated with shaking at 200 rpm at a temperature of 28°C for 12 hours.

2.7.3. Antibacterial Testing

The antibacterial activity of *Hibiscus sabdariffa* Linn. petal extract, silver nitrate, and silver nanoparticles was assessed utilizing the well diffusion method [21]. A total of 750 µL of the test bacterial culture (McFarland 0.5) was combined with 15 mL of nutrient agar (NA) media and poured into a 90 mm diameter and 15 mm high petri dish. Once solidified, the agar medium was perforated using a sterile hole tool with a 6 mm diameter. Subsequently, 25 µL of silver nanoparticles, 25 µL of *Hibiscus sabdariffa* Linn. petal extract, and 25 µL of silver nitrate were added to designated wells. Additionally, 20 µL of tetracycline (10 µg/well) served as the positive control, while 25 µL of distilled water functioned as the negative control. The solvent in the samples was allowed to evaporate, and after evaporation, the medium containing the samples was incubated at a temperature of 28°C for 24 hours. The presence of antibacterial activity was identified by forming a clear zone around the wells. The diameter of the clear zone formed was measured using a caliper.

3. Results and Discussion

3.1. Extraction of *Hibiscus sabdariffa* Linn. Petals

The extraction of *Hibiscus sabdariffa* Linn. petal extract was performed using the maceration method with methanol as the solvent. The maceration process was iteratively conducted until the originally red powder of *Hibiscus sabdariffa* Linn. petals exhibited a faded color, indicating the extraction of secondary metabolite compounds into the methanol solvent. The filtrate obtained from the maceration process was concentrated using a rotary evaporator, resulting in a dense, purplish-red extract. According to the research findings, the extract yield was determined to be 15.4% (w/w), signifying the weight of the obtained *Hibiscus sabdariffa* Linn. extract. This yield was then compared to the weight of dry powder samples derived from *Hibiscus sabdariffa* Linn. petals.

3.2. Phytochemical Qualitative Test

Phytochemical qualitative testing is a method employed to ascertain the presence of secondary metabolite compounds qualitatively. This testing approach involves observing color change reactions, foam formation, and precipitation reactions with specific reagents, providing identifiable visual changes. The outcomes of the phytochemical test for the methanol extract of *Hibiscus sabdariffa* Linn. petals revealed positive results for alkaloids, flavonoids, saponins, phenolics, and terpenoids while showing negative results for steroids. The presence of these secondary metabolites in the *Hibiscus sabdariffa* Linn. petal extract qualifies it for use as a reducing agent in the synthesis of silver nanoparticles.

3.3. Synthesis of Silver Nanoparticles

3.3.1. Determination of the pH of *Hibiscus sabdariffa* Linn. Petals Extract

The synthesis of silver nanoparticles involved a chemical reduction method utilizing the reducing agent derived from chemical compounds present in *Hibiscus sabdariffa* Linn. petal extract. The pH of *Hibiscus sabdariffa* Linn. petal extract was varied by adding 1 M sodium hydroxide to obtain pH variations of 10, 11, and 12.

Silver nanoparticles are generated in an alkaline environment, which is achieved by adjusting the pH of the *Hibiscus sabdariffa* Linn. petal extract. The addition of sodium hydroxide to the extract, before its reaction with silver nitrate, serves the purpose of deprotonating the hydroxyl group within the phenolic compounds present in the extract, forming phenoxide salt. Subsequently, the electron pair within the phenoxide can be transferred to the silver ion (Ag^+), facilitating the reduction process to Ag^0. Concurrently, the hydroxyl group undergoes oxidation to ketone [22]. The proposed mechanism involves the keto-enol tautomeric transformation of phenolics in flavonoids, facilitating the release of reactive hydrogen atoms crucial for reducing metal ions [23].

According to the research findings, the formation of silver nanoparticles occurs under mildly alkaline conditions in the *Hibiscus sabdariffa* Linn. petal extract solution. Figure 1 illustrates that silver nanoparticles are produced in an alkaline pH environment and are not formed when sodium hydroxide is absent from the extract. This lack of formation is attributed to the functional groups in the extract being unable to reduce Ag^+. The data in Figure 1 further indicates that at pH 10 and 11, a broad absorption peak is evident, with the maximum absorption peaks occurring at wavelengths of 410 nm and 414 nm, accompanied by a color change to yellow. As measured with a UV-Vis spectrophotometer, the yellow color reveals an absorption peak in the characteristic wavelength range of silver nanoparticles, specifically between 400-450 nm.
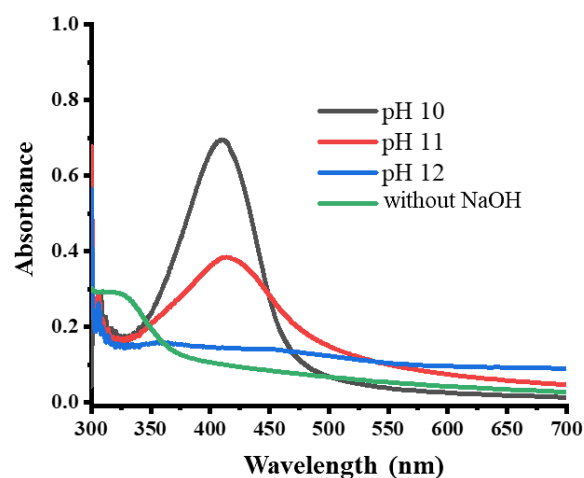

Figure 1. Spectra of silver nanoparticles with variations in extract's pH

Table 1. Phytochemical qualitative test results

Compound Classes	Reagent	Test results
	Dragendorff	++
Alkaloids	Meyer	–
	Wagner	++
Saponin	Distilled water + HCl	+++
Phenolic	FeCl₃ 1%	+++
Flavonoids	Zinc/magnesium powder + HCl	++
	NaOH 2%	++
Terpenoids	Lieberman-Burchard	+
Steroids	Lieberman-Burchard	–

3.3.2. Determination of Reaction Time

The determination of reaction time involved the reaction of silver nitrate solution with *Hibiscus sabdariffa* Linn. petal extract over various time intervals. This reaction was initiated by heating on a water heater with varying times of 5, 10, 15, 30, 45, and 60 minutes, and the heating temperature was maintained at 94°C. The elevated temperature was chosen to optimize and expedite the silver nanoparticle nucleation process. This is attributed to the impact of high temperatures on molecular bond tension, inducing kinetic vibrations that enhance the interaction between silver nitrate molecules and the extract, leading to a quicker reaction [24]. Observations of the reaction outcomes were conducted by examining the surface plasmon resonance spectra of silver nanoparticles using a UV–Vis spectrophotometer.

Figure 2 illustrates that the reaction, occurring at all variations in heating time, results in the formation of silver nanoparticles. While the peak intensity differs at each time interval, all peaks are consistently centered at 415 nm, a characteristic wavelength indicative of silver nanoparticles. The results indicate that the reduction process of Ag^+ to Ag^0 intensifies with increasing heating time, peaking at 45 minutes, as evidenced by the notably high absorption intensity compared to other durations. This heightened absorption intensity indicates a greater number of silver nanoparticles formed, signifying the extent of interaction between the silver nanoparticles and UV–Vis radiation. After heating for more than 45 minutes, the absorption intensity diminishes, indicating a stabilization in the reduction process as the precursor has completed its reaction with the reductant. Consequently, the reduction reaction and nucleation of silver nanoparticles cease to occur [25]. The detailed reaction mechanism for the reduction of Ag^+ to Ag^0 has been explained in Section 3.3.1.
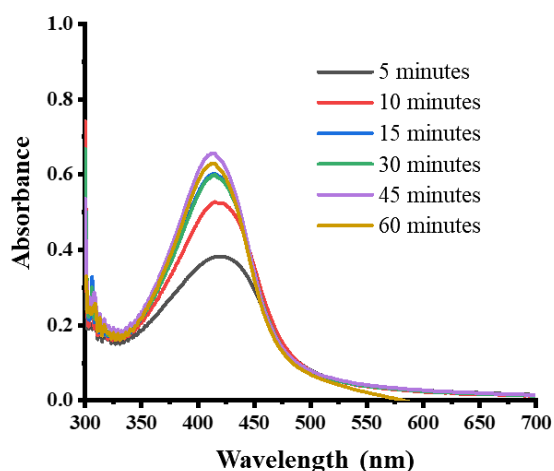

Figure 2. Spectra of silver nanoparticles with variations in heating time

3.3.3. Determination of Silver Nitrate Concentration

Determining silver nitrate concentration for the synthesis involved utilizing the pH reference of *Hibiscus sabdariffa* Linn. petal extract and preselected reaction time. Variations in concentration were explored as they are directly associated with the availability of Ag^+ for reduction. Figure 3 presents the surface plasmon resonance spectra of silver nanoparticles for each variation in silver nitrate concentration used. As depicted in Figure 3, all concentrations of silver nitrate employed successfully generated silver nanoparticles, as evidenced by the appearance of an absorption peak centered around the wavelength range of 406–425 nm.

The absorbance intensity demonstrates an increase with the elevated concentration of silver nitrate utilized. This phenomenon arises because the concentration of the reducing agent in this reaction remains sufficient to effectively reduce Ag^+ in solution, even as the concentration of silver nitrate steadily increases [26]. However, it is noteworthy that the surface plasmon resonance spectra at higher silver nitrate concentrations, specifically ranging from 1.5×10^{-4} M to 4×10^{-4} M, exhibiting the formation of two distinct maximum wavelengths. This occurrence is attributed to the heterogeneous shape and growth of silver nanoparticles towards larger sizes. The continuous nucleation process results in uncontrolled variations in the shape and size of silver nanoparticles [27]. Consequently, a silver nitrate concentration of 1.5×10^{-4} M was selected for utilization in the subsequent synthesis stage.
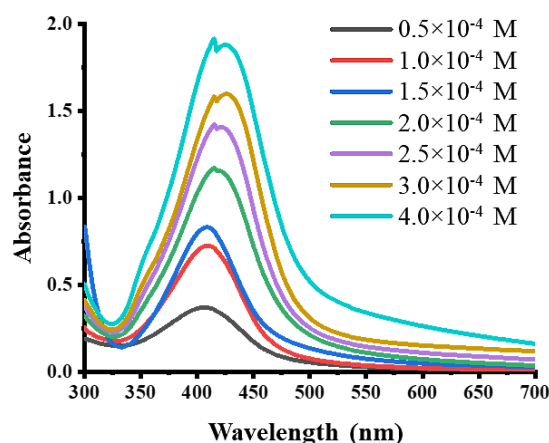

Figure 3. Spectra of silver nanoparticles with varying concentrations of silver nitrate

3.3.4. *Hibiscus sabdariffa* Linn. Petals Extract Concentration

The *Hibiscus sabdariffa* Linn. petal extract was synthesized by varying the extract concentration as a reducing agent to assess its efficacy in optimally reducing Ag^+. The reduction of silver ions is facilitated by the secondary metabolite compounds in the extract, utilizing the functional groups present in these compounds. According to the research outcomes, all concentrations employed successfully formed silver nanoparticles, ranging from the smallest concentration of 0.002% to the largest at 0.02%. In Figure 4, it is evident that the absorbance intensity reached its peak when the reaction employed an extract with a concentration of 0.004%. Selecting the appropriate concentration for the reducing agent in conjunction with silver ions is crucial for achieving optimal results in silver nanoparticle formation. Consequently, a concentration of 0.004% was chosen for utilization in the subsequent synthesis stage.
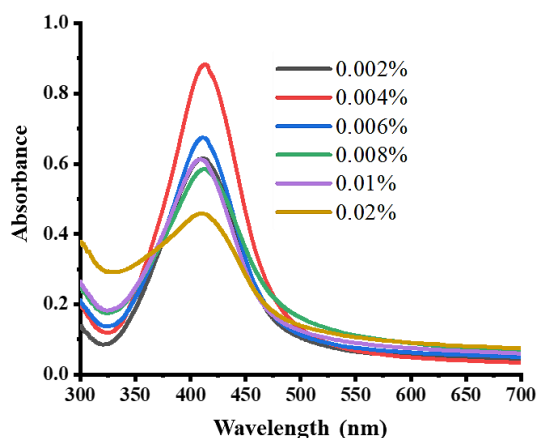
Figure 4. Spectra of silver nanoparticles with varying extract concentrations

3.4. Stability Test

Silver nanoparticles, synthesized using a reductant derived from *Hibiscus sabdariffa* Linn. petal extract, were subjected to a stability test over a 3-month period. The stability of these silver nanoparticles was assessed through three parameters: maximum wavelength (λ_{max}), absorption intensity, and absorption peak width or FWHM (full width half maximum). Table 2 shows a slight change in the data for maximum wavelength (λ_{max}), absorption intensity, and absorption peak width or FWHM over the 3-month storage duration. After 3 months, there was a 0.2% decrease in absorption intensity, a shift in the maximum wavelength by approximately 1 nm, and a 0.1% increase in the broadening of the absorption peak.

The visual observation of silver nanoparticles after 3 months of storage revealed that the color remained consistent with the initial synthesis, albeit with a slight decrease in intensity. Notably, no black precipitates were at the bottom of the storage container. The reduction in color intensity is accompanied by a decrease in absorption intensity, indicating a reduction in the number of silver nanoparticles during storage. This phenomenon may be attributed to the re-dissolution of silver nanoparticles into Ag^+ ions, facilitated by an oxidation reaction throughout the storage process [28].
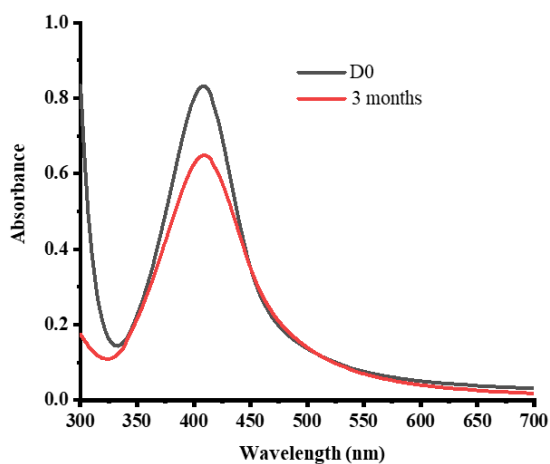

Figure 5. Spectra of silver nanoparticles after 3 months of storage

Table 2. Silver nanoparticle stability data for 3 months

Storage Time	λ_{max} (nm)	Absorbance	FWHM
Day 0 (D0)	409	0.832 ± 0.002	75 ± 0.6
Day 1	409	0.826 ± 0.003	77 ± 0.0
Day 2	409	0.830 ± 0.004	78 ± 0.0
Day 3	409	0.830 ± 0.005	80 ± 0.6
1st week	409	0.805 ± 0.006	79 ± 0.6
2nd week	410	0.799 ± 0.007	80 ± 0.6
3rd week	409	0.785 ± 0.009	80 ± 1.5
1 month	409	0.7623 ± 0.04	84 ± 2.0
2 months	409	0.704 ± 0.030	79 ± 3.5
3 months	409	0.698 ± 0.040	83 ± 3.5

Although there is a slight shift in the maximum wavelength, indicating a change in the size of the silver nanoparticles, it remains within the nanometer range, and the position of the maximum wavelength remains relatively constant [29, 30]. Additionally, a slight change in FWHM suggests a modification in the size distribution of silver nanoparticles. This alteration is likely caused by the aggregation or interaction of silver nanoparticles, leading to an increase in particle size that still falls within the nanoparticle size range, resulting in peak broadening [31]. Throughout the 3-month storage period, the FWHM value tends to increase, although, at a certain point in the storage time, it may decrease due to the dissolution (redissolving) process of silver nanoparticles.

The spectrum changes in surface plasmon resonance (SPR) after 3 months of storage are depicted in Figure 5. Notably, silver nanoparticles, even after 3 months of storage, exhibited stability, as evidenced by the minimal alterations in maximum wavelength, absorbance intensity, and FWHM. This stability data underscores the capability of silver nanoparticles to prevent agglomeration between particles, which could lead to an increase in nanoparticle size over time [32]. The level of size stability observed is quite commendable for silver nanoparticles synthesized using a reductant derived from *Hibiscus sabdariffa* Linn. petal extract. This stability is presumed to be influenced by *Hibiscus sabdariffa* Linn. petal extract, which remains attached to the surface of the silver nanoparticles. Without the addition of external stabilizing agents, secondary metabolite compounds are present in the petals of *Hibiscus sabdariffa* Linn. are believed to function as stabilizing agents, contributing to the production of more stable silver nanoparticles, in addition to their role as reducing agents.

3.5. Characterization of Silver Nanoparticles

The size and morphology of silver nanoparticles synthesized using *Hibiscus sabdariffa* Linn. petal extract were observed through transmission electron microscopy (TEM), as illustrated in Figure 6. The TEM results and the corresponding size distribution histogram confirm that the silver particles produced exhibit a high degree of homogeneity, displaying a round morphology evenly distributed in the colloidal phase.
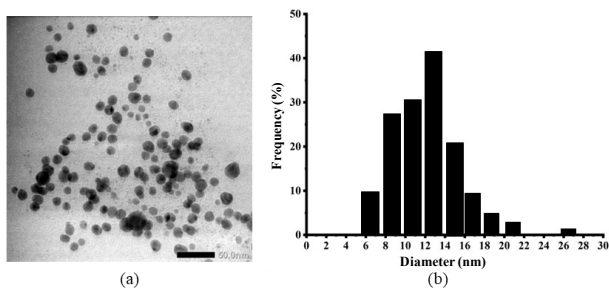
Figure 6. a) TEM test results and b) histogram of size distribution of silver nanoparticles
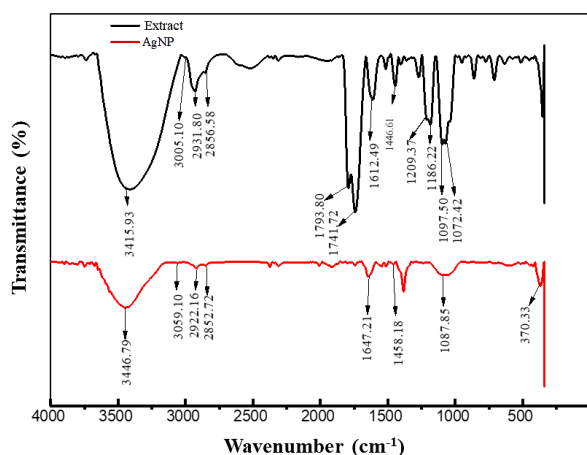

Figure 7. FTIR spectra of *Hibiscus sabdariffa* Linn. petals extract and silver nanoparticles

The FTIR spectrum in Figure 8 provides confirmation of the existence of an organic layer on the surface of silver nanoparticles synthesized using a reductant derived from *Hibiscus sabdariffa* Linn. petal extract. FTIR measurements were conducted to identify various functional groups of compounds in *Hibiscus sabdariffa* Linn. petal extract, which play a role in the reduction of Ag^+ and the stabilization of silver nanoparticles [33]. According to Figure 7, the appearance of a strong and broad absorption at the wavenumber 3415.93 cm⁻¹ indicates the OH stretching vibration characteristic of the hydroxyl group in alcohol or phenol compounds. Absorption bands at wavenumbers 3005.10 cm⁻¹, 2931.80 cm⁻¹, and 2856.58 cm⁻¹ signify the presence of aromatic CH stretching vibrations. Additionally, a strong absorption band is evident in the wavenumber range of 1700 cm⁻¹, representing the C=O stretching vibration, indicating the presence of a carbonyl group from the aldehyde group. The weak absorption peak at wavenumber 1612.49 cm⁻¹ indicates the presence of aromatic C=C vibrations. Peaks with moderate intensity at wavenumbers 1209.37 cm⁻¹, 1186.22 cm⁻¹, 1097.50 cm⁻¹, and 1072.42 cm⁻¹ originate from the CO strain absorption area [34].

The similarity in the position of the absorption peak in the FTIR spectrum between the extract and silver nanoparticles synthesized using a reductant from *Hibiscus sabdariffa* Linn. petal extract reveals the presence of phytochemical extracts from *Hibiscus sabdariffa* Linn. petals on the surface of the synthesized silver nanoparticles. The absorption of bond vibrations at a wavenumber in the range of 1700 cm⁻¹ in the silver nanoparticle spectrum has decreased in intensity, indicating the contribution of the carbonyl group in the formation of a cap of metabolite compounds on the surface of the silver nanoparticle [35]. Figure 7 illustrates that the secondary metabolite compounds in *Hibiscus sabdariffa* Linn. petal extract is involved in the synthesis process of silver nanoparticles both as a reducer and stabilizer. The complexity of the compounds in the extract predicts that more than one substance will be adsorbed on the surface of the silver nanoparticles as a stabilizing agent. A strong absorption band in the FTIR spectrum of the synthesized silver nanoparticles appears at a lower wavenumber, namely 370.33 cm⁻¹, indicating the vibration of the Ag-O bond [36].

3.6. Antibacterial Activity Test

The antibacterial activity of the samples is evident from the formation of a clear zone where bacteria do not grow around the well. The measured diameters of the clear zones in Table 3 confirm that silver nitrate and silver nanoparticles exhibit antibacterial activity against the bacteria *Staphylococcus aureus* ATCC 12600. Conversely, silver nitrate and silver nanoparticles do not demonstrate antibacterial activity against the bacteria *Escherichia coli* ATCC 11775, as no clear zone is formed. The results of the antibacterial activity test against *Staphylococcus aureus* and *Escherichia coli* bacteria are illustrated by the presence or absence of a clear zone, as depicted in Figure 8.

This phenomenon is attributed to the resistance of Gram-negative bacteria due to their cell structure. Gram-negative bacteria possess a thin layer of lipopolysaccharide on the outer membrane, which can restrict the penetration of silver nanoparticle solutions. In contrast, Gram-positive bacteria only have a peptidoglycan layer consisting of polysaccharide chains, which is more easily accessible for permeation by silver nanoparticle solutions [37]. The differences in the chemical composition of the cell walls of each bacterium lead to *Staphylococcus aureus* being more easily penetrated by colloidal particles, exhibiting a greater inhibitory effect compared to *Escherichia coli.*
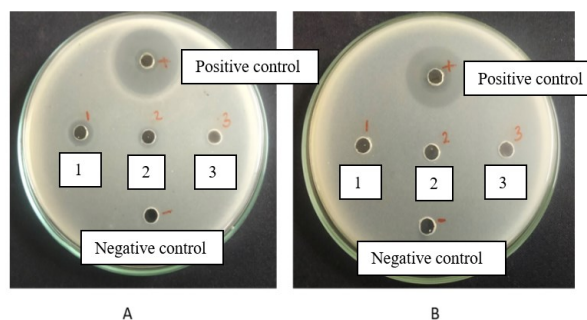

Figure 8. Clear zone diameter against (a) *Staphylococcus aureus* ATCC 12600 and (b) *Escherichia coli* ATCC 11775

Table 3. Antibacterial activity of silver nanoparticles

Gram-positive bacteria (*Staphylococcus aureus*)		Gram-negative bacteria (*Escherichia coli*)	
Test sample	Diameter of clear zone (mm) [21]	Test sample	Diameter of clear zone (mm) [21]
Silver nitrate solution	4.8	Silver nitrate solution	0
Silver nanoparticles	2.1	Silver nanoparticles	0
Extract	0	Extract	0
Control (+)	26.7	Control (+)	18.2
Control (−)	0	Control (−)	0

Another factor influencing the antibacterial activity of silver nanoparticles is the presence of other constituents, particularly secondary metabolite compounds from the reducing agent, which remain attached to the surface of the silver nanoparticles. This can impede the optimal dissolution process of Ag⁺ ions. However, these secondary metabolite compounds, still linked after the reduction process, also function as effective stabilizing agents, maintaining the size of the resulting nanoparticles. This is supported by the results of a stability test conducted over a 3-month period, demonstrating the continued presence of secondary metabolite compounds from *Hibiscus sabdariffa* Linn. petal extract. These compounds may influence the sustained antibacterial effect.

The research results provide evidence that small-sized silver (silver nanoparticles) with good stability exhibit antibacterial capabilities. Similar findings were reported in another study [38], which demonstrated that silver nanoparticles synthesized using *Eriobotrya japonica* extract displayed superior antibacterial properties against *Staphylococcus aureus* compared to *Escherichia coli*. Silver nanoparticles, owing to their small size and stable nature, can serve as a reservoir, reducing the need for excessive material usage while providing long-term antibacterial effects.

4. Conclusion

The secondary metabolite compounds present in *Hibiscus sabdariffa* Linn. petal extract has been demonstrated to effectively reduce silver nitrate, leading to the formation of silver nanoparticles. The optimal synthesis conditions involved a reaction time of 45 minutes, utilizing an extract concentration of 0.004%, pH of 10, and an $AgNO_3$ concentration of 1.5×10^{-4} M. The resulting silver nanoparticles, synthesized with the reductant properties of *Hibiscus sabdariffa* Linn. petal extract, exhibit a characteristic spherical shape with an average size of 12 nm. Additionally, an organic layer from *Hibiscus sabdariffa* Linn. petal extract envelops the surface of these silver nanoparticles, as confirmed by FTIR analysis. Furthermore, stability testing revealed that the synthesized silver nanoparticles maintained stability over a 3-month period of storage. Importantly, these nanoparticles demonstrated inhibitory effects against *Staphylococcus aureus*, highlighting their potential in antibacterial applications.

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