

Green Synthesis of Silver Nanoparticles using Aqueous Extract of *Prunus africana* and their Antimicrobial Activities

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Abstract

The alarming effect of antibiotic resistance prompted the search for alternative medicine to resolve the microbial resistance conflict. Over the last two decades, scientists have become increasingly interested in metallic nanoparticles to discover their new dimensions. *Prunus africana* is a traditional medicinal plant rich in phytochemicals. In this study, we expand this knowledge by synthesizing anti-bacterial silver nanoparticles (AgNPs) using *Prunus africana* stem bark extract as a reducing, capping, and stabilizing agent. The biosynthesis of AgNPs was carried out using 0.1 M silver nitrate and 2% w/v stem bark extract. The effect of temperature, contact time, and concentrations on the synthesis of AgNPs was examined using UV-Vis spectra. The formation of AgNPs was indicated by the development of a dark-brown color from red-brown. Using a UV-Vis spectrophotometer, the surface plasmon resonance observed at 432.5 nm indicated the formation of silver nanoparticles. Probable vibrational stretches that are characteristic of silver nanoparticles, such as OH and C = O vibrations, were identified using an FT-IR spectrophotometer. The characteristic peaks of the XRD pattern confirmed the synthesis of pure AgNPs with an average crystalline size of 17.07 nm. The TEM (transmission electron microscopy) analysis confirmed that the synthesized AgNPs were spherical with sizes ranging from 15.95 nm to 43.04 nm. The DLS analysis confirmed the stability of AgNPs in solution at -12.44 mV. The synthesized silver nanoparticles (AgNPs) exhibited antibacterial activity against four bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*) and one fungus (*Candida albicans*).

1. Introduction

The field of nanotechnology is expanding, and it can be utilized to make structures at the nanoscale. The approach and formulation of particles with a diameter ranging from 1 to 100 nm are the focus of nanoparticles. Since nanoparticles (NPs) are not simple molecules, they are separated into three layers: (1) the shell layer, which is physically and chemically different from the core; (2) the surface layer, which can be made stable with a range of special polymers, emulsifiers, metal ions, and chemicals; and (3) the core, which may be the nanoparticle's central component [1]. Innovations in nanobiotechnology have led to fascinating findings in materials science. This method now allows for the biosynthesis of environmentally and economically advantageous metal nanomaterials for use in the food, agricultural, cosmetics, defense, environmental safety, and health sectors [2].

The biological pathways are quick, easy, affordable, and, most importantly, environmentally benign as compared to physical (pyrolysis, high-energy irradiation, laser ablation, etc.) and chemical (microwave-assisted, electrochemical deposition, photo-induced reduction, etc.) procedures [3]. Whilst these conventional techniques of producing nanoparticles are efficient, they are generally quite costly. To keep the nanoparticles from aggregating, capping reagents or surface passivators are usually required. However, several organic passivators, including mar-capto acetate, thiophenol, and thiourea, are hazardous enough to pollute the environment if large-scale nanoparticles are formed [4]. The biological method reduces metal ions to generate corresponding nanoparticles by applying bioactive chemicals

from fruit peel waste, bacteria, and primarily plants [5]. Plants and microorganisms are safe, reproducible biological resources that do not harm people or the environment. They can be a better alternative to physical and chemical approaches for the development of silver nanoparticles (AgNPs) [6].

Furthermore, natural extracts act as a rich supply of bioactive molecules that can be used for the green fabrication of metallic NPs as well as anti-oxidant medications and dietary supplements. The components of the natural product can function as potent reducing and capping substrates, ensuring the stability of the produced nanoparticles [7]. Biosynthesis has been effected in recent publications for the synthesis of silver nanoparticles, where they have used medicinal plant extracts (*Scutellaria barbata*, *Abelmoschus esculentus*, etc.) [8] [9], among others. All these studies agree that biomolecules present in them prove the reduction of silver ion (Ag^+) to zerovalent silver (Ag^0).

AgNPs' small particle sizes and high surface area provide them unique biological, physical, and chemical characteristics [10]. Their optical characteristics, which result from localized LSPR (surface plasmon resonance), make them interesting [11]. As catalysts, biosensors, or antibacterial agents, they have a wide range of uses. Apart from their efficaciousness against inflammation, they find use in antiseptic sprays, topical creams, and wound dressings in biomedicine. Their application in the detection and treatment of cancer is showing success [12]. In addition to silver nanoparticles, noble metals such as iron, zinc, and gold exhibit a wide variety of material behaviors as well as amazing characteristic features dependent on size, shape, and application in a wide range of industries [13].

Silver nanoparticles are most frequently utilized in the textile industry as an antibacterial agent for water treatment. Being non-toxic to animal cells and highly poisonous to bacteria makes them safe and efficient antibacterial agents [14]. Antimicrobial resistance is becoming a global issue with regard to the management of viral illnesses due to the improper and excessive use of antibiotics. The application of nanotechnologies in the production of potent antimicrobials is a novel and promising solution in response to a demand for novel antimicrobial drugs that can either eradicate or limit the growth of a broad spectrum of microorganisms [15]. Through the production of ions or superoxide radicals that can obstruct cell granules or damage membrane proteins, metallic nanoparticles have an antibacterial impact. These nanoparticles may prevent the production of advanced products of glycation, which makes them useful for anticancer treatments [16].

The species *Prunus africana* (Hook f.) is a member of the genus *Prunus*, which includes around 400 species. There are roughly 98 species that are very significant. A mature *Prunus africana* stem can have a diameter of up to 1 m, and the plant can reach a height of about 40 m. The leaves of the plant are alternate, oval-shaped, and simple between a deep green topside and a shining, light green underside. The bark of the plant has a dark brown color. The flowers range in color from green to white. The fruits have a pink-brown hue [17]. As a therapeutic plant, *Prunus africana*'s high-value bark has led to harsh exploitation over time. It has several difficulties, including the intransigence of its seed and erratic fruiting, which hinders its ability to regenerate sexually. Thus, the proper channel for its domestication has been determined to be asexual regeneration [18]. The findings revealed that aqueous/acetonic

Prunus africana stem and root bark extracts outperformed other extracts and the reference antibiotic in their considerable ($p < 0.05$) antibacterial activity against *S. aureus* at 800 $\mu\text{g/ml}$ [19]. The bark has been used traditionally to treat various illnesses, including malaria, renal abnormalities, stomach pain, urinary tract infections, and cardiac issues. One traditional way to consume the bark is to chew it or crush it into a fine powder for use in tea [20].

The current study aimed to investigate the potential of the medicinal plant *Prunus africana* in the green synthesis of silver nanoparticles. Biogenic silver nanoparticles were explored for their antimicrobial potential on multi-drug-resistant (MDR) bacterial strains.

2. Materials and Methods

Silver nitrate (AgNO_3 , 99.8%) precursor salt, distilled water, Whatman (No.1) filter papers, Nutrient Agar, Petri dishes, bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*), one fungus (*Candida albicans*), antibiotics (ampicillin/gentamicin) and *Prunus africana* stem bark. All the microbiological media and reagents used for the chemical analyses and extraction process are of pure analytical and laboratory grade and were obtained from HiMedia.

2.1. Extraction of Biomolecules

The extraction method is adopted from [21] with minor modifications. The *Prunus africana* stem barks were washed using tap water, rinsed with distilled water, and left to dry for 15 days at room temperature. A mechanical grinder was used to pulverize the dried sample into a powder. 10 g of powdered plant sample was dissolved in 500 mL of distilled water, in a 1000 mL conical flask and heat macerated at 60°C for 2.5 hrs on a hot plate with a magnetic stirrer. The extract solution was filtered through a filter paper (Whatman No. 1). The filtrate was refrigerated at 4°C for further use.

2.2. Synthesis of AgNPs using *Prunus africana* Extracts

The *Prunus africana*-capped silver nanoparticles (AgNPs@PA) were synthesized using various protocols with minor modifications. The effects of temperature, reaction duration, and concentration of AgNO_3 solution as well as plant extract were studied in the synthesis of AgNPs to determine their impact during the reaction process. 18 mL of the 2% (w/v) aqueous bark extract was added to 1 mL of (0.01, 0.1, 1 M) AgNO_3 solution and the formulation of AgNPs was observed using a UV–Vis spectrophotometer (Shimadzu model, UV–Vis 1800 series) in the 200–800 nm range [22]. In addition, the experiment was conducted at room temperature, 35, 40, 45, 50, 60, 65, 70, 80, 90, and 100°C for 40 minutes, to assess the significance of temperature during synthesis [23]. The transition of the colorless solution to dark brown will indicate that AgNO_3 has been bio-reduced to AgNPs. The mixture was centrifuged for 25 minutes at 10,000 rpm [24]. The solid masses were then dried in a vacuum oven at 60°C overnight after being individually rinsed with distilled water ($\text{DI-H}_2\text{O}$) in triplicate to remove unreacted precursor salts and biological particles [25].

2.3. Characterization of AgNPs

The absorption spectrum and SPR of the formed Ag-NPs were recorded using a UV-Vis (UV-1800 series, Shimadzu model). The functional groups in the AgNPs samples and *Prunus africana* extract were investigated via Fourier transform infrared (FTIR) spectroscopy ((IRAffinity-1S, SHIMADZU model)), using ATR technique. Transmission electron microscopy (TEM) (JEOL-JEM-1011, Japan) was used to examine the size and morphology of the biosynthesized Ag-NPs.

2.4. Anti-bacterial activity

The method was adopted from [26] with significant adjustments. Nutrient agar medium was prepared by dissolving 14 g of agar powder in 500 mL of distilled water and then autoclaved. A total of 20 mL of prepared agar was poured into each Petri dish, which was left to stand for 15 min for the agar to solidify, then the plates were inoculated overnight with human pathogens, such as the Gram-negative strain, *Escherichia coli*, and the gram-positive strains, *Staphylococcus aureus* obtained from MKU Laboratory, Kenya. All organisms were tested simultaneously by the disc diffusion method. The green synthesized Ag-NPs, positive control (ampicillin) and pure extract of *Prunus africana* were added steadily until the wells were full, followed by incubation at 37°C for 24 h. The diameter of the zone of inhibition was measured.

3. Results and Discussion

3.1. Green Synthesis of AgNPs

Green fabrication of silver nanoparticles is a fascinating work nowadays. The aqueous solution of silver nitrate precursor salt and aqueous *Prunus africana* stem bark aqueous extract was used for the synthesis of silver nanoparticles. The mixture was uniformly mixed using a mechanical shaker at regulated temperatures and time. The formation of AgNPs was indicated by colour changes from red-brown to dark-brown (Fig. 1) as also reported by [22].

3.2. Optical Analysis

UV–Visible spectral analysis was done using a UV-Visible spectrophotometer (UV-1800 series, Shimadzu model). The analysis was carried out in a scan range of 200–800 nm with a resolution of 1 nm. Using distilled water as a blank, 300 microliters of the biogenic AgNPs sample solution was pipetted out and scanned with dilution in 3 mL standard quartz cuvettes. The monitoring process for the absorption pattern of the extracts and mixture of extracts with silver nitrate was achieved at varied concentration ratios, synthesis temperatures, and contact times. The peak broadening decreases as you increase the concentration of the reducing and capping agents in the plant extract (Fig. 2). The reaction time of Ag^+ ion reduction to Ag^0 by biomolecules present in *Prunus africana* aqueous extracts is concentration, temperature and time -dependent (Figs. 2, 3 and 4), and therefore, as also reported by [15], the

enhancement of the reaction temperature to 60°C increases the reduction rate and shortens the reaction time necessary for AgNPs synthesis (Fig. 3), [27]. Figure 4 shows that the maximum reaction time was 55 minutes, since, at 65 minutes, peak broadening was noted, which can be a result of agglomeration [28]. A blue shift was revealed by the SPR (surface plasmon resonance) peak at (435-437.5 nm) (Figs. 2, 3 and 4) shifting to a lower wavelength (432.5 nm) (Fig. 6) signifying particle decrease at optimized synthesis parameters i.e. a temperature of 60°C, 0.1 M AgNO₃ and a reaction time of 55 minutes, whereas the red shifts show the production of large-sized particles or the presence of agglomerates [29]. The tauc plot shows biosynthesized AgNPs possessed a bandgap energy of 4.16 eV (Fig. 6). Increasing the temperature above 60°C results in agglomeration, disappearance of peaks, and peak broadening of the nanoparticles (Fig. 3). The common peak located at 278.5 nm (Figs. 2, 4, and 6) was a result of the chemical constituents present in *Prunus africana* aqueous extract.

The formation of AgNPs can be monitored by the increased intensity of the absorbance band with respect to time (Figs. 4 and 5). The particle characteristics, including size, shape, kind of metal, and dielectric compound around the medium, affect the surface plasmon resonance band wavelength and intensity [16].

3.3. FT-IR Analysis

The phytochemical constituents present in the stem bark extracts of *Prunus africana* that are responsible for reducing, capping, and stabilizing AgNPs were determined using FT-IR spectroscopy at 4000–400 cm⁻¹ scan range [30]. The FT-IR spectrum of plant extracts and formulated AgNPs is shown in Figs. 7(a) and 7(b), respectively. The presence of a strong, broadband spectrum (Fig. 7(a)) at 3209 – 3028 cm⁻¹ in *Prunus africana* stem bark extract can be attributed to hydrogen-linked O-H stretching vibrations of phenol, alcohol, carboxylic groups, and other compounds [31].

The biosynthesized AgNPs' vibrational bands at 3446 cm⁻¹ and 1369 cm⁻¹ were also observed (Fig. 7(b)), which are assigned to O-H bands of carboxylic groups, phenolics, and alcohols [32][33]. The C–O stretching vibrations at 1060 cm⁻¹ signify the presence of carbohydrates, terpenoids, and flavones in the *Prunus africana* extract [34]. When compared to the spectra of *Prunus africana* stem bark extract, the intensity of AgNPs spectrum at 769 cm⁻¹, 1060 cm⁻¹, 1211 cm⁻¹, 1369 cm⁻¹, 1600 cm⁻¹, and 3209 cm⁻¹ is reduced [35]. The reason for the reduction and alterations of the spectra is that the phytochemicals, such as alcohols, amides, and carboxylic groups, are involved in the redox reactions during the synthesis of AgNPs [36]. The absorption peak at 1211 cm⁻¹ is assigned to the stretching of N-O [3].

A strong absorption spectrum for *Prunus africana* stem bark extract (Fig. 7(a)) was observed at 1600 cm⁻¹, which may be attributed to C = O bands and the N–H group of proteins and enzymes [37]. The spectra of C = O bands and the N–H group of proteins and enzymes for the biosynthesized AgNPs shift to 1597 cm⁻¹, and the intensity is significantly decreased, confirming the role of enzymes, proteins, and other biomolecules in the bio-reduction [38], stabilization, and capping of the silver nanoparticles

(Fig. 7(b)) [31]. The presence of saturated aliphatic esters is attributed to the sharp absorption band at 1741 cm^{-1} corresponding to the carbonyl $\text{C}=\text{O}$ bonds [39]. The reduced stretching vibration of Ag-O with an absorption band at 769 cm^{-1} (Fig. 7(b)), confirms the production of silver nanoparticles (the stretching was attributed to the metal-ligand frequency that formed due to the interaction between biomolecules and the AgNPs surfaces) [40].

3.4. Structural analysis of AgNPs

The XRD pattern for the prepared AgNPs using *Prunus africana* is shown in Fig. 8. The XRD analysis was conducted to determine the purity, size, and crystalline structures of the biosynthesized AgNPs. In the XRD spectrum, three prominent diffraction bands were observed at $2\theta = 38.26^\circ$, 44.45° , and 64.76° which could be indexed to (111), (200), and (220) diffraction planes, respectively [41]. All the diffraction peaks were attributed to the cubic structure of pure Bragg's reflections of the FCC (face-centered cubic) structure of the metallic silver powder phase. These planes confirmed the crystalline nature of the green-synthesized AgNPs. The highest peak intensity of the (111) plane with a narrow full width at half maximum (FWHM) illustrates the good crystalline nature of the synthesized AgNPs as observed from the XRD patterns. The resulting peaks and their corresponding Bragg reflections strongly agreed with the Joint Committee on Powder Diffraction Standards (JCPDS, file no. 04-0783) [42]. The prominent characteristic peaks of the green synthesized silver nanoparticles indicate the purity of the synthesized nanoparticles without any additional diffraction peaks [43]. The average crystallite sizes of the particles were calculated by using Debye-Scherrer's equation (Eq. 1):

$$x = \frac{K\lambda}{\beta \cos \theta} \text{ Eq. 1}$$

where, x is the estimated crystal size in nano-meter (nm) from XRD patterns, θ is the Bragg's angle (in radians), λ is the wavelength of X-ray maximum of the diffraction peak (in radians) and K is the shape factor or source used ($\text{CuK}\alpha = 1.5419\text{ \AA}$), β is the angular width at the half Scherrer constant (0.9) of Debye-Scherrer's equation [34]. The estimated average crystalline size (x) of the synthesized silver nanoparticles is found to be 17.07 nm.

3.5. Transmission electron microscopy analysis of AgNPs

The TEM image of the AgNPs synthesized using *Prunus africana* showed that the nanoparticles are predominantly spherical and of different sizes (Fig. 9 (a)). In addition to the spherical shape, a few of other shapes such as oval and triangular were also observed. Aggregation of particles was also seen. A study by [44] also reported spherical AgNPs synthesized using *Aloe vera* gel extract. The clear boundaries seen around the nanoparticles signify for the occurrence of phytochemicals as capping agents stabilizing the silver nanoparticles. Figure 9 (b) showed the particle size distribution of the synthesized AgNPs. The synthesized nanoparticles are polydisperse and range in size from 15.95 nm to 43.04 nm, with an average size of 32.04 nm. Our findings followed previous reports, where plant extract

as a reducing and capping agent was utilized in the synthesis of AgNPs, and almost similar results have been reported for AgNPs with the size of a nanoparticle ranging from 18.23 to 53.68 nm [43].

3.6. EDS Analysis

The EDS spectrum mainly identifies the purity and the elemental composition of the biosynthesized AgNPs, as shown in Fig. 10. The strong signal for Ag, with higher atom percentages, was located at 3 keV, confirming the formation of silver nanoparticles biosynthesized with an aqueous extract of *Prunus africana*. Additionally, a few weaker signals of O, and C were also obtained, signifying the existence of biomolecules capping and stabilizing AgNPs [3].

3.7. DLS Analysis

The zeta potential, which provides important information about nanoparticle dispersion through the magnitude of the charge, reflects the mutual repulsion between particles. The particle sizes range from 100 to 1000 nm in diameter. The measured zeta potential of AgNPs was – 12.44 mV (Fig. 11), preventing agglomeration and improving stability in solution [35].

3.8. Antibacterial activity

The in vitro antibacterial potential of the synthesized AgNPs was examined against two types of selected human pathogenic microbes, namely, fungus (*Candida albicans*), gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) (Table 1 and Fig. 12). The clear zones of inhibition around the discs impregnated with the biosynthesized AgNPs, plant extract, and erythromycin (positive control), as shown in Fig. 12. Erythromycin is a commonly used antibacterial against superficial and deep infections caused by human pathogens [45]. AgNPs exhibited superior antibacterial properties against all the test microbes, as evidenced by the calculated zones of inhibition (Table 1), which may be due to the synergistic effects of bioactive capped nanoparticles. The structures that make up bacterial cells include proteins, DNA, and cell membranes. These structures contain phosphorus and sulfur. Since silver is a Lewis acid and these substances are Lewis bases, sulfur proteins and silver ions are attracted to each other electrostatically, which could be the silver nanoparticles' possible antibacterial action mechanism [46]. Therefore, AgNPs can bind to the cell wall and penetrate bacterial cells. The internalization of silver nanostructures disrupts respiratory function, inactivating respiratory enzymes and generating reactive oxygen species (ROS). This overproduction of ROS damages intercellular components, including DNA, lipids, and proteins. The destruction of the cellular membrane causes loss of cytoplasm, leading to cell death [33]. In addition, cell wall thickness affects how effectively different bacteria respond to silver nanoparticles. Gram-negative *E. coli* has a thinner cell wall, making it more vulnerable to silver nanoparticle penetration than Gram-positive bacteria like *S. aureus*, which have a thicker cell wall [47]. In general, nanoparticles have a high surface area to volume ratio that enables them to interact more with microbes compared to larger particles, resulting in improved microbial activity [48].

Table 1

Zones of inhibition (mm) of AgNPs, plant extract, and erythromycin (positive control) impregnated discs against: *Pseudomonas aeruginosa*; *Bacillus subtilis*; *Escherichia coli*; *Staphylococcus aureus*, and *Candida albicans* (Fungus) by the Disc Diffusion Method

Sample Name	AgNPs	Plant extract	Positive Control (erythromycin)
Microbial Strains			
<i>Bacillus subtilis</i>	10.67 ± 1.25	7.67 ± 0.94	10.67 ± 0.47
<i>Pseudomonas aeruginosa</i>	9.00 ± 0.82	7.533 ± 0.47	7.67 ± 0.47
<i>Escherichia coli</i>	11.00 ± 0.82	8.33 ± 0.47	7.83 ± 0.24
<i>Staphylococcus aureus</i>	11.33 ± 01.25	5.33 ± 0.47	7.33 ± 0.47
<i>Candida albicans</i>	10.00 ± 0.82	9.33 ± 0.47	9.83 ± 0.24

3.9. Statistical Analysis: One-Way ANOVA

Table 2

The ANOVA analysis of antibacterial and antifungal (Zones of Inhibition) results.

SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	5	52	10.4	0.85445		
Column 2	5	38.33	7.666	2.50668		
Column 3	5	41.33	8.266	3.49768		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	20.64785	2	10.32393	4.51562	0.034506	3.885294
Within Groups	27.43524	12	2.28627			
Total	48.08309	14				

The acquired anti-bacterial and anti-fungal results are significant because the p-value is less than 0.05 (Table 2).

Conclusions

The silver nanoparticles were successfully fabricated via a simple and eco-friendly green synthesis method using *Prunus Africana* aqueous extract. The AgNPs formation was confirmed by the change in

color of the reaction mixture and the appearance of the SPR band at 432.5 nm. The biomolecules, which acted as reducing, capping, and stabilizing agents, were recognized in the FTIR spectrum. The synthesized AgNPs were stable and smaller in size, as described in XRD and TEM analysis. The XRD pattern showed an fcc crystal structure of AgNPs. The synthesized silver nanoparticles were found to be highly stable, as shown in DLS analysis, crystalline, poly-dispersed, and mostly spherical in shape as determined using TEM analysis. The AgNPs showed excellent antimicrobial activity against the microbial strains studied and therefore can be considered as a promising opportunity for developing antimicrobial medications.

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

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Figures

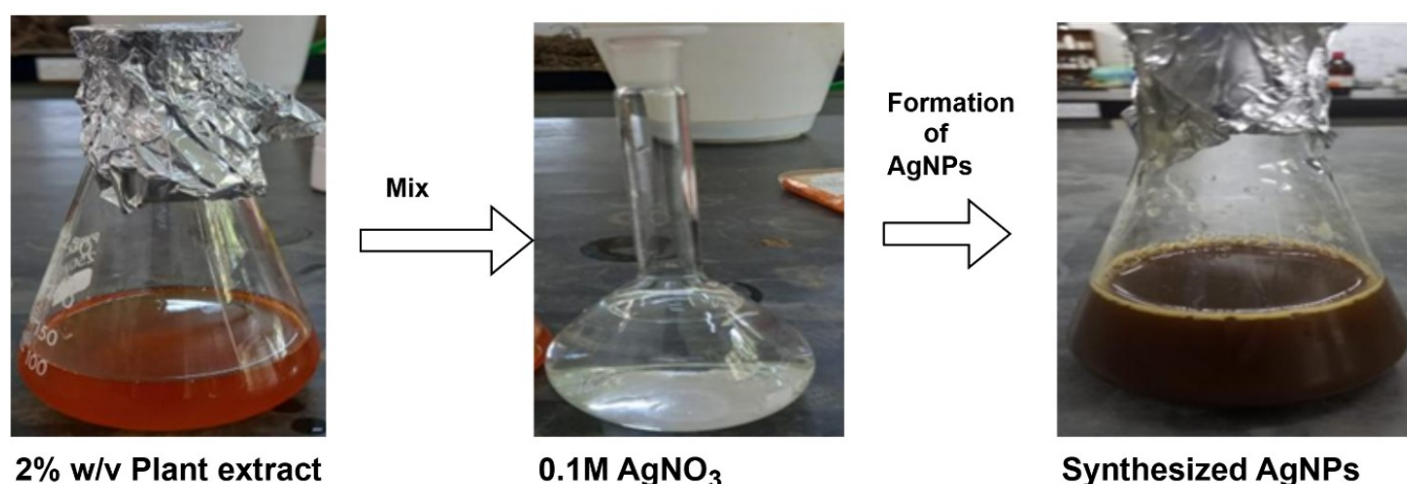


Figure 1

The graphical flowchart for the green synthesis of AgNPs using *Prunus africana* stem bark extract.

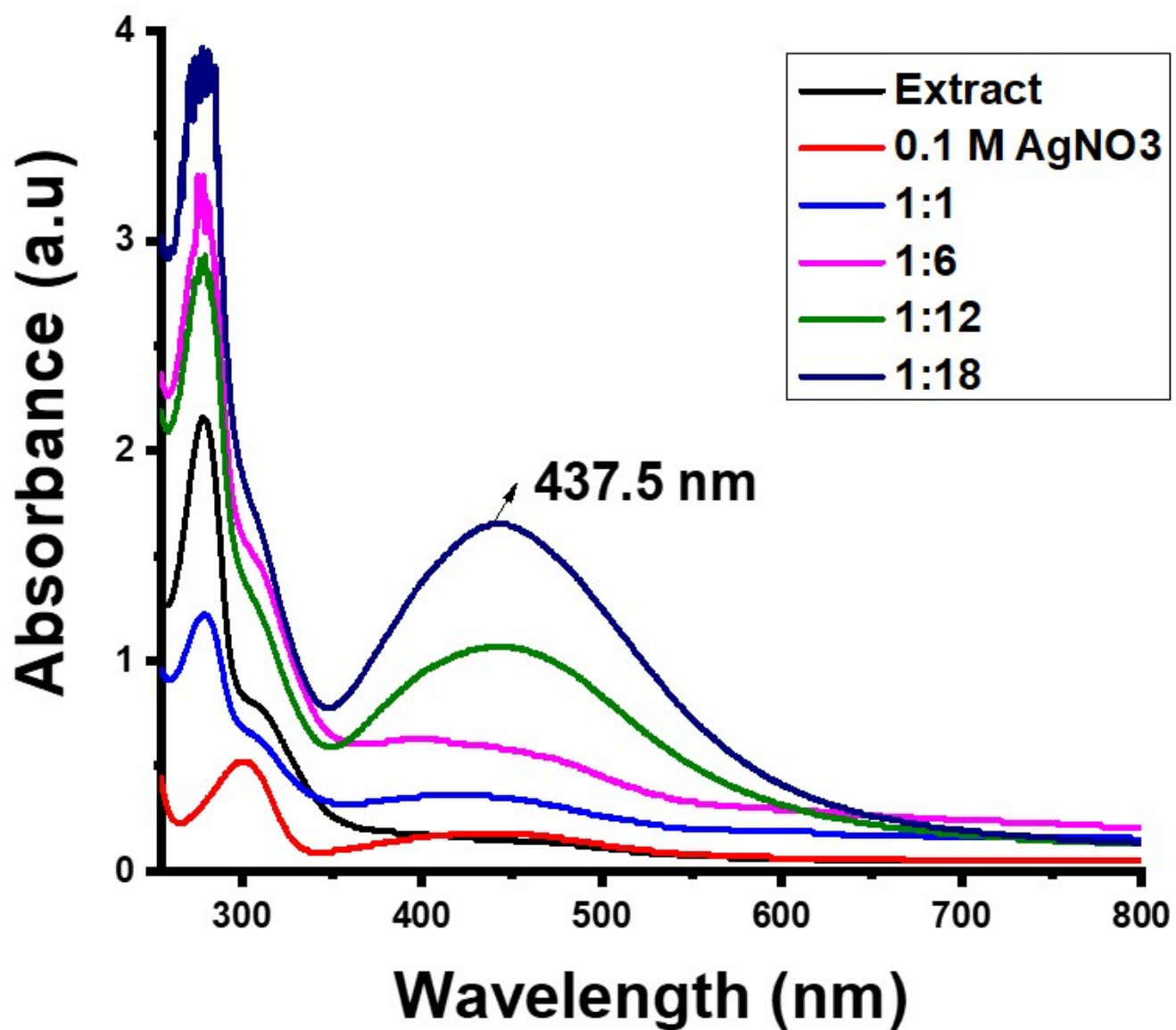


Figure 2

Effect of Extract concentration on Synthesis of AgNPs.

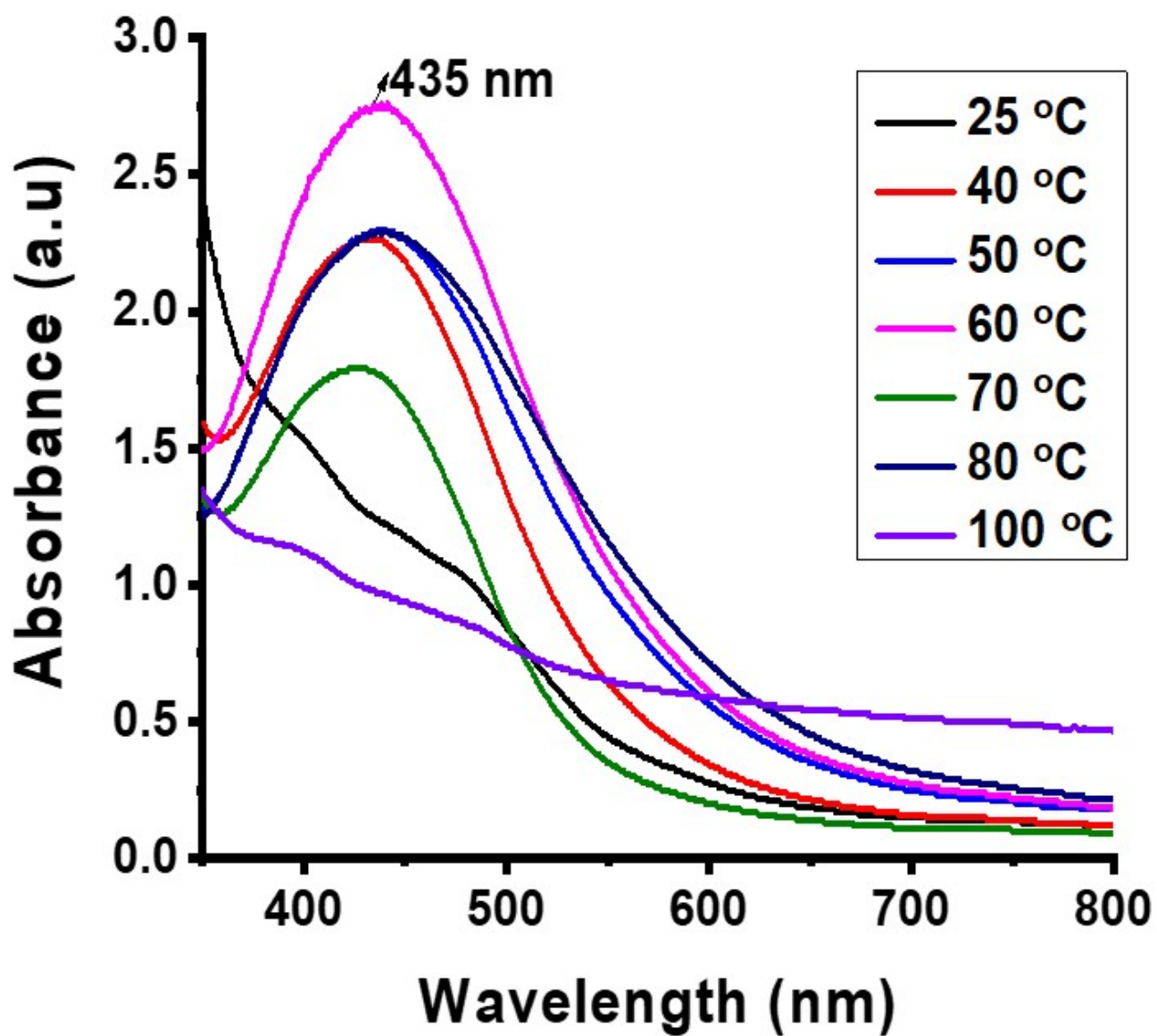


Figure 3

Effect of Temperature Variation on Synthesis of AgNPs

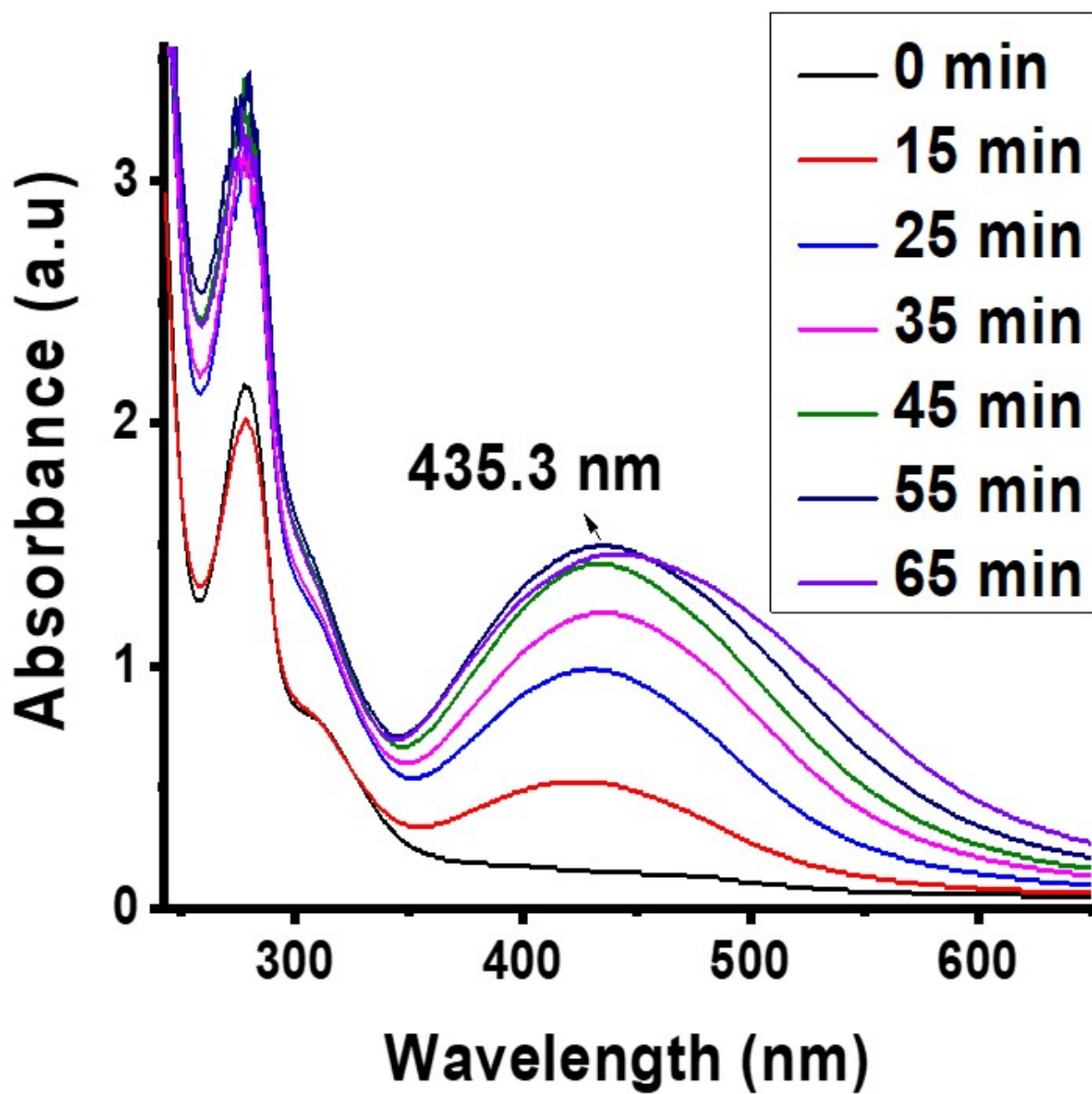


Figure 4

Effect of Time Variation on Synthesis of AgNPs.

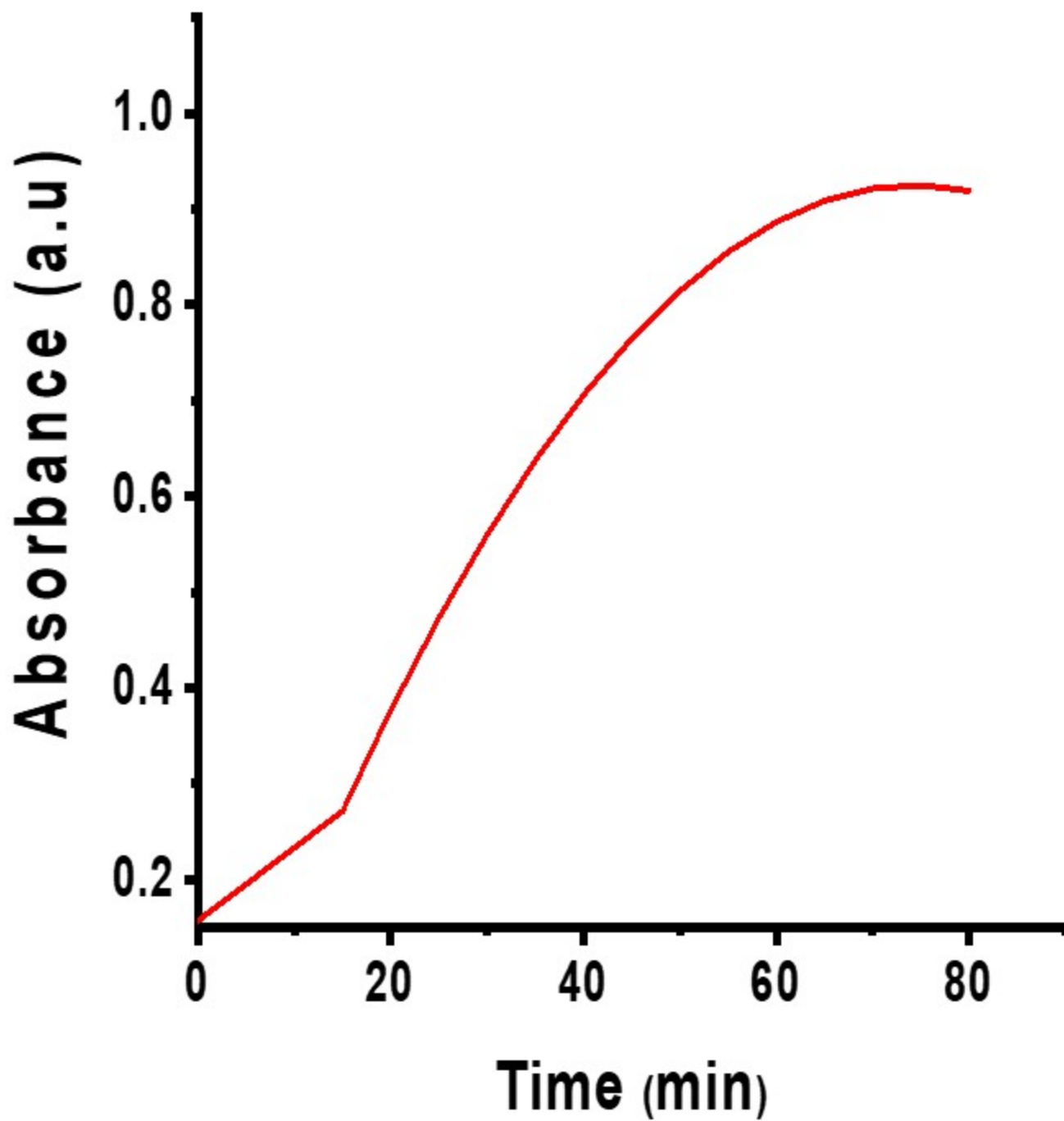


Figure 5

The curve of Absorbance versus Temperature.

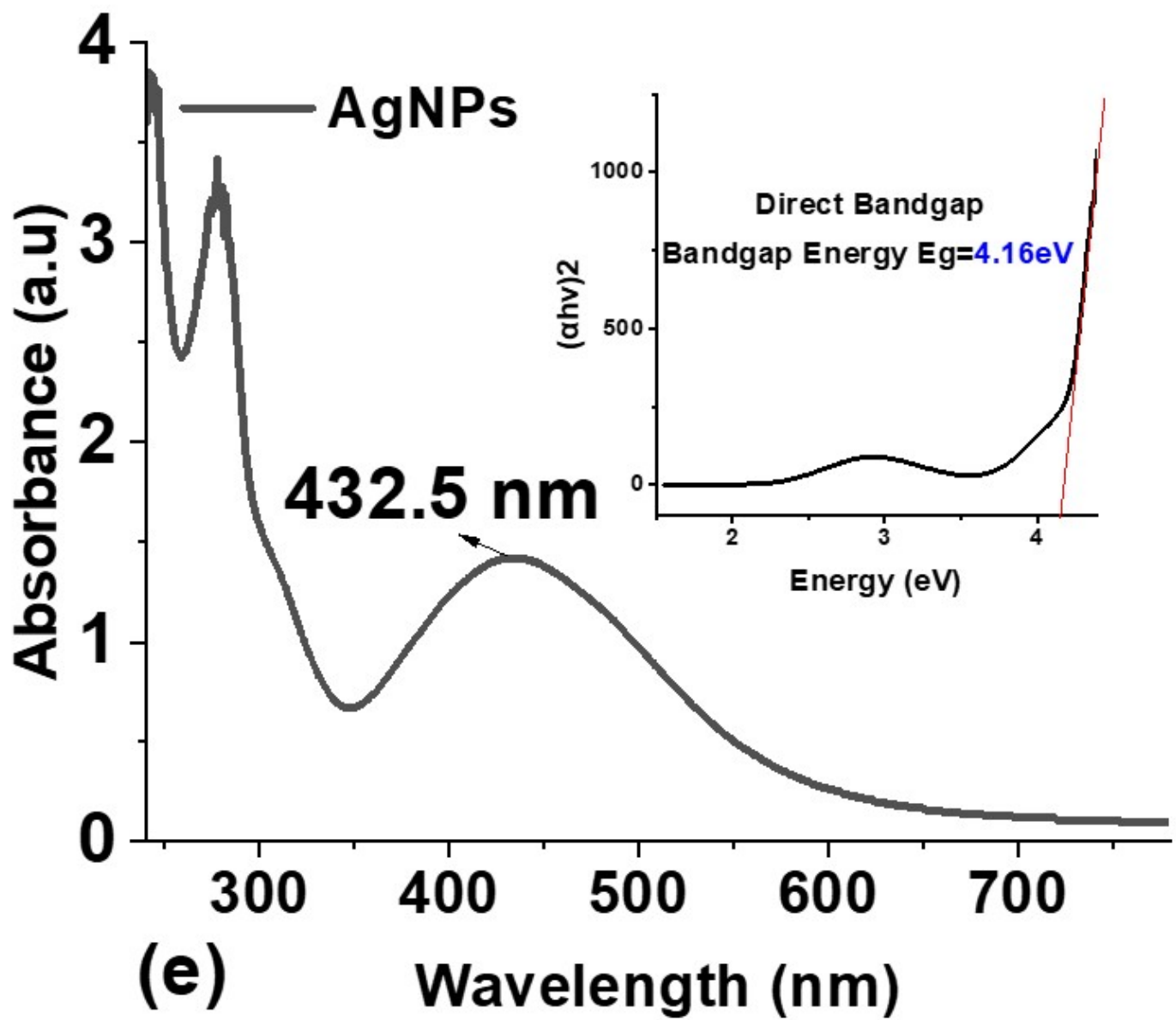


Figure 6

Optimized plot of AgNPs formation peak.

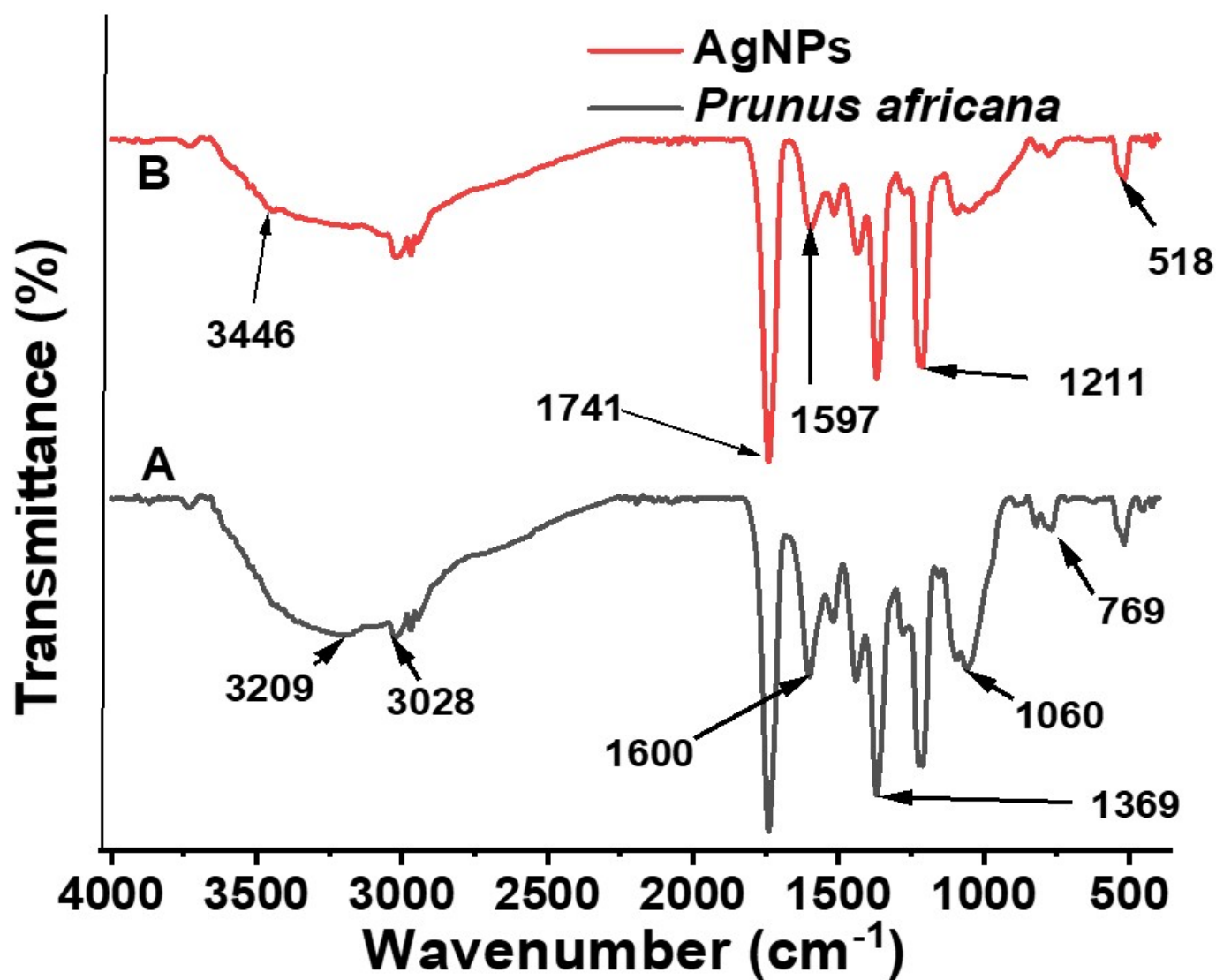


Figure 7

FT-IR spectra of (a) *Prunus africana* stem bark extract and (b) AgNPs.

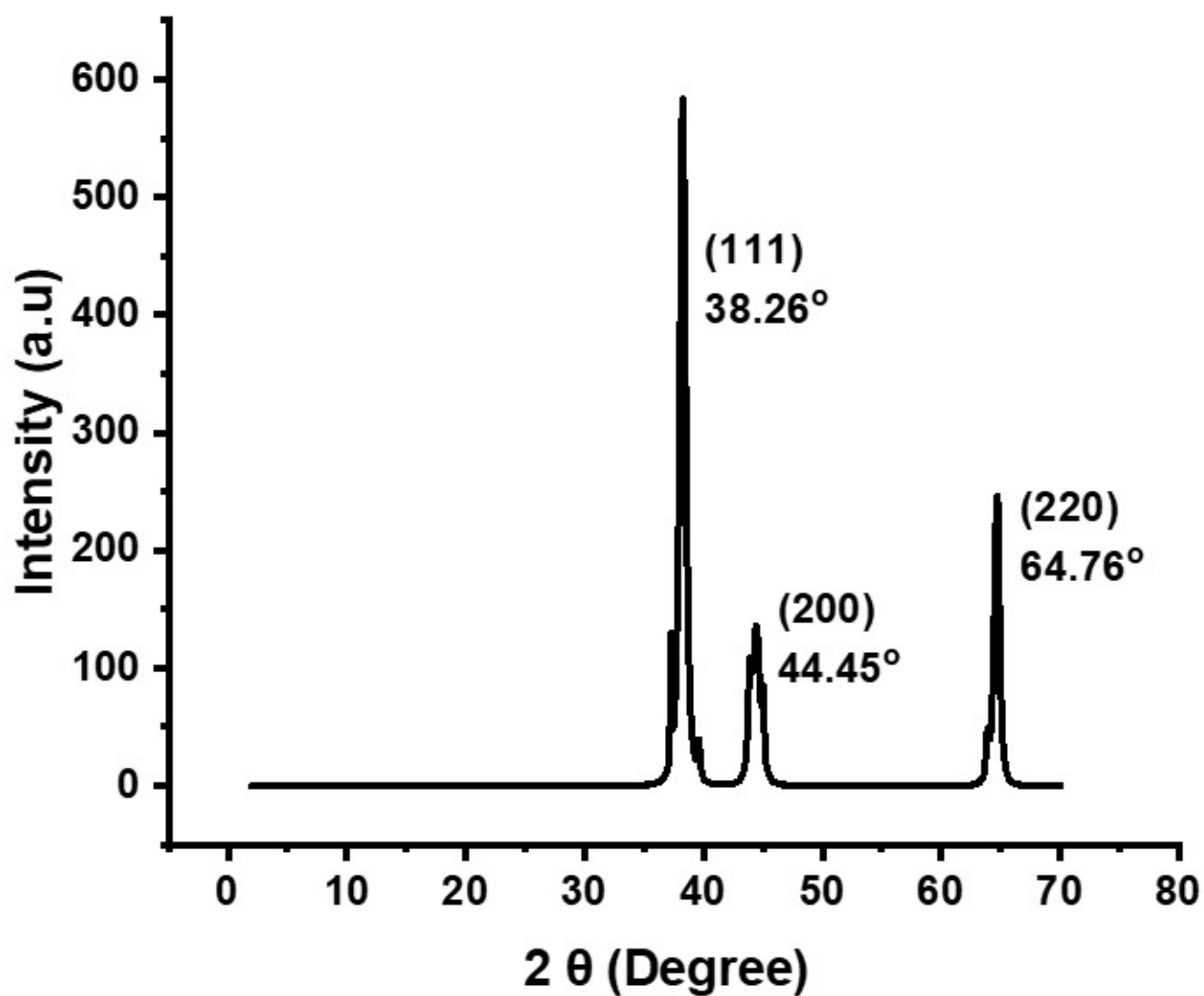


Figure 8

X-ray diffraction pattern of biosynthesized AgNPs.

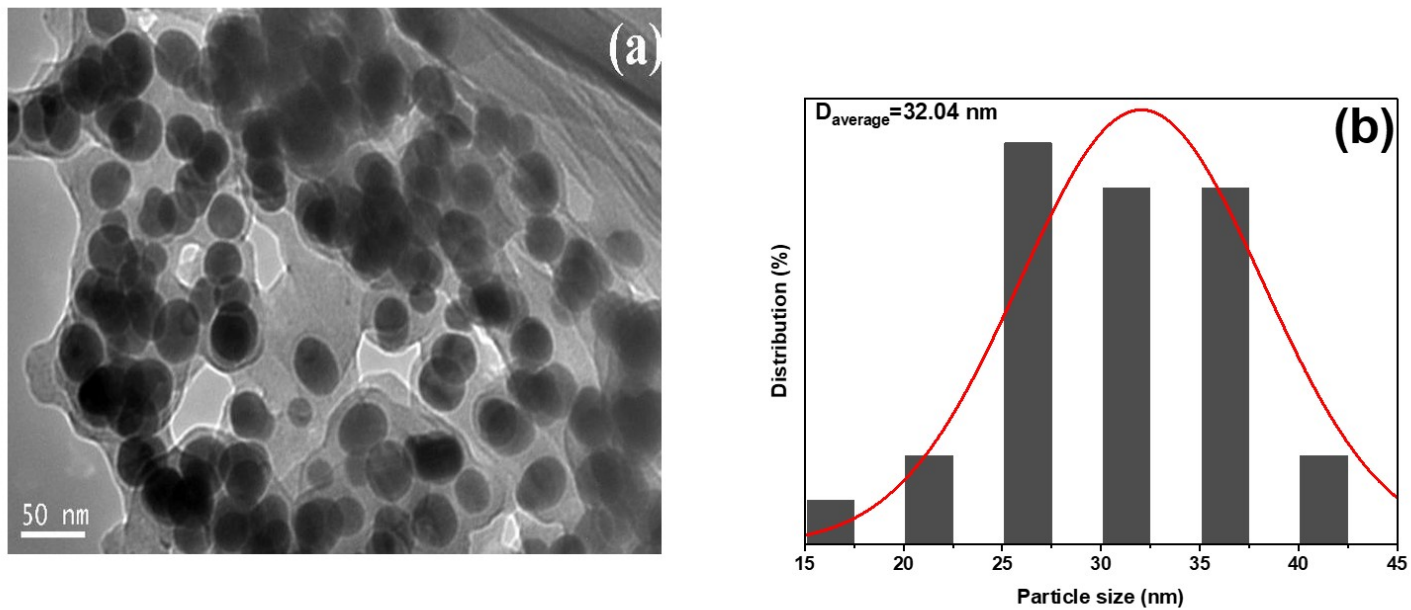


Figure 9

The TEM micrograph of the synthesized AgNPs includes (a) an analysis of the morphology of AgNPs and (b) a histogram that shows the size distribution of AgNPs.

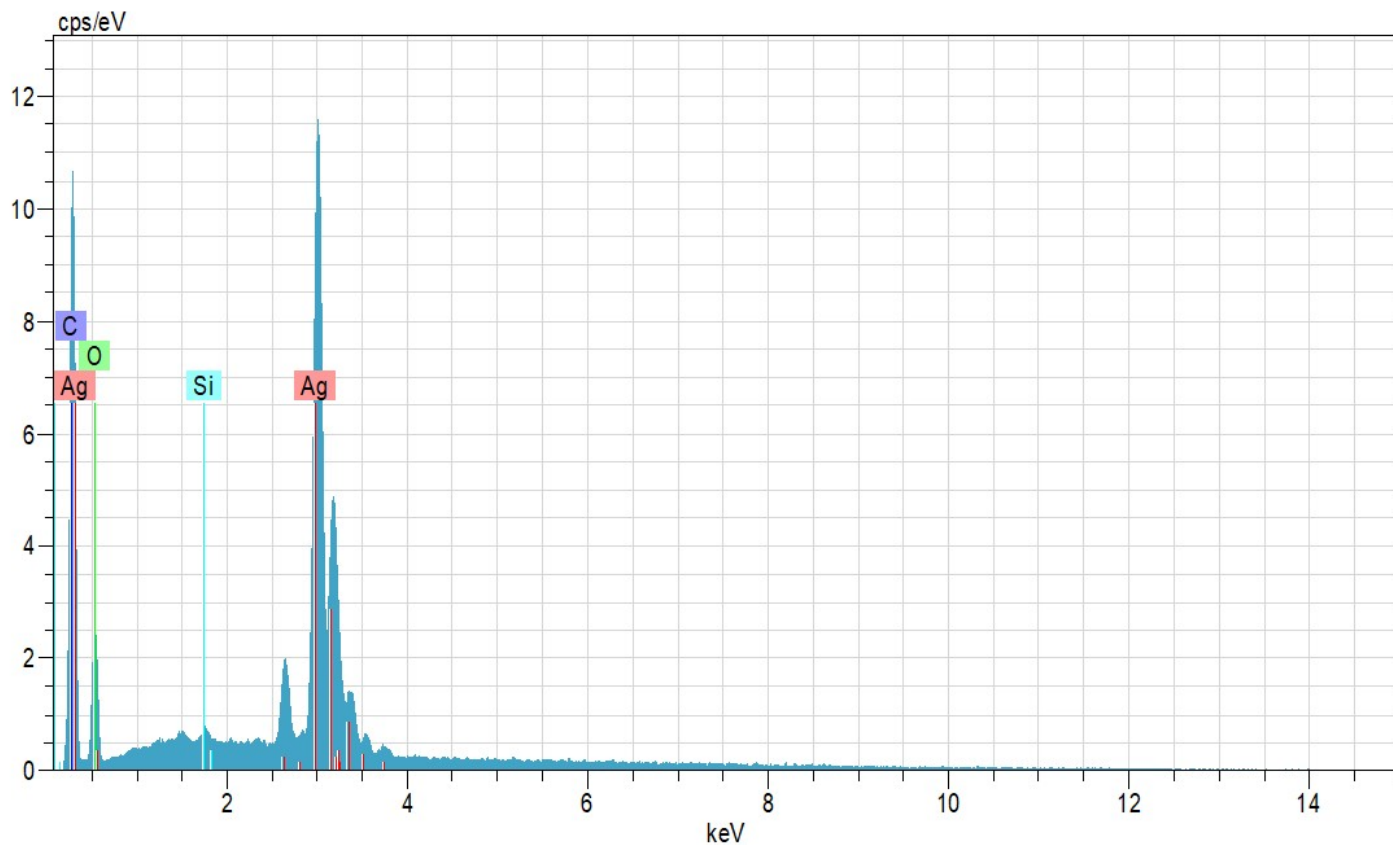


Figure 10

The EDS spectrum analysis showing major peaks of the synthesized AgNPs at 3 keV.

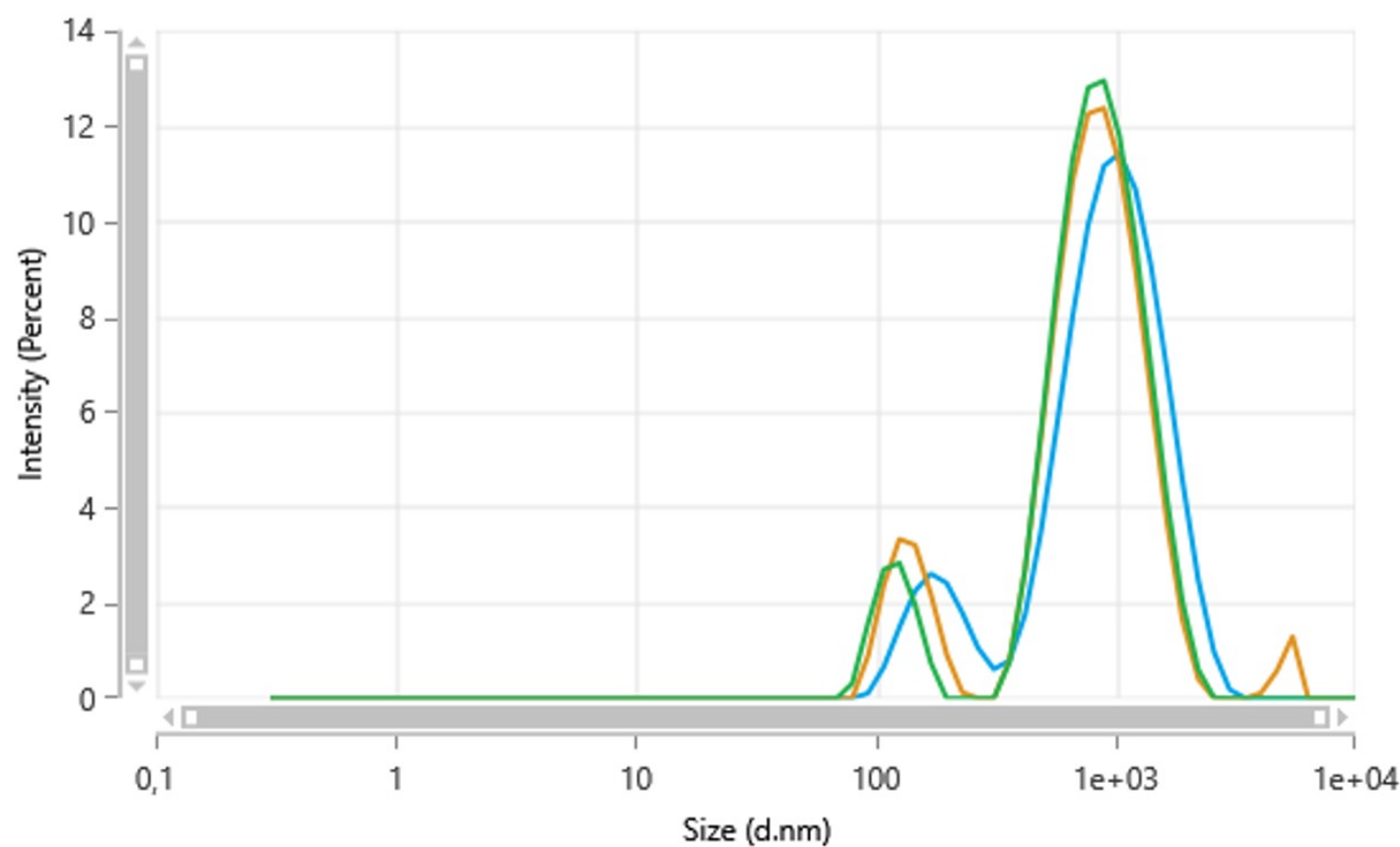


Figure 11

Particle size distribution of AgNPs

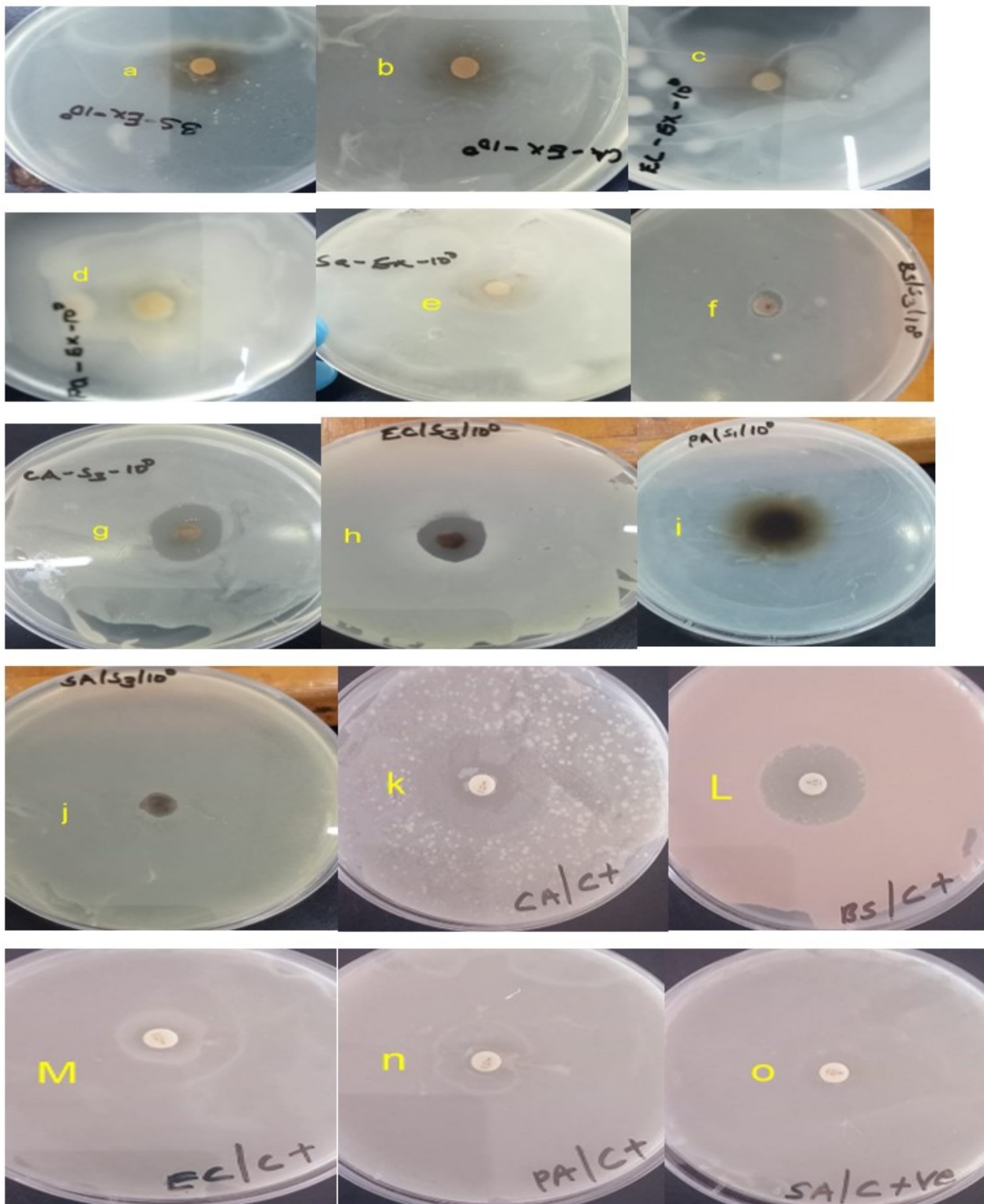


Figure 12

Zones of inhibition of (i) plant extract against: (a) *Bacillus subtilis*; (b) *Candida albicans* (Fungus); (c) *Escherichia coli*; (d) *Pseudomonas aeruginosa*; (e) *Staphylococcus aureus* and (ii) AgNPs against: (f) *Bacillus subtilis*; (g) *Candida albicans* (Fungus); (h) *Escherichia coli*; (i) *Pseudomonas aeruginosa*; (j) *Staphylococcus aureus*, and (iii) erythromycin against: (k) *Candida albicans* (Fungus), (l) *Bacillus subtilis*; (m) *Escherichia coli*; (n) *Pseudomonas aeruginosa*; and (o) *Staphylococcus aureus*

