Biosynthesis of silver nanoparticles using *Endod* (*Phytolacca dodecandra*) leaf extract and evaluation of their antibacterial activity

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Received: November 1, 2022 **Accepted:** January 14, 2023 **Published:** January 2023

ABSTRACT

Nowadays, the applications of metal nanoparticles are growing rapidly in different fields due to their unique properties such as size and shape. Among the different metal nanoparticles, silver nanoparticles (Ag NPs) have attracted considerable attention. In recent years, biosynthesis of Ag NPs has gained much interest from researchers. Ethiopian flora has a potential to provide cost-effective non-hazardous reducing and stabilizing compounds in preparing Ag NPs. In this study, leaf extracts of endod (*Phytolacca dodecandra*) were used as a reducing and stabilizing agent for the synthesis of Ag NPs because it is cost effective, eco-friendly and has medicinal property. During the synthesis of the nanoparticles, we used 8 % (w/v) of 25 mL *P. dodecandra* leaf extract and 5 mM of 50 mL silver nitrate solution. The synthesised Ag NPs were characterized by using UV–Vis absorption, FTIR and XRD spectroscopy. Antibacterial susceptibility of green-produced Ag nanoparticles was examined against Gram-positive and Gram-negative microorganisms. The UV-Visible spectrum of the colloidal solutions has a maximum absorbance observed at 424 nm. FTIR spectrum analysis revealed the presence of functional groups in the leaf extract which are responsible for the reduction of Ag^{+} to Ag^{o}. XRD spectrum showed the crystallinity and face-centered cubic (FCC) structure of the synthesized Ag nanoparticles. The particle size of silver nanoparticles calculated using the Debye-Scherrer formula was found to be equal to 22 nm. The antibacterial susceptibility test showed an inhibition zone of 22 mm against *S. aureus* and an inhibition zone of 21 mm against *S. typhi*.

Keywords: Ag NPs; Antibacterial activities; Green synthesis; P. *dodecandra* leaf extract; UV-Vis
DOI: https://dx.doi.org/10.4314/ejst.v16i1.5

INTRODUCTION

Nanotechnology is one of the most contemporary and flourishing fields of science which allows manipulation of materials at the nanometer (nm) scale either by scaling up from single groups of atoms or by refining or reducing bulk materials (Jemal *et al.*, 2017; Pluta et *al.*, 2017). Nanoparticles within the size range of 1 and 100 nm can undergo fundamental changes in their chemical, physical and biological properties (Ahmed *et al.*,

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2016a*;* Jemal *et al.,* 2017). Nowadays, the applications of metal nanoparticles are growing rapidly in different fields due to their completely new or enhanced properties such as shape, size and morphology (Banerjee *et al.,* 2014*;* Jain *et al.,* 2021). Among the different metal nanoparticles, silver nanoparticles (Ag NPs) have attracted considerable attention due to their large number of applications and their nontoxicity towards humans (Ali et *al.,* 2016). Ag NPs are applicable in different areas, such as in drug delivery (Jain *et al.,* 2021; Pahal *et al.,* 2022), dye degradation and clothing (Aritonang *et al.,* 2019), anticancer and antimicrobial activity (Hemlata *et al.,* 2020), biomedicine (Pahal *et al.,* 2022), selective coatings for solar energy absorption (Ali *et al.,* 2016; Jemal *et al.,* 2017) and biosensors (Jemal *et al.,*2017). Research findings carried out on Ag NPs reveal that they possess high antibacterial activity due to their large surface area-to-volume ratio. Such a property of Ag NPs is of great interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Heydari *et al.,* 2014*;* Ahmed *et al.,* 2016b*;* Logeswari *et al.,* 2015; Praba *et al.,*2015). Many researchers have developed a keen interest in the synthesis of silver nanoparticles due to their enhanced antimicrobial activity, and also their use as solutions to many technological and environmental challenges in different fields (Jain and Mehata, 2017).

Ag nanoparticles can be synthesized using various approaches including chemical, physical, and biological methods. Chemical methods of nanoparticles synthesis and stabilization require toxic chemicals and lead to non-eco-friendly byproducts (Aritonang *et al.,* 2019). The need for environmental nontoxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for "green nanotechnology." Many green synthesis approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi, and plants (Yasir *et al.,* 2018; Masum *et al.,* 2019). Plant - mediated synthesis of Ag NPs would have been appropriate because it is cheaper and environmentally friendly (Basavegowda *et al.,*2015; Kumar *et al.,*2017; Pluta *et al.,* 2017; Aritonang *et al.,* 2019). It may be concluded from a detailed evaluation of recent studies on the biosynthesis of nanoparticles using green chemistry that the presence of reducing agents in plant leaves makes them useful for synthesizing silver nanoparticles (Verma *et al.,* 2016; Masum *et al.,* 2019*;* Vanlalveni *et al.,* 2021).Various researchers have reported that plant extracts can act as a reducing, capping, and stabilizing agent for the synthesis of nanoparticles (Banerjee *et al.,* 2014*;* Aritonang *et al.,* 2019; Jain *et al.,* 2021*;* Pahal *et al.,* 2022). Figure 1 presents the mechanism for the synthesis of Ag NPs from plant extracts.

Based on the previous literature reports, Ag nanoparticles have been synthesized from various plant extracts such as extract of *Parkia speciosa* Hassk pods (Fatimah *et al.,*

2016), phlomis leaf (Allafchian *et al.*, 2016), *Averrhoa bilimbi* fruit (Isaac *et al.*,2013), *Azadirachta indica* leaf (Roy *et al.*, 2017), lemon peel (Jahan *et al.*, 2021), *Pechuel-loeschea leubnitziae* (Mofoloa *et al.*, 2020), *Vitis vinifera* (Gnanajobitha *et al.*,2013), *Clitoria ternatea* and *Solanum nigrum* (Krithiga *et al.*, 2015), leaves of *Hibiscus Rosa sinensis* (gurhal) (Tyagi *et al.*, 2021), *Azadirachta indica* (Ahmed *et al.*, 2016b), *Lycopersicon Esculentum* (Maiti *et al.*, 2014), etc.

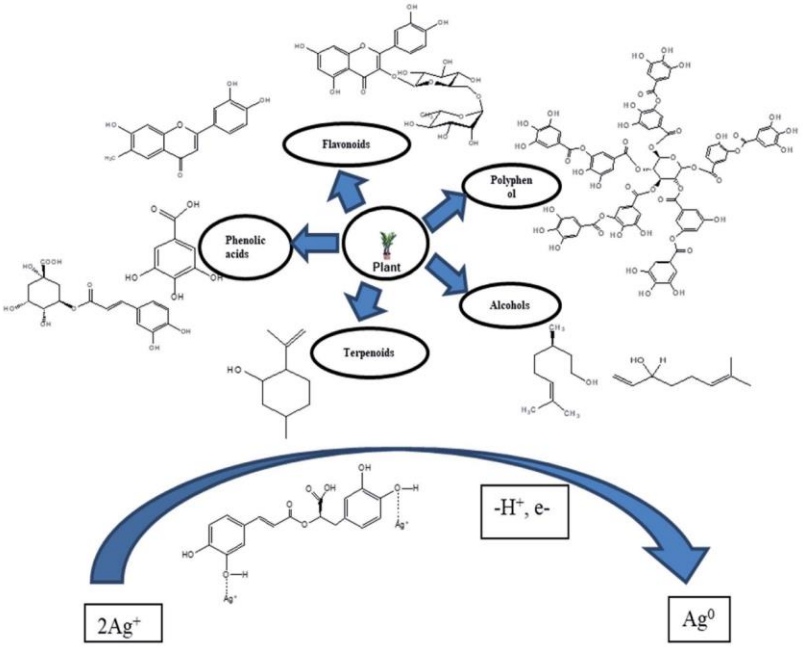

Figure 1. Mechanisms for the synthesis of Ag NPs from plant sources (Tarannum *et al.*, 2019).

Hence, in this study, we used *P. dodecandra* leaf extract as a reducing and stabilizing agent for the synthesis of silver nanoparticles. As far as our knowledge is concerned this is the first work to use *P. dodecandra* leaf extract for the synthesis of silver nanoparticles. The move towards the synthesis of silver nanoparticles using *P. dodecandra* leaf extract appears to be cost effective and eco-friendly compared to that of conventional methods of silver nanoparticles synthesis.

P. dodecandra is a plant having a long stem or climber (Figure 2) native to tropical Africa, Southern Africa, and Madagascar. *P. dodecandra* is used as a soap and shampoo as well as plant pesticides because of its high toxicity to snails, and low toxicity to

mammals (Adams *et al.,* 1989; Zeleke *et al.,* 2017*;*). The plant produces fruit (berries) twice a year, in January and July, when the climate is favorable (Adams *et al.,* 1989). *P. dodecandra* berry extract has been used for centuries in Ethiopia as soap to wash cotton clothing (Adams *et al.,* 1989). Leaves of *P. dodecandra* can also be used as soap to wash cotton clothing, but are less effective compared to berries.

Figure 2. Image of *P. dodecandra*

On the other hand, the plant is used for various medicinal purposes because of its larvicidal effect. It can be used against the larvae of mosquitoes and other insects such as the housefly (Adams *et al.,* 1989*;* Esser *et al.,* 2003). *P. dodecandra* can also produce a series of triterpenoid saponins that possess very potent and useful biological properties, including antifungal, anti-protozoan, spermicidal, and insecticidal properties (Tegegn *et al.,* 2019).

MATERIALS AND METHODS

Chemicals such as silver nitrate ($AgNO_3$), nutrient broth, Mueller Hinton agar, iodine, potassium iodide (KI), sodium hydroxide (NaOH), dilute ammonia solution, (NH_3(aq)), glacial acetic acid (CH_3COOH), ferric chloride ($FeCl_3$), lead acetate $Pb(C_2H_3O_2)_2$ and chloroform ($CHCl_3$) (wag tech International Ltd., UK), sulfuric acid (H_2SO_4), nitric acid (NHO_3) and hydrochloric acid (HCl) (Lam mark chemicals Pvt., India) were used in the experiment. All the chemicals used were analytical grade. Freshly prepared double-distilled water was used throughout the experiment. *Salmonella typhi (S. typhi)* (gram-

negative) and *Staphylo coccus aureus (S. aureus)* (gram-positive) were purchased from IMTECH, Chandigarh, India.

Preparation of *P. dodecandra* leaves extract

Fresh *P. dodecandra* leaves were collected from nearby rural communities around diaspora village, Bahir Dar city, Ethiopia. The leaves were cleaned twice, once with tap water followed by distilled water, to get rid of dust and other contaminants. A knife was used to cut the leaves into little pieces. Eight grams of finely chopped leaves was combined with 50 ml of distilled water in a 100 ml beaker to create the aqueous leaf extract. The mixture was then heated at 60 $^{\circ}$C for 20 minutes. After the extract has cooled down to room temperature, it was filtered with Whatman No. 1 filter paper to get rid of any last-remaining impurities and produce a clear solution. The filtrate was collected and stored at 4 °C in the refrigerator for the next use.

Photochemical test of *P. dodecandra* leaves extract

Preliminary phytochemical tests were carried out to check the presence of proteins, tannins, flavonoids, terpenoids, saponins, quinones, and phenols in *P. dodecandra* leaf extract (Karunamoorthi *et al.*, 2008*;* Rao *et al.,* 2016*;* Sankhalkar *et al.,* 2016; Gul *et al.*, 2017).

Test for proteins
About 2 mL of 8 % (w/v) *P. dodecandra* leaf extract and a drop of concentrated nitric acid were mixed. The formation of a yellow color shows the presence of proteins.

Test for tannins
About 2 mL of 5% ferric chloride was added to 1 mL 8 % (w/) of *P. dodecandra* leaf extract. The formation of a white colored layer confirms the presence of tannins.

Test for flavonoids
About 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of *P. dodecandra* leaf extract followed by addition of concentrated sulfuric acid. The appearance of yellow precipitate coloration indicates the presence of flavonoids.

Test for terpenoids
About 2 mL of chloroform and concentrated Sulfuric acid was added to 0.5 mL 8% (w/) of *P. dodecandra* leaf extract. A dark brown color formation at the interface indicates the presence of terpenoids.

Test for saponin

About 2 mL of distilled water was added to 2 mL of 8% (w/) *P. dodecandra* extract and shaken for 15 minutes. The formation of a layer of foam confirms the presence of saponins.

Test for quinones

About 1mL of concentrated sulphuric acid was added to 1 mL 8% (w/) of *P. dodecandra* leaf extract. A formation of a deep red color indicates the presence of quinones.

Test for phenols

About 2 mL of distilled water was added to 1 mL 8% (w/) of *P. dodecandra* leaf extract followed by a few drops of 10% ferric chloride. A formation of a dark green color indicates the presence of phenols.

Synthesis of silver nanoparticles (Ag NPs)

About 50 mL of aqueous 5 mM $AgNO_3$ was taken in a beaker and diluted in 100 mL of distilled water for the synthesis of Ag nanoparticles. To this solution 25 mL of 8% (w/v) *P. dodecandra* leaf extract was added. The solution was kept at room temperature until the color of the solution changed to red, confirming the formation of Ag NPs (Figure 2).

Characterization of silver nanoparticles

Ag Nps synthesized by reducing silver ions in the presence of leaf extract of *P. dodecandra* were characterized using UV-Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA), FTIR machine (Perkin Elmer, USA), and X-ray diffraction (PAN analytical X'Pert PRO MPD, USA) techniques. The UV- Visible spectroscopy is used to measure the absorbance of the synthesized Ag NPS. Identification of the functional groups acting as reducing and stabilizing agents in the plant extract was done using FTIR spectroscopy. The crystalline nature of Ag nanoparticles was investigated by subjecting the samples to X-ray diffraction operating at 40 KV and a current of 30 mA with Cu Kα radiation. The Debye–Scherrer equation was employed to calculate the average particle size of the Ag NPs.

Antibacterial activity of Ag NPs

Antibacterial activity of the synthesized Ag nanoparticles was investigated using the disc-diffusion method against human pathogens such as *S. typhi* (Gram- Negative bacteria) and *S. aureus* (gram-positive bacteria). Preparation of the bacteria stock was done to reproduce and rejuvenate bacteria. This was done by inoculating each inoculation loop pure culture of *S. typhi* and *S. aureus* into nutrient broth solution and

then incubated at 37 °C for 24 hours in the incubator. All the equipment and growing media were sterilized by autoclaving at a temperature of 115 °C for 30 minutes. An overnight culture of inoculum was spread over the Mueller Hinton Agar (MHA) plates by a non-toxic cotton swab on an applicator stick which was dipped into the standardized suspension of bacteria. Subsequently, the filter paper disc of 6 mm in diameter was soaked in a 30 µl of Ag nanoparticle colloidal solution and in a 30 µl solution of a positive control drug chloramphenicol. These discs were placed over the Mueller Hinton Agar (MHA) plates using sterile forceps. Each disc was gently pressed down with sterile forceps to confirm complete contact with the Mueller Hinton Agar (MHA) plate's surface. Next, it was then incubated at 37 °C for 24 hours. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a digital electronic caliper and expressed in millimeters.

RESULTS AND DISCUSSION

Phytochemical analysis of *P. dodecandra* leaf extract

Bioactive substances known as phytochemicals are produced by various plant parts. They are produced naturally in the leaves, stems, roots, flowers, fruits, seeds, and other parts of the plant (*Karunamoorthi et al.*, 2008*;* Rao *et al.*, 2016*;* Gul *et al.*, 2017). In this section we carried out qualitative phytochemical analysis of P. *dodecandra* leaf extract that are responsible for silver ion reduction and stabilization for the formation of Ag NPs. We used standard methods to check the phytochemical constituents in *P. dodecandra* leaf extract based on color changes and precipitate formation (Rao *et al.*, 2016*;* Sankhalkar *et al.*, 2016; Gul *et al.*, 2017). The qualitative phytochemical analysis result of *P. dodecandra* leaf extract is shown in Figure 3. From the result, it is possible to conclude that *P. dodecandra* leaf extract was found to contain phytochemicals listed on Table 1.

Table 1. Qualitative analysis of phytochemicals in *P. dodecandra* leaf extract.

No.	Phytochemicals	Results	Colors observed
1.	Terpenoids	+	Red color at the interface
2.	Flavonoids	+	Yellow precipitate
3.	Tannin	+	White color
4.	Phenols	+	Dark Green color
5.	Saponin	+	Layer foam
6.	Quinones	+	Dark black color
7.	Proteins	+	Yellow color

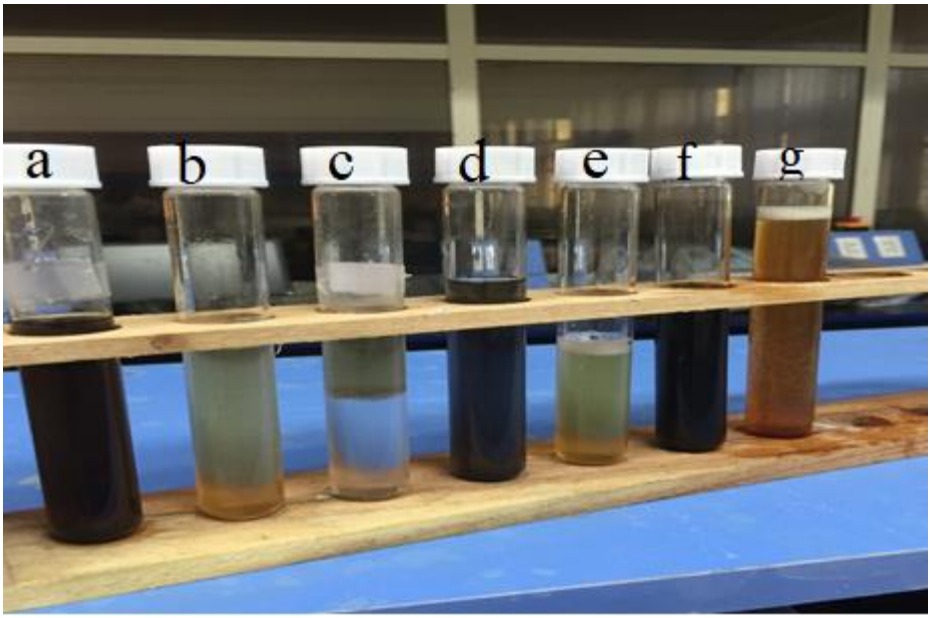

Figure 3. Phytochemical test of *P. dodecandra* leaves extract for a) Terpenoids, b) Flavonoids, c) Tannins, d) Phenol, e) Saponin, f) Quinones, g) proteins.

UV–Vis spectral analysis

Confirmation of Ag NPs formation in colloidal solution was easily observed due to a change in the color of the colloidal solution after the addition of $AgNO_3$ to the fresh leaf extract of *P. dodecandra*. As the reaction time progressed, the colorless $AgNO_3$ solution turned red due to mixing with yellow color *P. dodecandra* leaf extract (Figure 4). The color change of this solution confirms the formation of Ag NPs, which is in agreement with the previous work (Heydari *et al.,* 2014; Ahmed *et al.,* 2016). Such characteristic color change is possible because of the collective oscillation of electrons at the surface of nanoparticles, which results in Surface Plasmon Resonance (SPR) (Ali *et al.,* 2016; Jain *et al.*, 2021).

The SPR spectra of the synthesized Ag NPs showed a distinct maximum absorbance at 424 nm (Figure 5), indicating that silver ions were bio-reduced into silver nanoparticles. The maximum absorbance of UV-Vis spectra is in agreement with previous findings (Krithiga *et al.,* 2015; Ahmed *et al.,* 2016b*;* Aritonang *et al.,* 2019; Jain *et al.,* 2021).

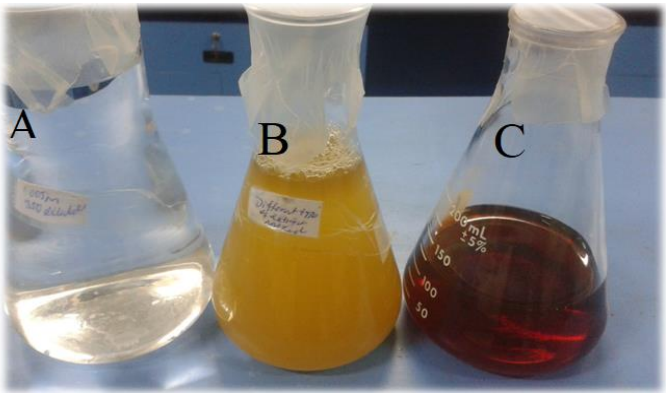

Figure 4. Color changes observed before and after the formation of Ag NPs: (a) silver nitrate solution, (b) leaf extract of *P. dodecandra*, and (c) Ag NPs.

The reduction of silver ions to atoms by *P. dodecandra* leaf extract could account for the result of UV-Vis spectra. The atoms produced by the reduction processes then form tiny clusters, which eventually agglomerate into nanoparticles. During the reduction process, the size and shape of nanoparticles are controlled by the availability of atoms. The properties of the nanoparticles can be controlled by optimizing the different parameters such as Ag NO_3 concentration, amount of *P. dodecandra* leaf extract, pH and time of the reaction.

Effect of silver nitrate concentration

Figure 6 shows the UV-visible spectra measured at various silver nitrate concentrations with a constant amount of *P. dodecandra* leaf extract. The concentrations of silver nitrate solution used for the measurement were 4, 5, 6, 7, and 8 mM in 50 mL. As the concentration of silver nitrate solution increased from 4 to 5 mm, the intensity of the spectra was increased. Further increasing the concentration of silver nitrate solution from 5 mM to 8 mM gives a broader spectrum and the intensity of the spectra decreases. The main reason is that the concentration of *P. dodecandra* leaf extract employed as a limiting reagent in the reaction decreases as the reaction advances. Because of this, the amount of Ag NPs produced during the reaction decreases. As a result, the UV-Vis spectrum intensity decreases and becomes broader. The UV-Vis spectrum of Ag NPs obtained using 5 mM concentrations of $AgNO_3$ solution was strong and intense. Thus, 5

mM concentration of $AgNO_3$ solution was chosen for further work.

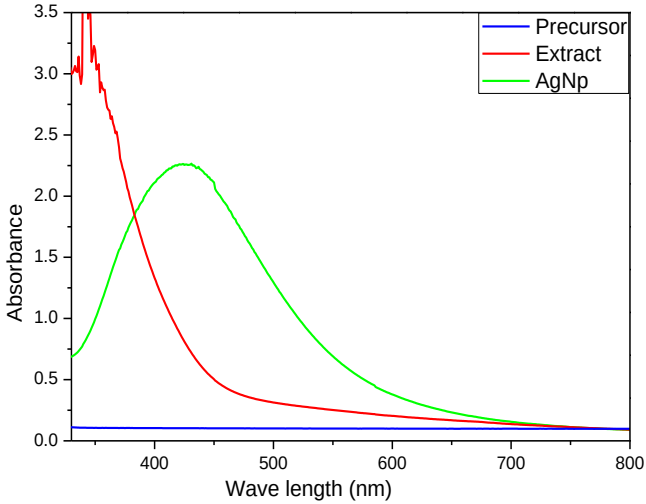

Figure 5. UV-Vis spectra of silver nitrate solution (red), *P. dodecandra* leaf extract (blue) and Silver Nanoparticles (Ag NPs) (green).

Effect of *P. dodecandra* extract amount

The reaction of different concentrations of *P. dodecandra* leaf extract with 50 mL of 5 mM silver nitrate solution was used to study the effect of *P. dodecandra* leaf extract concentration on the synthesis of silver nanoparticles. During the study, 25 mL of 5, 6, 7, 8, 9, and 10% (w/v) of *P. dodecandra* leaf were dissolved in 50 mL of 5 mM silver nitrate solution. Figure 7 shows UV-Visible spectra of silver nanoparticles synthesized using varied concentrations of leaf extract and a constant concentration of silver nitrate solution. The intensity of the spectra was low, and it was also quite broad at lower amounts of *P. dodecandra* leaf extract, showing the formation of a smaller number of Ag NPs in the solution.

The intensity of the absorption band increases with increasing the amount of *P. dodecandra* leaf extract to 8% (w/v) because of increases in the production of more Ag NPs. Because of the limited amount of silver ions and the abundant functional groups of *P. dodecandra* leaf, further increasing the extract concentration resulted in lower absorption peaks. As a result, the best value for this investigation was 25 mL of 8% (w/v) *P. dodecandra* leaf extract.

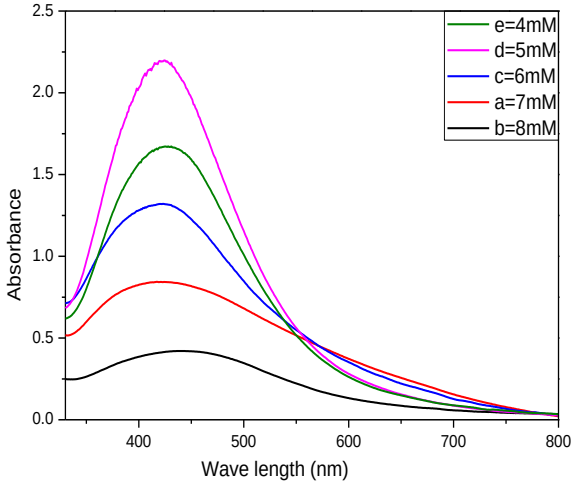

Figure 6. UV-Vis spectra of Ag NPs showing the effect of variation of $AgNO_3$ concentration.

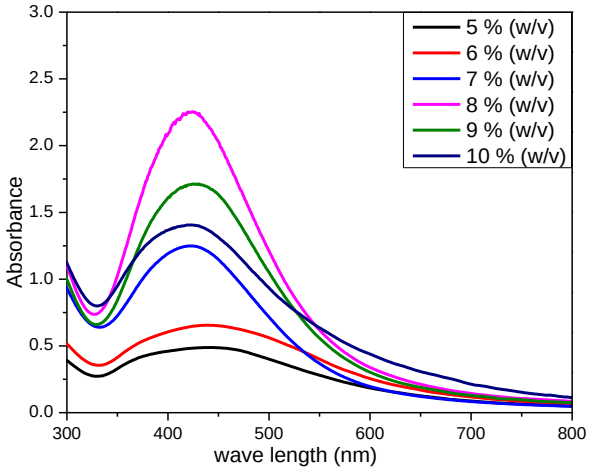

Figure **7.** UV-Vis spectra of Ag NPs showing the effect of variation of P**.** ***dodecandra*** extract amount.

Effect of pH

By changing the solution pH value using HCl and NaOH, the influence of pH on the synthesis of Ag NPs was investigated at 4, 6, 8, 10, and 11 pH values. The UV-visible spectra of silver nanoparticles produced at various pH levels are shown in Figure 8. UV-Vis absorption spectra increase its intensity as the pH increases from pH 4 to pH 10 due to reduction in size of particles from large size to small size. This indicates that the Ag NPs size become smaller with increase in the pH value. At higher pH, a large number of Ag nanoparticles formed with smaller diameters (Ndikau *et al.;* 2017). But as shown in the Figure 8, the particles become unstable and agglomerated at very high pH, such as pH 11 (Logeswari *et al.*, 2015; Verma *et al.*, 2016). This result confirmed the vital role played by pH in controlling the shape and size of the Ag NPs. The optimum pH chosen for this work was pH 10.

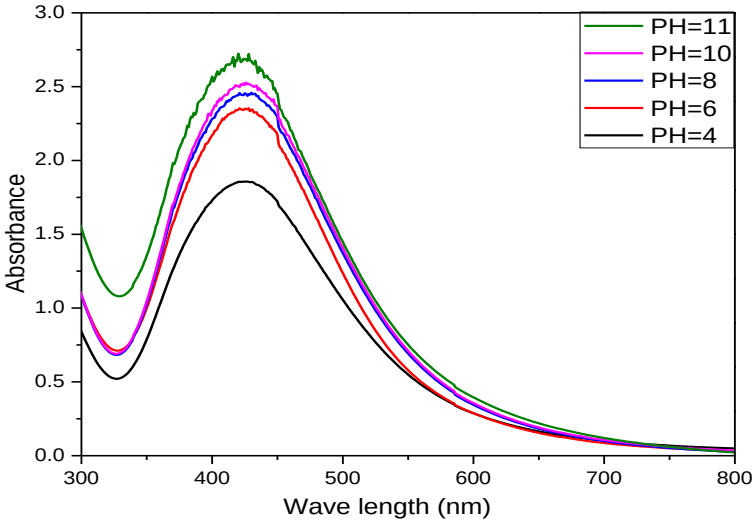

Figure 8. UV-Vis spectra of Ag NPs showing the effect of variation of pH on Ag NPs synthesis.

Effect of reaction time

Figure 9 shows the UV-Visible spectra obtained at various time intervals. UV-Visible spectroscopic measurements were conducted at different time intervals, ranging from 4 hrs to 72 hrs as shown in the figure below. The intensity of the absorption band increases with increasing reaction time. The maximum intensity of the spectrum was

recorded at a reaction time of 48 hours. After the optimum reaction time, an agglomeration of silver nanoparticles occurred due to the instability of the silver nanoparticles (Krithiga *et al*.; 2015). This agglomeration results in larger particle sizes and a smaller number of Ag NPs. Thus, a decrease in peak intensity was observed when the reaction progressed beyond 48 hours as shown in figure 8. As a result, 48 hrs was used as the optimum time required for the completion of the reaction.

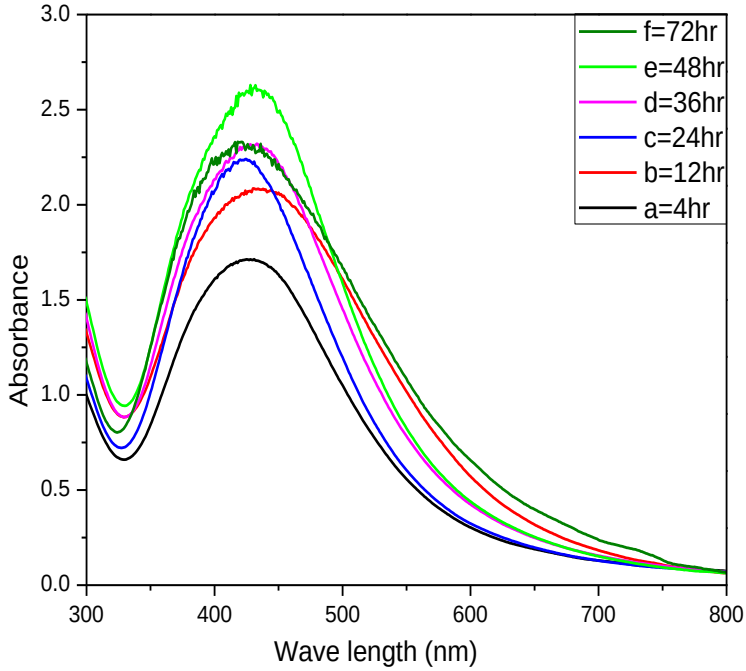

Figure 9. UV-Vis spectra of Ag NPs showing the effect of variation of time on Ag NPs synthesis.

Fourier transformation infrared (FTIR) spectroscopy

The biomolecules in the *P. dodecandra* leaf extract that are responsible for reducing, capping, and stabilizing the biosynthesized silver nanoparticles were identified using FT-IR spectroscopic measurements. The FTIR spectra of *P. dodecandra* leaf extracts and manufactured Ag NPs are shown in Figure 10 (A and B), respectively. *P.*

dodecandra leaf extract's FTIR spectrum showed major peaks at 693, 1081, 1643, and 3427 cm^{-1}. The occurrence of a major broad spectrum in *P. dodecandra* leaf extract centered at 3427 cm^{-1} can be attributed to hydrogen bounded O-H stretching vibrations of phenol, alcohol, carboxylic groups, and other compounds (Krithiga *et al.,* 2015*;* Hassan *et al.,* 2020; Aminuzzaman *et al.,* 2018). The synthesized Ag NPs showed a strong absorption spectrum at 693, 1081, 1643, and 3427 cm^{-1}. The intensity of Ag NPs spectra was reduced when compared to the spectra of *P. dodecandra* leaf extract (Narendhran *et al.,* 2016). This is because phytochemicals such as alcohols, flavones, and carboxylic groups are consumed during the reaction for the synthesis of Ag nanoparticles (Banerjee *et al.,* 2014). The strong absorption spectrum of plant extracts observed at 1643 cm^{-1} could be attributed to C= O and N – H groups of proteins and enzymes present in the plant (Akintelu *et al.,* 2020). The intensity of this spectrum is also reduced for the synthesized Ag NPs, indicating the participation of functional groups in the reduction process. The presence of N–H and O–H bands in the FTIR spectrum revealed that proteins, phenolic and flavonoid compounds in the leaf extract are responsible for the bioreduction and stabilization process of Ag^{+} ions in to Ag NPs (Akintelu *et al.,* 2020). The stabilization of the synthesized Ag NPs may be due to the coordination of Ag NPs with – OH and C= O groups (Akintelu *et al.,* 2020; Banerjee *et al.,* 2014).

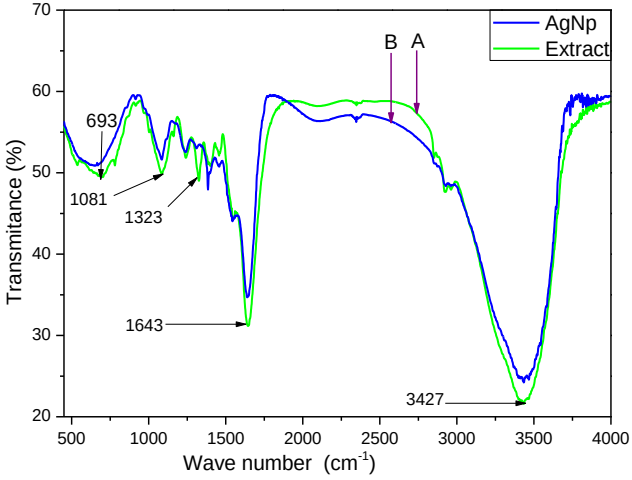

Figure 10. FT-IR spectra of pure *P. dodecandra* leave extract and synthesized silver nanoparticle.

X-ray diffraction analysis

In this study, the crystalline nature of Ag NPs synthesized using *P. dodecandra* leaf extract was confirmed by the analysis of XRD patterns, as shown in Figure 11. The spectrum clearly indicated that the synthesized Ag NPs are crystalline in nature (Jemal *et al.,* 2017; Logeswari *et al.,* 2015; Vanlalveni *et al.,* 2021*;* Niluxsshun *et al.,* 2021).

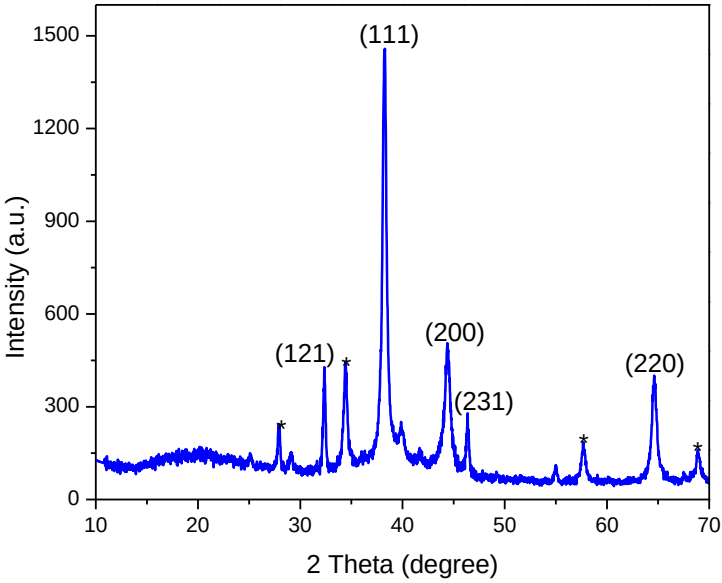

Figure 11. X-ray diffraction patterns of synthesized Ag NPs

The diffraction peaks of the XRD spectra were found in the 32.42°, 38.20°, 44.54°, 46.50°, and 64.61° ranges, respectively, corresponding to the (121), (111), (200), (231), and (220) planes of metallic silver's face centered cubic (FCC) structure. The measured spectra match the standard diffraction data for silver provided by the Joint Committee on Powder Diffraction Standards (JCPDS) File No. 04-0783 (Khan *et al.,* 2017; Masum *et al.,* 2019; Dhar *et al.,* 2021; Vanlalveni *et al.,* 2021; Malik *et al.,* 2022). The particle size of silver nanoparticles was determined using the Debye -Sherrer's equation (Bharathi *et al*., 2018; Yasir *et al.,* 2018):

$$D = \frac{0.94\lambda}{\beta \cos\theta} \quad (1)$$

Where D is the average crystalline size (A^o), λ is the x-ray wavelength (λ = 0.154 A^o), β is the full width at half maximum (FWHM) and θ is the Bragg diffraction angle.

The full width at half maximum was measured using the Gaussian curve for the highest peak. Thus, the crystalline size of the synthesized Ag NPs calculated using the Debye-Scherrer formula was found to be equal to 21 nm. A small number of un-assigned peaks (marked with stars) were recorded that might be due to the crystallization of bioorganic phases present in *P. dodecandra* leaf extract on the surface of the silver nanoparticles (Banerjee *et al.,* 2014; Bharathi *et al*., 2018; Tyagi *et al.,* 2021).

Antibacterial activity of synthesized Ag NPs.

Using the agar well-diffusion method, the antibacterial activity of Ag NPs produced from *P. dodecandra* leaf extract was tested against Gram-negative and Gram-positive pathogenic bacteria. The pathogenic bacteria used for the test were *S. typhi* and *S. aureus*. Antibiotic chloramphenicol was used as a positive control. The antibacterial effect of Ag NPs was determined on the basis of zone of inhibition as shown in Figure 12. The Figure shows Ag nanoparticle protection levels against two bacterial strains. *S. aureus* showed a maximum zone of inhibition, while *S. typhi* showed a moderate zone of inhibition.

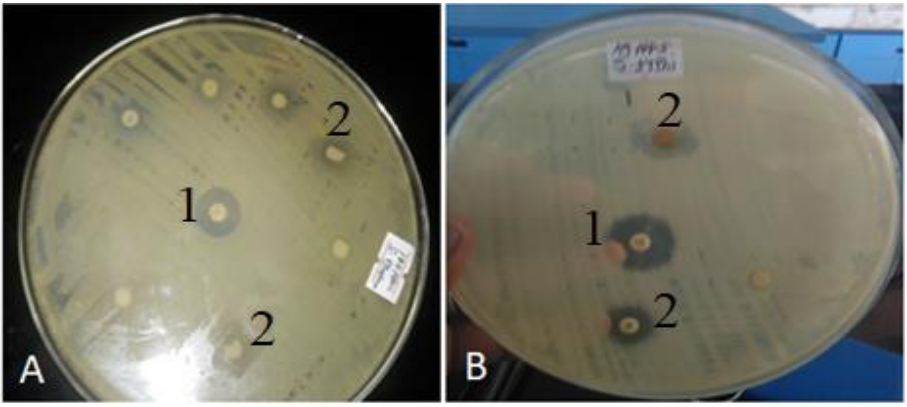

Figure 12. Antimicrobial activities testing for different human pathogenic bacterial strains

a) *S. typhi* and b) *S. aureus* (in both cases 2 is for Ag NPs and 1 is for chloramphenicol).

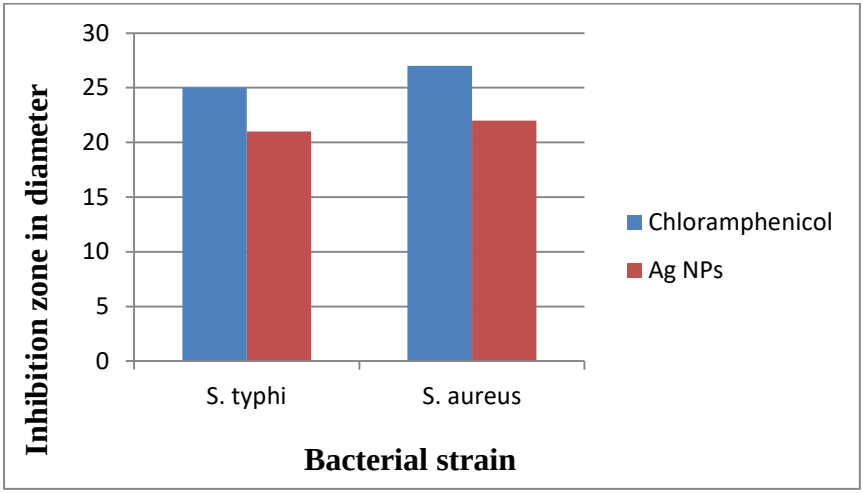

Figure 13. Antibacterial activity of Ag nanoparticles against bacterial strains.

Several studies propose that there are several mechanisms by which silver nanoparticles could kill microorganisms; (i) destroying the cell wall and ceasing the cell permeability (ii) formation of free radicals, (iii) inactivating important enzymes by interacting with thiols, (iv) interruption of DNA replication and dephosphorylating the tyrosine residues on peptides inhibiting the signal transduction and growth in bacteria (Maiti *et al.*, 2014). It is also possible that Ag NPs not only interact with the surface of the membrane, but can also penetrate inside the bacteria (Maiti *et al.*, 2014; Banala *et al.*, 2015). According to general agreement, Ag nanoparticles bind to proteins that contain sulfur in bacterial cell membranes, making the membrane significantly more permeable and inducing bacterial death (Morones *et al.*, 2005; Prabhu and Poulose, 2012). The antibacterial investigations of Ag NPs revealed substantial antibacterial action against both Gram-positive and Gram-negative pathogens, as shown in Figure 13.

CONCLUSION

In this study, we used an aqueous solution of *P. dodecandra* leaf extract to successfully synthesize Ag NPs. The work focused on the green synthesis of Ag NPs using *P. dodecandra* leaf extract, which has not yet been explored to its full potential in terms of phytochemistry. The results obtained using different characterization techniques showed

that phytochemicals present in the extracts of *P. dodecandra* leaf are mainly responsible for the reduction of silver ions to Ag NPs. Phytochemical screening using FTIR spectroscopic analysis showed that the plant extract is rich in important phytochemicals. The synthesized Ag NPs exhibited a strong absorption peak centered at 424 nm due to surface Plasmon resonance. The average particle size of synthesized Ag NPs obtained using the green approach is 21 nm. Moreover, the green synthesized Ag NPs demonstrated effective antimicrobial activity against gram-positive and Gram-negative bacteria. The antibacterial activity of Ag NPs was apparent from the inhibition zone.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Department of Chemistry, Bahir Dar University, for providing laboratory facilities.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publishing of this work.

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