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Green synthesis, characterization and antibacterial activities of silver nanoparticles using *Sida schimperiana Hochst. ex A. Rich* (*Chifrig*) leaves extract

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Abstract
The use of metal nanoparticles as antibiotics for resistant pathogens has become a current topic of research. Biosynthesized nanoparticles are promising drugs as antibacterial agents by inhibiting bacteria from infectious diseases. This study aimed at the synthesis and characterization of silver nanoparticles (AgNPs) from $AgNO_3$ solution and *Sida schimperiana Hochst. ex A. Rich* leaves extract. The synthesized AgNPs were characterized by Fourier Transform Infrared (FTIR), Ultraviolet–Visible (UV–Vis) and X-ray diffraction (XRD) spectroscopic techniques and evaluated for antibacterial activities against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli* and *Klebsiella Pneumonia*) bacteria by the disc diffusion method. The result indicated an initial color change that was observed below 400 nm for the leaves extract and 429 nm for AgNPs in the UV–Vis band supporting the synthesis of silver nanoparticles. The FTIR peaks at 3447, 1638 and 1413 cm^{-1} predict the hydroxyl, carbonyl and unsaturated C–C bonds, respectively, in the plant leaves extract. The XRD analysis showed that the synthesized AgNPs were crystalline in nature and face-centered cubic (FCC) structure with an average particle size of 26.27 nm. Furthermore, the synthesized nanoparticles showed antimicrobial activity to both types of bacteria, more significant being on Gram-negative bacteria (*E. coli*). In conclusion, the leaves extract of *Sida schimperiana Hochst. ex A. Rich* plant contains bioactive molecules that are used in the reduction and stabilization of AgNPs as potential antibacterial agents.

Keywords Biosynthesis · Phytochemicals · Antimicrobial resistance · Medicinal plant · Resistant pathogens

1 Introduction

Recent works on metal nanoparticles (MNPs) are used in many fields, including pharmaceuticals, therapeutics and antimicrobial products. More focus was on MNPs for controlling diseases due to their wide activities against various microorganisms, low toxicity, long-term activity and also very effective action against phytopathogenic bacteria [1].

There are numerous physical, chemical and biological methods for synthesizing metallic nanoparticles. Since chemical and physical processes are time-consuming, expensive and produce toxic substances [41, 42] a simplistic, eco-friendly and adaptable means is the biological synthetic route. The green synthesis method has a significant impact on the effectiveness, including lower production costs, and the potential for trouble-free manufacturing without the use of hazardous chemicals or high temperatures and pressures and bio-compatibility,. Many secondary metabolites are present in plant

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extracts, and they reduce and stabilize the NPs during their synthesis [43]. Antioxidant, antimicrobial, anticancer and other biological properties of the synthesized RsFb-AgNPs, exhibited dose-dependent activities [45].

Biosynthesis of AgNPs uses biomaterials such as bacteria, yeast, algae, fungi and plants. Of these, the use of medicinal plants attracted the attention of many researchers because of simple and efficient processes due to the content of bioactive molecules and therapeutic properties, and biosynthesized AgNPs show very interesting anti-bacterial effect [2]. Besides, the synthesis of nanoparticles using plant extracts is advantageous over other biological processes, because it eliminates the elaborate process of maintaining cell cultures and it enables a large-scale synthesis of nanoparticles [3].

Metal nanomaterials of Au, Ag, Se…, and the oxides have been exploited as antibacterial agents, targeted drug delivery vehicles, antimycotic, antioxidant and anticancer agents [4–9]. In this regard, Au and AgNPs are highly significant due to their unique properties [10]. Hence, noble metallic nanoparticles including silver nanoparticles (AgNPs) differ from others due to their attractive physical and chemical characteristics in relation to biological systems [11]. By comparison, AgNPs demonstrated to be very effective antimicrobial, antioxidant and antifungal agents due to their powerful cytotoxic activities toward a broad range of microorganisms [1, 2]. For instance, Silver nanoparticle synthesized from the fruit extracts of Solanum nigrum (Sn AgNPs) had greater inhibition of *B. pumulis* and *E. faecalis* compared to *P. vulgaris* and *V. parahaemolyticus* [39]. Similarly, Silver nanoparticles from the fruit extracts of *Momordica charantia* (*Mc*-AgNPs) showed greater inhibition of Enterococcus faecalis compared to Aeromonas *hydrophila* [40]. Antimicrobial activity of Plumeria alba -AgNPs against selected human pathogenic microbes and the in effective anticancer activity against the glioma U118 MG cancer cells was reported [44].

Over centuries, people around the world have relied on natural resources and developed their locally-specific knowledge of plant use including traditional medicines [12]. From their traditional use, a remarkable number of modern drugs have been isolated from plants [13]. *Sida schimperiana Hochst. ex A. Rich* (*Chifrig*) is a Perennial plant under shrubs or herbs [14], which is a traditional medicinal plant used for the treatment of Prenatal abortion, killing worms, Amoebic dysentery, Cough, Influenza and Liver disease [15]. In Ethiopia, this plant is also used in the treatment of wound and Tumors around Debark [16], for breast cancer in Nekemte [17], for dysentery in Gozamin [18] and for the treatment of intestinal parasites in Harari [19]. Therefore the objective of this study was to synthesize and characterize AgNPs from the leaves extract of *Sida schimperiana Hochst. ex A. Rich* (*chifrig)* plant and $AgNO_3$ solution and evaluate its antimicrobial action.

2 Materials and methods

2.1 Apparatus and instruments

Apparatus and instruments used in the experiment were centrifuge, oven, weighing balance, pH meter, refrigerator, mechanical shaker, UV–Vis, FTIR and XRD spectroscopy.

2.2 Chemicals and reagents

The chemicals used were all Analytical grade from Abron Chemicals India: 99.8% $AgNO_3$, $CHCl_3$, $FeCl_3$, HCl, NH_3, H_2SO_4 and NaOH. Muller agar solution and the antibiotic ampicillin was used as a control.

2.3 Sample collection and preparation of the extract

The fresh leaves of *Sida schimperiana Hochst. ex A. Rich* were collected from Debre Markos University area at coordinates of 10°32′N, 37°73′E, Ethiopia [20]. The plant was authenticated by the botanical science member (Haimanot Retta) of Debre Markos University. It grows in the university compound as a wild species. The leaves of the plant were washed and cleaned and allowed to dry under shadow for 7 days. The dried leaves were ground using a grinding machine. A 40 g of powdered leaves of *Sida schimperiana Hochst. ex A. Rich* was placed in a 500 ml Erlenmeyer flask containing 400 ml of distilled water. Later, the flask was covered with aluminum foil to prevent the effect of light. Then the mixture was shaken using a mechanical shaker for 30 min and warmed at 50 °C for 1 h. on a magnetic stirrer. The mixture was allowed to cool down to room temperature overnight. The prepared jelly-like solution was filtered through folded nylon mesh. The filtrate was stored in the refrigerator at 4 °C for analysis [21].

2.4 Phytochemical screening

The aqueous leaves extracts were subjected to preliminary phytochemical (alkaloids, terpenoids, steroids, flavonoids, saponins, phenols, glycosides, tannins and anthraquinones) screening following the methods shown in Table 1.

2.5 Synthesis of AgNPs

Following the procedure of [25, 26] and with slight modifications, a 1 mM of 450 ml aqueous $AgNO_3$ solution was mixed with 50 ml of the plant leaves extract (1:9) ratio, drop wise in an Erlenmeyer flask under constant stirring (Fig. 1). The pH of the solution was maintained to 9 by using 0.1 M NaOH solution. The mixture was allowed to react in the dark at room temperature for 24 h. The color change was observed after 30 min and then the solution was centrifuged for 20 min at 10,000 rpm. The solid was filtered and washed with distilled water to remove any impurities. Then the sample was allowed to dry and ground for future use [21].

2.6 Characterization of silver nanoparticles

The AgNPs synthesized above were characterized by FTIR, UV–Vis and XRD spectroscopic methods. The reduction of silver ions was analyzed for surface plasmon resonance (SPR) (Perkin Elmer Lambda 35 Germany UV–Vis spectrophotometer) in the wavelength range of 200–700 nm, by dissolving the samples in ethanol. The FTIR analysis was carried out using FT/IR-6600 in the wavenumber range of 4000–400 cm^{-1} by grinding the sample in KBr pellets. The XRD analysis was carried using an X-ray Diffractometer (XRD-6100, Shimadzu, Japan) with Cu-Kα radiation of wavelength 1.5406 Å and scanned in the range of angle 2θ from 3° to 80° at a scanning rate of 5° min^{-1}. Samples were finely ground and homogenized. Then the average bulk composition was analyzed. The average crystallite sizes of the particles were calculated using Debye–Scherrer's equation.

$$D = \frac{K\lambda}{\beta\cos\theta},$$

where, D is the estimated crystal size in nanometer (nm) from XRD patterns, θ is the Bragg angle in degree, λ is the wavelength of the X-ray source used (CuKα = 1.5406 Å), β—the angular width at the half maximum of the diffraction peak in degree and K is the shape factor or Scherrer constant (0.94) of Debye–Scherrer's equation.

2.7 Antibacterial evaluation of AgNPs

The disc diffusion method was used to study the antibacterial activity of the synthesized silver nanoparticles. Broth medium was used to subculture bacteria and was incubated at 37°Cfor 24 h. The bacterial strains were Gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) and Gram-positive (*staphylococcus aureus* and *staphylococcus epidermidis*). Ampicillin as a standard, AgNPs, plant extract, and silver nitrate were evaluated for the antibacterial activity [28]. The antibacterial test was carried out at Bahir Dar University microbiology laboratory center, Bahir Dar, Ethiopia.

3 Results and discussion

3.1 Phytochemical analysis of the plant leaves extract

Qualitative tests of some useful bioactive compounds such as alkaloids, terpenoids, steroids, flavonoids, saponins, phenols, glycosides, tannins, and anthraquinones in the leaves extract of *Sida schimperiana Hochst. ex A. Rich* revealed color changes, precipitate formation, and other physical changes as a confirmatory test. The phytochemical screening of the *Sida schimperiana Hochst. ex A. Rich* is summarized in Table 2 below.

3.2 UV–Vis analysis

A change in color may confirm the reduction of silver ions into silver nanoparticles. The initial mixture having pale yellow color (Fig. 2A) changed into reddish-brown (Fig. 2B) after 30 min of reaction time. The color variation is as a result of the excitation of the Surface Plasmon Resonance (SPR) in the metal nanoparticles (Fig. 2). Such color change might indicate

Table 1 Qualitative phytochemical screening of *Sida schimperiana Hochst. ex A. Rich* leaves extracts

No.	Phytochemicals	Test	References
1	Alkaloids	A 3 ml of plant leaf extract was added to 3 ml of 1% HCl solution and it was heated for 20 min in a water bath. The resulted reaction mixture was cooled at room temperature and used to perform Mayer's test. To the cooled reaction mixture in a test tube, 1 ml of Mayer's reagent was added drop by drop	[22, 23]
2	Terpenoids	A 5 ml of plant leaf extract solution was mixed with 2 ml of chloroform, and 3 ml of concentrated sulfuric acid to form a layer	[22, 23]
3	Steroids	A 3 ml of plant leaf extract solution was mixed with 2 ml of chloroform, and 3 ml of concentrated sulfuric acid to form a layer	[22, 23]
4	Flavonoids	A 3 ml of plant leaf extract was treated with 1 ml of 10% NaOH solution	[22, 23]
5	Saponins	A 0.2 ml of the plant leaf extracts was shacked with 5 ml of distilled water and then heated to a boil	[22, 23]
6	Phenols	A 2 ml of 5% solution of $FeCl_3$ was added to 1 ml of plant leaf extracts	[22, 23]
7	Glycosides	A 3 ml of leaf extract was added to 5 ml distilled water and 5 ml an aqueous NaOH solution	[24]
8	Tannins	To 0.5 ml plant leaf extract solution, 1 ml distilled water and 2 drops of ferric chloride solution were added to it	[22, 23]
9	Anthraquinones	To 0.2 ml of plant leaf extract, 5 ml of chloroform, and 5 ml of ammonia solution were added	[24]

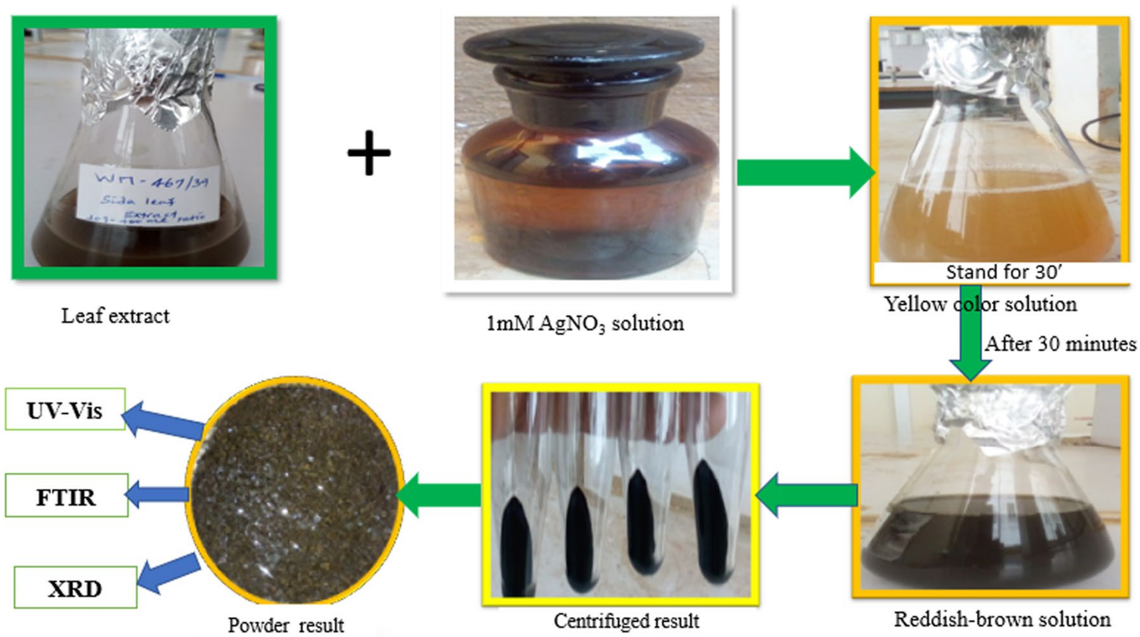


Fig. 1 Synthesis scheme for AgNPs

that the active molecules present in the *Sida schimperiana Hochst. ex A. Rich* aqueous extract have reduced the silver ions to the stable AgNPs. A similarly work had a color change from yellow to reddish-brown confirming the formation of AgNPs [2, 26, 27].

The UV–Vis spectrum in Fig. 3 indicated that the leaves extract show three peaks at 235,273 and 320 nm. Absorption bands that occur at 234–676 nm are characteristic of alkaloids, and phenolic compounds [58], which suggests the presence of secondary metabolites in the leaves extract that reduced silver ions and stabilize AgNPs. *Sida schimperiana Hochst. ex A. Rich* leaves extract alone did not show any peak between 400 and 700 nm.

The band of silver nanoparticles was observed at 429 nm in the case of *Sida schimperiana Hochst. ex A. Rich* leaves extract (Fig. 3), which are potential agents to reduce Ag^+ ions into AgNPs. Absorption bands of plant extracts of *Moringa oleifera* leaf in the wavelength from 440–450 nm [29], *Brillantaisia patula* aqueous leaf extract at 434 nm [31], *Prunus persica* plant extract at 440 nm by [31] and *Tropaeolum majus* plant extract at 430 nm showed the potentials of phytochemicals in the reduction of silver and other metal ions.

3.3 FT-IR analysis of AgNPs and leaves extracts

The FTIR spectrum of the leaves extract of *Sida schimperiana Hochst. ex A. Rich* (Fig. 4) showed major absorption broad peaks at 3447 (3740 to 2894) cm^{-1}, 2080 (2310 to 1930) cm^{-1}, 1638 (1746 to 1480) cm^{-1}, 1413 cm^{-1}, 1041 cm^{-1}, 632 cm^{-1} and 450 cm^{-1}. The broad and strong peak at 3740 to 2894 cm^{-1} confirm the presence of O–H and/or N–H, functional groups of alcohols, carboxylic acids, or amines and overlapped C–H for alkanes. Small and medium intensity peaks at 2080 (2310 to 1930) cm^{-1} indicate the presence of C ≡ N, C ≡ C stretching bands. A strong peak at 1638 (1746 to 1480) cm^{-1} revealed the C = O functionality of aldehydes, esters, ketones, amides and C = C functionality of alkenes, aromatic rings, CH_2 or N–H bending. The peak at 1413 cm^{-1} may be due to the C = C aromatic ring and 1041 cm^{-1} implied an N–C bending, C–O alkoxy or the C–O–H stretching. The majority of the peaks correspond to the phenolic groups of the phenols, terpenoids, steroids, and tannins, adequately present in the leaf extract, which helped in the formation of AgNPs. A broad peak of the plant extract shifts from 3447 cm^{-1} to a relatively narrow peak of 3434 cm^{-1} due to the involvement of the O–H or N–H stretching of phenolic or amine compounds that are present in the leaves extract. The bands at 2080 cm^{-1} to 2060 cm^{-1}, 1638 cm^{-1} to 1634 cm^{-1}, 1413 cm^{-1} to 1375 cm^{-1}, showed the involvement of triple bond, carbonyl, alkenyl, or aromatic C–C stretching respectively. The band from 1041 cm^{-1} to 982 cm^{-1}, show the C-O stretching of phenol or tertiary alcohols and N–C bending in the reduction and stabilization process, and peaks below 600 cm^{-1} may be assigned to the stretching vibration of the Ag–O [37, 38]. It implies that the hydroxyl, aliphatic hydrocarbon, nitro compounds, and amine functional groups on the surface of *Sida schimperiana Hochst. ex A. Rich* leaves extract were involved in the

Table 2 Results of qualitative phytochemical screening of *Sida schimperiana Hochst. ex A. Rich* leaves extract

No.	Bioactive phytochemicals	Expected outcome	Observation	Result	References
1	Alkaloids	Formation of a greenish-colored cream precipitate	A green color cream precipitate was formed	+	[23, 24]
2	Terpenoids	Formation of reddish-brown coloration of the interface	The reddish-brown color at the interface was observed	++	[23, 24]
3	Steroids	Formation of the red color at the lower surface	Red color on the lower surface was observed	++	[23, 24]
4	Flavonoids	Formation of an intense yellow color	The greenish color was observed	+	[23, 24]
5	Saponins	Formation of Froth	Froth was formed	++	[23, 24]
6	Phenols	Formation of black color in the reaction mixture	The black color of the reaction mixture was formed	++	[23, 24]
7	Glycosides,	Formation of yellow color	The yellow color was formed	++	[25]
8	Tannins	Formation of blue-black color	A blue-black color was observed	+	[23, 24]
9	Anthraquinones	The presence of bright pink color in the aqueous layer	The yellow color was formed	–	[25]

++, present in high amount; +, present, −, absence of the bioactive molecule

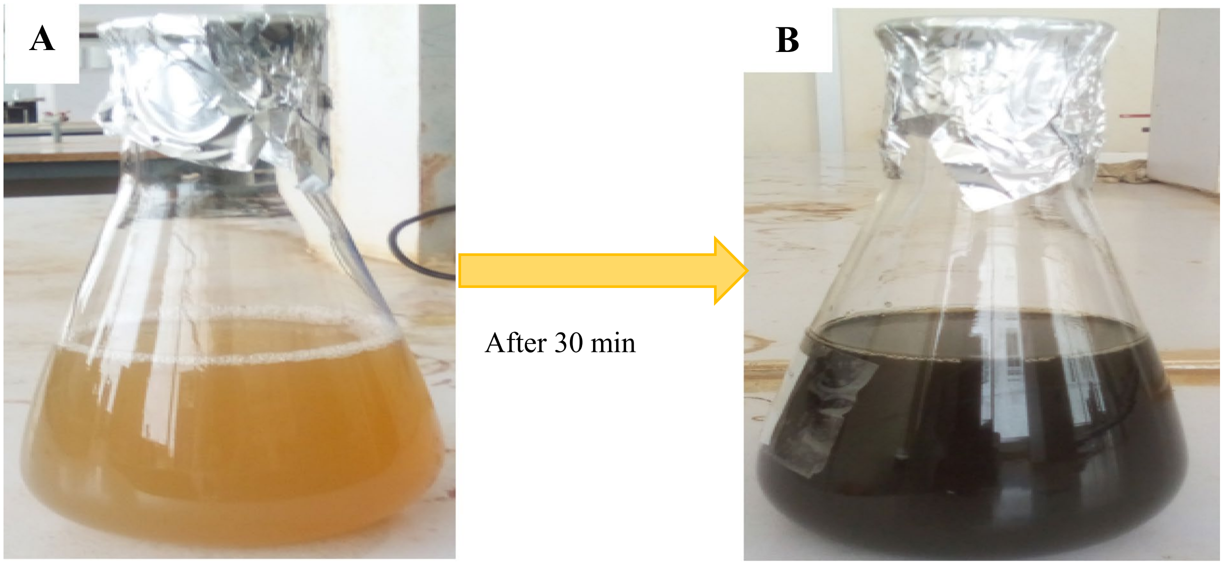


Fig. 2 The synthesis of AgNPs from silver nitrate solution and the plant leaves extract. **A** Before synthesis. **B** After the formation of AgNPs

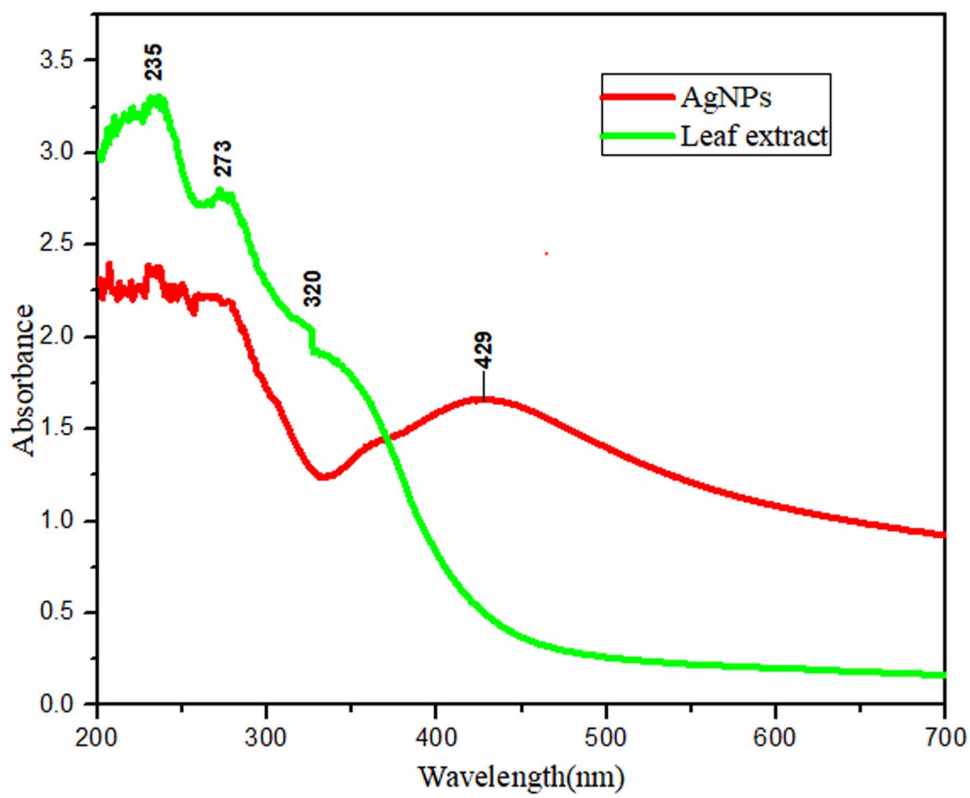


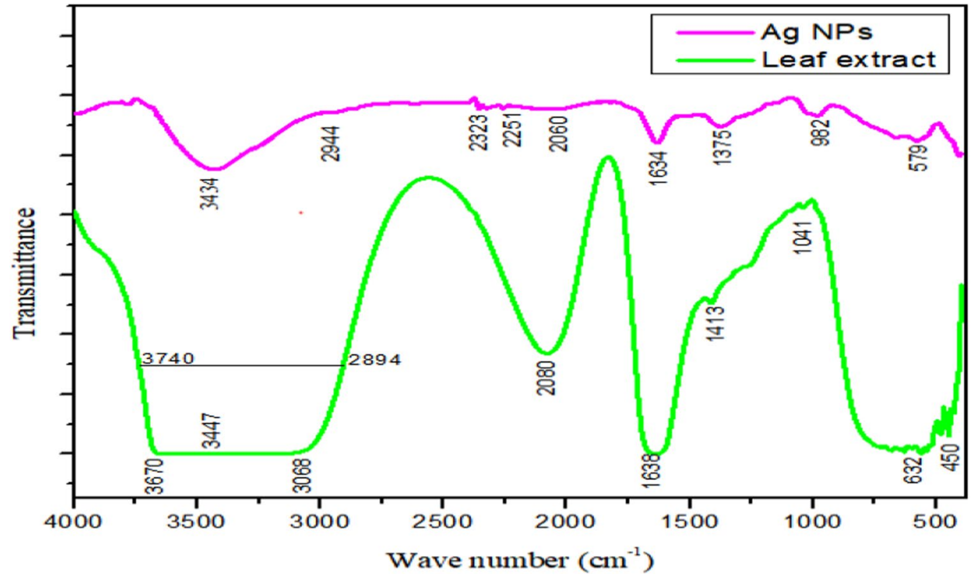
Fig. 3 UV–Vis absorption spectra of the synthesized AgNPs and *Sida schimperiana Hochst. ex A. Rich* leaves extract

reduction, stabilization as well as capping agents in the synthesis of silver nanoparticles. Major shifts of these biomolecules in the FTIR spectrum in Fig. 4 of the plant leaves extract and the AgNPs indicated that they were involved in the synthesis of AgNPs. Similar results to this were also reported [33–35].

3.4 XRD analysis

Figure 5 shows the AgNPs analysis of the XRD pattern. Four prominent diffraction peaks were observed at 2θ of 38.00°, 44.16°, 64.50° and 77.36° that correspond to the diffraction planes of (111), (200), (220) and (311), respectively. The

Fig. 4 FTIR spectra of *Sida schimperiana Hochst. ex A. Rich* leaves extract and AgNPs



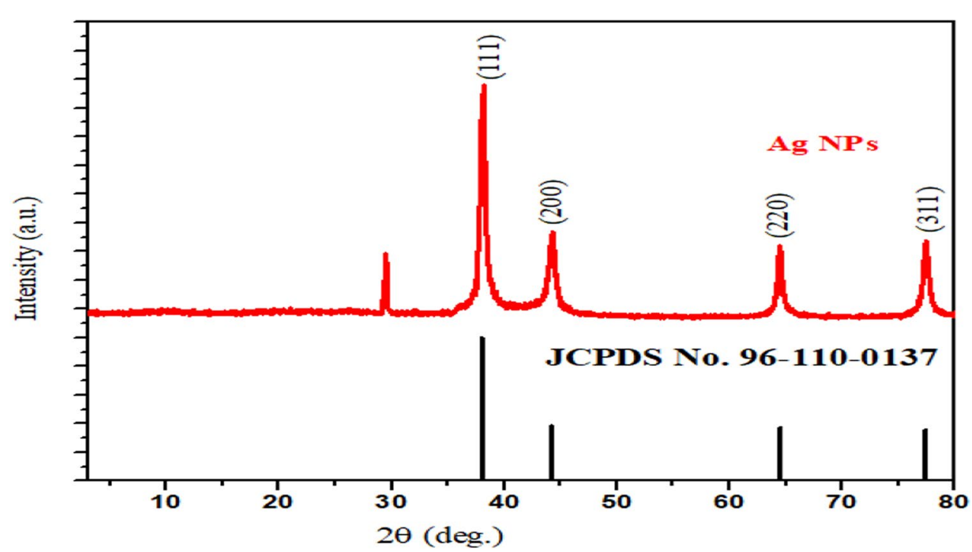
diffraction peaks were face-centered cubic (FCC) structure in the powdered phase of metallic silver. The peaks along with their Bragg's reflections agree with the Joint Powder Diffraction Standards Committee (JCPDS, file no. 96–110-0137), as closely matched results were reported [22, 30, 31, 36]

The estimated average crystalline size of AgNPs was 26.27 nm. The peak at 2θ 29.57° showed the presence of an organic compound in the extract that is responsible for the reduction of silver ions and stabilization of resultant nanoparticles. [57, 58] had very close results to the current finding.

3.5 Anti-bacterial studies of green synthesized AgNPs

The AgNPs, *Sida schimperiana Hochst. ex A. Rich* leaves extract alone and silver nitrate were evaluated for their antibacterial activity against two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and two Gram-positive bacteria (*Staphylococcus aureus* and *staphylococcus epidermidis*) by the agar disk diffusion method (Fig. 6I–IV). The positive control Ampicillin had comparable zone of inhibition (ZOI) with AgNPs in the tested bacteria except for Gram-negative bacteria *E. coli* as described in Fig. 6I and Table 3. The antibacterial activity of AgNPs was maximum with ZOI of 26.33 mm against Gram-negative (*E. coli*) bacteria at a concentration (200 μg/ml) and the plant leaves extract against Gram-negative (*E.coli*) bacteria had a maximum ZOI (17.33 mm) at a concentration of 200 μg/ml. A higher antibacterial activity by AgNPs may be

Fig. 5 XRD pattern of the synthesized AgNPs from *Sida schimperiana Hochst. ex A. Rich* the leaves extract and $AgNO_3$ solution



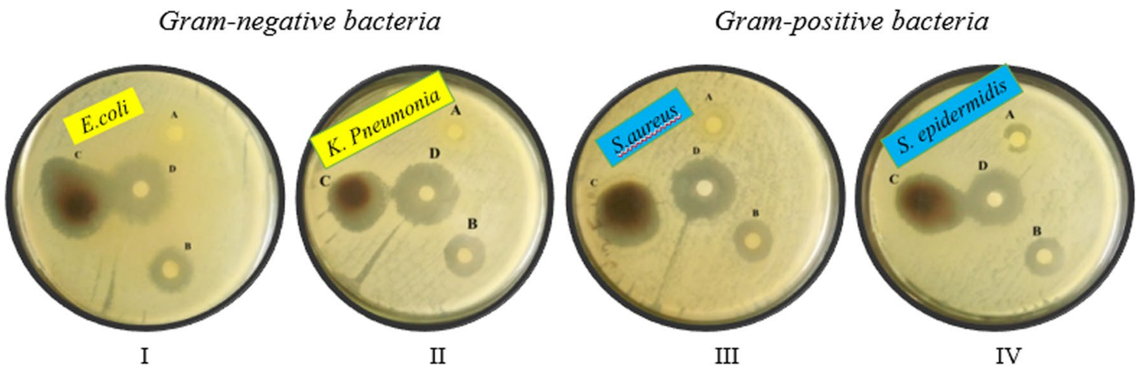


Fig. 6 Antibacterial activity of **A** $AgNO_3$, **B** plant extract, **C** AgNPs, **D** Ampicillin

Table 3 Antibacterial activity of AgNPs *Sida schimperiana Hochst. ex A. Rich*, $AgNO_3$ solution, and Ampicillin against clinically isolated human pathogenic bacteria

Bacterial species	Tested components (mm)			
	A = $AgNO_3$	B = plant extract	C = AgNPs	D = Ampicillin
E. coli (gram -ve)	9.33 ± 0.6	17.33 ± 0.6	26.33 ± 3.2	23.00 ± 1
K. Pneumonia (gram –ve)	9.33 ± 0.6	17.00 ± 1	22 ± 1	24.33 ± 0.6
S. aureus (gram + ve)	10.33 ± 0.6	16.67 ± 0.6	23.33 ± 0.6	23.33 ± 0.6
S. epidermidis (gram + ve)	10.33 ± 0.6	16 ± 1	22.33 ± 0.6	22.67 ± 0.6

because of the nanoparticles large surface area that provides better contact with the microorganisms. AgNPs having large surface area will have a greater chance of interacting with bacterial cell membranes that leads to stronger antibacterial effects. As silver ions are involved to the antibacterial effects, the larger surface area allows them to bind more effectively to bacteria. The negative charge of the bacterial cell wall will be attracted to the silver ions and nanoparticles will move and get attached to the cell wall, changing the cell wall composition and affecting its permeability [30]. Similarly, the antimicrobial potential of synthesized AgNPs from aqueous leaf extract of I. brachypoda effectively inhibited the growth of pathogens and it can be used as broad-spectrum antimicrobials against multi-drug resistant microbial pathogens [46]. The synthetically obtained Ls-AgNPs displayed good antimicrobial activity against clinical pathogens [47].

4 Conclusion

AgNPs were synthesized from silver nitrate solution and *Sida schimperiana Hochst. ex A. Rich* plant leaves extract as a reducing, capping and stabilizing agent. The AgNPs were characterized by FTIR, UV–Visible and XRD spectroscopic methods. Qualitative tests on bioactive molecules of the leaves extract of the plant was identified by phytochemical screening. The FTIR spectrum of the *Sida schimperiana Hochst. ex A. Rich* extract of leaves had peak shifts in the AgNPs mainly because of these molecules were involved in capping, reduction and stabilization of the nanoparticles. The XRD analysis confirmed that the AgNPs were crystalline with FCC geometry having an average crystallite size of 26.27 nm. The plant leaves extract and the AgNPs showed a high antibacterial activity against Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative (*E. coli* and *K. pneumonia*) bacteria. The high antibacterial activity of the AgNPs is due to the large surface area of the nanoparticles that enables to bind them with the bacteria and the presence of silver ions. There was a stronger inhibition zone by the synthesized AgNPs in Gram-negative bacteria compared to the plant leaves extract on the same tested pathogenic bacteria strain.

Further research may be required on the optimization of reaction parameters such as boiling time, the volume of extract, the concentration of $AgNO_3$, temperature and pH in the synthesis of AgNPs from *Sida schimperiana Hochst. ex A. Rich* plant leaves extract and $AgNO_3$ solution.

Author contributions Both authors Wendmnew Moges and Yohannes Misskire have prepared manuscript from the start to the end of the whole work. i.e. Both involved in planning, worked in the lab and reviewed the manuscript.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Competing interests The authors declare no competing interests.

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