Nanomed Res J 5(3):276-287, Summer 2020

RESEARCH ARTICLE

Eco-friendly synthesis of silver nanoparticles from *Clerodendrum viscosum* leaf extract and its antibacterial potential

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ARTICLE INFO

Article History:
Received 17 May 2020
Accepted 13 July 2020
Published 01 Aug 2020

Keywords:
Eco-friendly Synthesis
Clerodendrum viscosum
Silver Nanoparticles
Spectral Observation
Antibacterial Activity

ABSTRACT

Objective(s): Recently, nanoparticles have occupied the leading stage for the development of nanotechnology. Silver nanoparticles (AgNPs) have received significant concentration for their potent chemical, electrochemical, physical, antimicrobial and wound healing applications. This paper represents effortless, cost effective and eco-friendly method for the synthesis of AgNPs from silver nitrate solution by using aqueous leaf extracts of *Clerodendrum viscosum*.

Methods: The synthesis of nanoparticles was carried out by using the aqueous extract of *Clerodendrum viscosum* leaf and silver nitrate solution. The formation of nanoparticles was identified by the change of colour from colourless to dark brown owing to surface plasmon resonance phenomenon. The synthesized nanoparticles were identified by UV-Vis spectrophotometer, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), X-ray diffraction (XRD) and Fourier Transform Infra-red Spectroscopy (FTIR) methods. In addition, the antibacterial activity was tested by agar disc diffusion assay against some bacteria.

Results: The appearance of silver nanoparticles was indicated by the presence of an absorption peak at 422 nm. The images of the electron microscope indicated that the nanoparticles were spherical and the average size about 56 nm at 25ºC and 53 nm at 60ºC. The X-ray diffraction was distinctly proven that the nanoparticles were crystalline nature. The results of microbial test also indicated that the synthesized nanoparticles had significant antibacterial activity.

Conclusions: This experiment showed that leaf extracts of *Clerodendrum viscosum* can be used for eco-friendly synthesis of nanoparticles, which also have potential antibacterial effect.

How to cite this article
Nahar K., Yang DC., Rupa EJ., Khodeza Khatun Mst., Al-Reza SM. Eco-friendly synthesis of silver nanoparticles from Clerodendrum viscosum leaf extract and its antibacterial potential. Nanomed Res J, 2020; 5(3): 276-287. DOI: 10.22034/nmrj.2020.03.008

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INTRODUCTION

Recently, nanotechnology takes the leading edge of material science. A nanoparticle is a small particle that exhibit strengthened or different effects when considered with bulk material. The necessity of nanoparticles is growing very fast for their various applications. Therefore, there is silent demand for cost effective, commercially visible as well as eco-friendly synthesis route for nanoparticles [1].

It has been suspected that the synthesis of nanoparticles depends on various factors, such as plant source, organic compounds and even the pigments, polyphenols, alkaloids and proteins, etc. in the plant extract. Number of biomolecules exists in plant sample decrease the monovalent silver ions to neutral atoms and these atoms accumulated to gain nano size, other biomolecules act as envelope or "cap" them to stop extra assembling [2]. It also reported that the negatively charged functional groups may conduct the reduction of metal ions and effective stabilization of synthesized nanoparticles [3].

Despite reducing and capping agents, the various process parameters are equally responsible for preparation of AgNPs. The shape and size morphology of NPs may vary in accordance to the reaction conditions such as incubation temperature, time period, concentration of reagents, pH, ratio of $AgNO_3$ solution and plant extract, concentration of plant extracts etc.

Clerodendrum viscosum belongs to Lamiaceae family which is locally known as Vita and enjoy an abundant distribution in Bangladesh. In folk medicine, *Clerodendrum viscosum* is widely used as blood purifier though there is no pharmacological evaluation in support of anti-hemolytic activity of *Clerodendrum viscosum* [4]. It is a shrub having quadrangular stem, large leaves of ovate shape, acuminate apex, entire or denticulate margin, cylindrical petiole and hairy leaves. The plant is of 0.9-2.4 meter in height and flowers are whitish pink in color with long pubescent pedicels in stalked cymes and the fruits are four lobed drupes of 8 mm in diameter. This plant is common throughout India, Bangladesh, Myanmar, Thailand and Indonesia. The leaf and root have been used in Indian traditional medicine for the treatment of asthma, fever, bronchitis, skin diseases, epilepsy, inflammation, tumors, worm infestation and snake bite [5,6]. The fresh leaf juice is used as verminfuge, bitter tonic, febrifuge in malaria fever, especially in children. The various parts of the plant are reported to have many biological activities like, antimicrobial, cytotoxic, anthelmintic, antioxidant and antinociceptive [7,8]. Recently, Sahoo et al., [9], reported the antiplasmodial and anticancer activity of nanosilver particles from *Clerodendrum viscosum* Vent leaf extract.

However, there is no record on the synthesis of silver nanoparticles from *Clerodendrum viscosum* leaves in Bangladesh. So, we take the first step for the synthesis of silver nanoparticles from silver nitrate solution by using aqueous extract of *Clerodendrum viscosum* leaf as reducing agent. The nanoparticles were characterized by using various spectral analyses. They were also assessed for their antibacterial activity.

MATERIALS AND METHODS

Preparation of plants extract

Clerodendrum viscosum leaf collected from the area of Islamic University, Kushtia, Bangladesh. This raw material was washed with distilled water to take out dirty materials and dried to remove remain moisture. The plant samples were homogenized to fine powder by using electric blender. 1 g of powder samples in 100 ml deionized water were boiled for 5 minutes at 80ºC and then cool and filtered the aqueous leaf extract through Whatman filter paper no.1. Then the filtered was kept in 4ºC for further experiment.

Synthesis of Silver Nanoparticle

An aqueous solution of 80 ml 0.01M $AgNO_3$ was added to 20 ml of leaf extract and stirred with a magnetic stirrer at 25ºC and 60ºC temperature, respectively. The colour of the mixture was transformed from colourless to light yellow and then to dark brown. Silver nanoparticles formation was primarily identified by colour change. The silver nanoparticles were separated from the dispersion by centrifugation following swept with distilled water and acetone to take out water soluble impurities. The nanoparticles were lyophilized and stored in dry bottle for remain examination.

Standardization

For better formation of silver nanoparticles, effect of concentration of leaf extract, effect of reaction time, effect of pH, effect of temperature, effect of ratio of leaf extract to $AgNO_3$ solution, and effect of concentration of $AgNO_3$ on AgNPs formation were investigated and the optimum conditions for the reaction were selected.

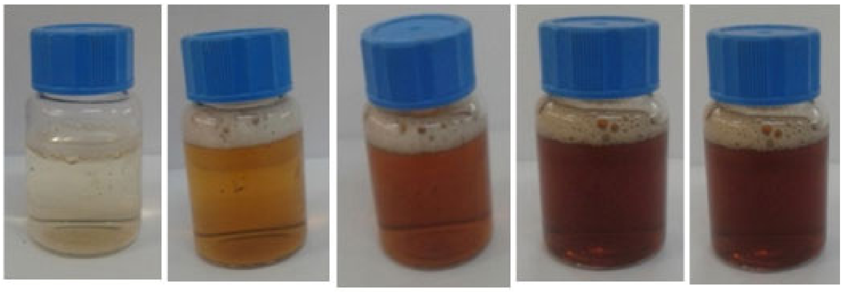

Fig. 1. Color change at different time interval of *Clerodendrum viscosum* leaf extract with 0.01M $AgNO_3$ solution

Characterization of silver nanoparticles

Silver nanoparticles were characterized by using visual observation and different spectral analyses. In visual observation, colour change of the solution was confirmed by naked eye. For confirmation of AgNPs in solution Shimadzu UV-Visible 2900 spectrometer was used in the wavelength (λ) range 300-600 nm. The surface character of AgNPs and biomolecules in *Clerodendrum viscosum* solution were determined by Fourier transform infrared spectroscopy (Shimadzu FTIR spectrophotometer, FTIR 8400 Shimadzu, Japan). The FTIR spectra were recorded from 4000-600 cm^{-1}. The surface morphology and particle size was identified by transmission electron microscopy (TEM) (Jenama/ model: philip/TEM CM12, Malaysia). The size, shape and surface of the synthesized AgNPs were analyzed by scanning electron microscopy (SEM) with high-resolution images and selected area. For SEM analysis, thin films of the sample were developed on a carbon coated copper grid by dropping a small quantity of the sample on the grid, extra solution was separated with a blotting paper and then the film were kept under a mercury lamp for 5 min for drying, followed by microscopic examinations (JSM- 6490, JEOL C0, Ltd., Japan). X-ray diffraction (XRD) (D8-Advance, Bruker, Germany) was done to find out the phase distribution, crystallinity and purity of the synthesized nanoparticles. Dynamic light scattering (DLS) (Malvern, UK) studies estimated the nanoparticle size in colloidal system without any aggregation and the hydrodynamic size.

Phytochemical screening

The qualitative phytochemical analysis of *Clerodendrum viscosum* extract was performed to determine the presence of alkaloids (Dragendorff's), triterpenes (Liberman test), phenolics (lead acetate, alkaline reagent test), saponins (foam test), flavonoids (alkaline reagent), tannins (Few $FeCl_3$) and carbohydrates (Molisch test) [10-12].

Antibacterial activity assay

The antibacterial assay was done by disc diffusion method [13] taking 100 µl of standardized inoculums suspension carrying 10^7 CFU/ml of bacteria. Two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria were used in this study. The strains were collected from the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh. After weighing the dried AgNPs were dispersed in deionized water to produce a stock solution with final concentration of 1 µg/µl. Dried and sterilized filter paper discs (6 mm diameter) were then soaked with different concentration of AgNPs. Standard reference antibiotics, tetracycline (Sigma-Aldrich Co., St. Louis, MO, USA), was used as positive control. The plates were incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the diameter of inhibition zones against the tested bacteria.

RESULTS AND DISCUSSION

Visual Observations

Silver nanoparticles formation was primarily confirmed by colour change (Fig. 1). *Clerodendrum viscosum* leaf extract was mixed with 0.01M silver nitrate solution. After 5 min a colour change from colourless to light yellow was shown then it changes from light yellow to brown and finally it was converted to dark brown after 60 min at 25°C. The colour intensity increased with time of incubation. The yellow coloured solution became

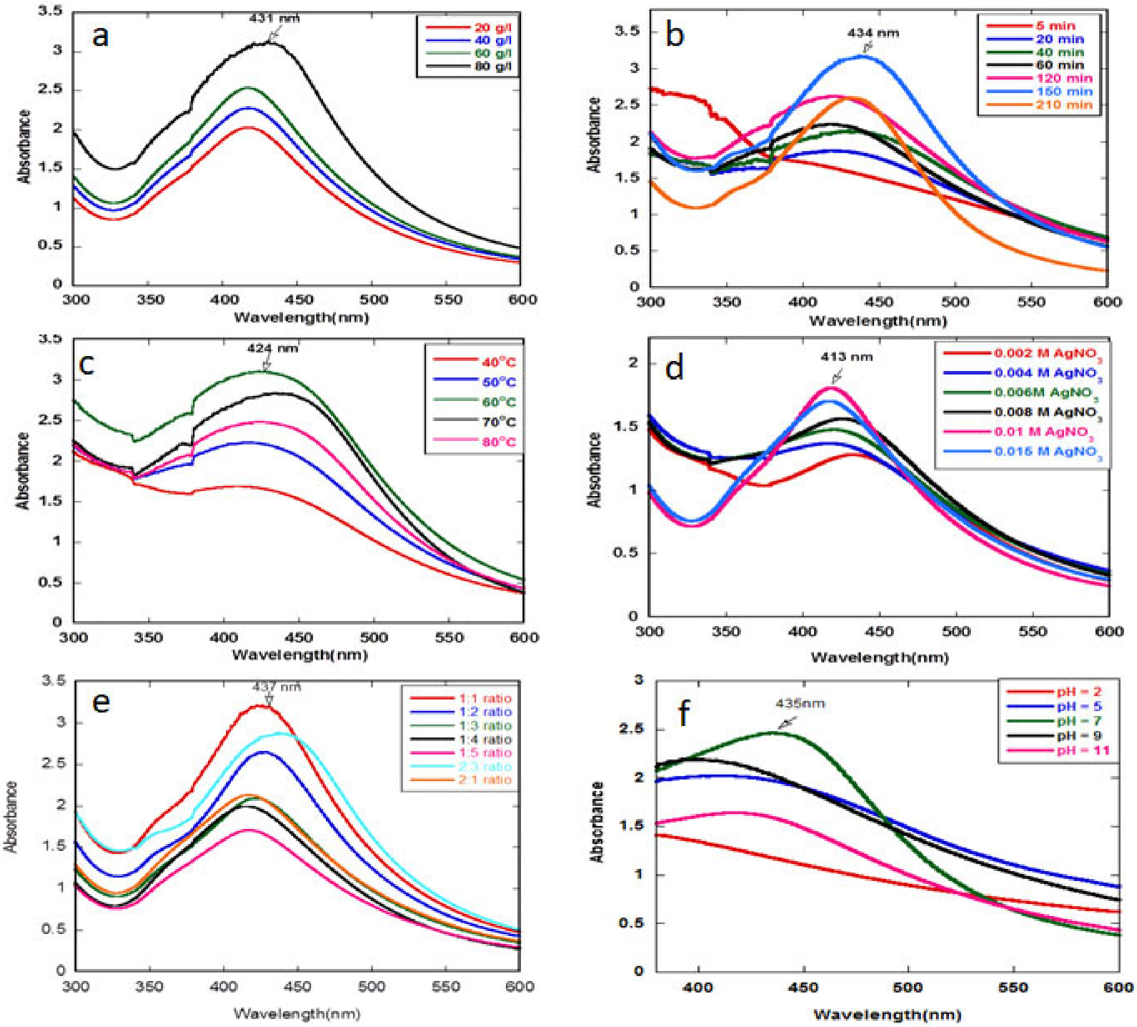

Fig. 2. UV-Vis absorbance spectra of silver nanoparticles synthesis (a) by different concentration of *Clerodendrum viscosum* leaf extract; (b) by leaf extract of *Clerodendrum viscosum* on different reaction time; (c) at different temperature; (d) at different concentration of $AgNO_3$ solution; (e) different ratios of *Clerodendrum viscosum* leaf extract and silver nitrate solution; (f) at different pH.

dark brown by 60 min which may be owing to the increased concentration of nanoparticles. After 24 hrs no further colour was changed. This brown colour of silver nanoparticles appears owing to surface plasmon resonance in the solution [14].

Standardization

Optimization of some parameters was essential for the efficient silver nanoparticles synthesis.

Concentration of Clerodendrum viscosum leaf extract

There was a difference in the formation of AgNPs by increasing the concentration of leaf extract (Fig. 2a). Different concentrations of *C. viscosum* leaf extract (20 g/l, 40 g/l, 60 g/l and 80 g/l) were utilized for the maximum production of AgNPs. The absorbance peak becomes more confined and high with the increasing of concentration. The absorption intensity increases monotonically with increasing concentration of leaf extract. However, the high concentrations of polyphenols in the *C. viscosum* may successfully reduced the Ag^+ ions to Ag^0 which provided sufficient capping agent for the stabilization of the synthesized nanoparticles through steric hindrance and prevent their aggregation [15]. The highest absorption peak was observed when using 80 g/l *C. viscosum* leaf extract.

Reaction time

The reaction time was varied from 5 min to 210 min for the synthesis of nanoparticles. The UV-Vis spectra showed no peak within 5 min. UV-Vis spectra showed peak after 20 min. It can be inferred that at between 20 to 60 min the Surface Plasmon Resonance band is broadened due to slow conversion of Ag^+ to Ag^0 nanoparticles. Maximum production of nanoparticles was confirmed by maximum absorption in the UV-Vis spectra. A broad peak with increasing absorbance was observed at 150 min with 434 nm (Fig. 2b). However, additional increase in reaction time leads to distinguishable decrease in absorption intensity and wavelength which may be due to some aggregation of silver nanoparticles [16,17].

Temperature

Temperature is an important factor since it controls the nucleation process of nanoparticles configuration. The silver salt was reduced excellently with the increase of temperature, as confirmed by quick change in the color of the solution. The temperature of the reduction reaction was maintained at 40ºC, 50ºC, 60ºC, 70ºC and 80ºC. The absorbance of the resulting solution was measured spectrophotometrically. Fig. 2c shows that the AgNPs formation start at 40ºC and maximum absorbance observed at 60ºC. An increase in the yield of AgNPs was remarked when the temperature of the reaction was increased up to 60ºC, though beyond this there was a fall in absorbance. However, it was found that increasing the temperature of the reaction to 80ºC showing a decrease in absorption due to the development of crystals around the nucleus [18].

Concentration of silver nitrate solution

The silver ion concentration also affects the formation of silver nanoparticles. In this study different concentrations of $AgNO_3$ solution (0.002M, 0.004M, 0.006M, 0.008M, 0.01M and 0.015M) were utilized in order to maximize the yield to AgNPs, and the absorbance of the silver colloidal solution was monitored using the UV-Visible spectrophotometer. As shown in Fig. 2d, the peak intensity increased with the increase of silver nitrate concentration from 0.002M to 0.01M indicating faster rate of bioreduction with increased concentration of parent salt [19]. But more increase of silver nitrate concentration to 0.015M showed the peaks with lower intensity which may be for the formation of agglomerated nanoparticles and their settling. The nanoparticles settle down at higher concentrations due to the large number of silver particles present in small volume of solution which creates higher coalescence between silver nanoparticles and hence results in agglomeration [20]. The UV-Visible absorption spectra showed high absorbance peak intensity when the concentration of $AgNO_3$ solution was 0.01M.

Ratio of leaf extracts and silver nitrate solution

The advantage of change in volume of *C. viscosum* extract to silver nitrate was showed. The reaction was carried out at the optimum time and temperature. Fig. 2e shows the ratios of *C. viscosum* leaf extract and silver nitrate in the ranges of 1:1, 1:2, 1:3, 1:4, 1:5, 2:1 and 2:3 were utilized in order to find out the optimum composition for the preparation of AgNPs. Since reactants ratio was an important aspect affecting AgNPs fabrication and symmetrical nanoparticles preparation. The absorption intensity increases with increasing extract concentration. The small variations in the absorbance values signify changing particle size. When the concentration of *C. viscosum* extract to silver salt is in the ratio 1:5, a weak absorption band was found. The maximum silver nanoparticle formation observed in 1:1 ratio which was confirmed by the formation of highest peak in spectroscopy.

Effect of pH

pH of the solution is also very important factor for biosynthesis of AgNPs. The solution was regulated in various pH and the concentration of $AgNO_3$ was fixed at 0.01M and ratio of extract and $AgNO_3$ was 1:1. During this experiment, the solution pH was varied from pH 2 to 11 and nanoparticles formation process was monitored by UV–Vis spectrophotometer (Fig. 2f). The result showed that the rate of AgNPs formation increases with increasing pH up to 7 and then decreases. At neutral pH i.e. pH 7, the reaction started as soon as the silver nitrate was added (as observed by the change in colour) to the reaction medium and the AgNPs formation was completed within few minutes. At acidic pH i.e. pH 2, pH 5 the synthesis was very low [21]. But enhancing pH agglomeration was noticed at pH 9 and 11 in which decrease in absorption was observed. This result confirms that pH 7 is the most convenient for the formation of AgNPs using *C. viscosum* leaf.

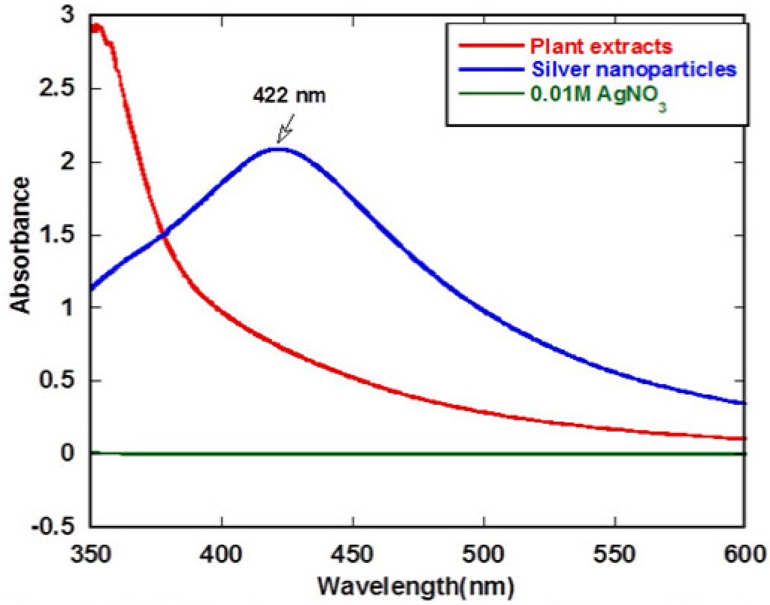

Fig. 3. UV-Vis absorbance spectra of the synthesized AgNPs using aqueous extract of *Clerodendrum viscosum* leaf

Characterization

UV-Visible absorption spectrum

UV-Vis spectroscopy is usually used to confirm the formation of AgNPs through green synthesis. The synthesized AgNPs give reddish brown colour due to the excitation of Surface Plasmon Resonance in AgNPs. In particular, absorbance in the range of 400 nm to 450 nm has been used as standard to indicate the reduction of Ag^+ to metallic Ag^0 [22]. The reduction of $AgNO_3$ into nanoparticles was showed an absorbance peak at around 422 nm with high absorbance which is very specific for the formation of silver nanoparticles Fig. 3.

Fourier Transform Infra-red

Fourier Transform Infra-red gives the information about the biofabrication of plant mediated silver nanoparticles [23]. FTIR spectrum of *C. viscosum* leaf extract was shown different major peak positions at 3462, 2896, 2037, 1654 and 729 cm^{-1} and FTIR spectrum of *C. viscosum* nanoparticles was shown different major peak positions at 3470, 2998, 2062, 1625, 1387, and 722 cm^{-1} (Fig. 4). The spectra with some minor shifts in peak position clearly specify the presence of the plant extract in the sample as a capping agent to the silver nanoparticles. The broad and intense peak at 3457 cm^{-1} corresponds to OH stretching vibrations of phenol/carboxylic group present in extract, a peak at 2093 cm^{-1} can be assigned to alkyne group present in phyto-constituents of extract. A peak at 2998 cm^{-1} is due to C-H stretching of alkanes. The peak located at 1635 cm^{-1} could be assigned to C=O stretching or amide bending. The peak at 1384 cm^{-1} assigned to nitro N-O bending and 733 cm^{-1} assigned to C–H alkenes stretch. The observed peaks were mainly attributed due to presence of some secondary metabolites like flavonoids, triterpenes, tannins and saponins excessively present in plants extract (Table 1) as also reported by other researchers [24,25].

X-ray Diffraction

XRD is very important instrument to determine the crystal structure and calculate the crystalline nanoparticle size [26]. Fig. 5(A) shows the silver nanoparticles synthesis at 25°C having three intense peaks were 38.3, 43.82 and 63.66 corresponding to the planes of (111), (200) and (220), respectively, which were in good agreement with reference to the unit cell of face centered cubic (FCC) structure of metallic silver (Joint Committee for Powder Diffraction Standards, JCPDS File No. 04-0783). Fig. 5(B) shows the silver nanoparticles synthesis at 60°C display three intense peak at 38.48, 44.38 and 64.62 corresponding to the planes of (111), (200) and (220), respectively. The average size of crystalline silver nanoparticle was determined from the XRD peaks by using the Debye-Scherrer formula [27]:

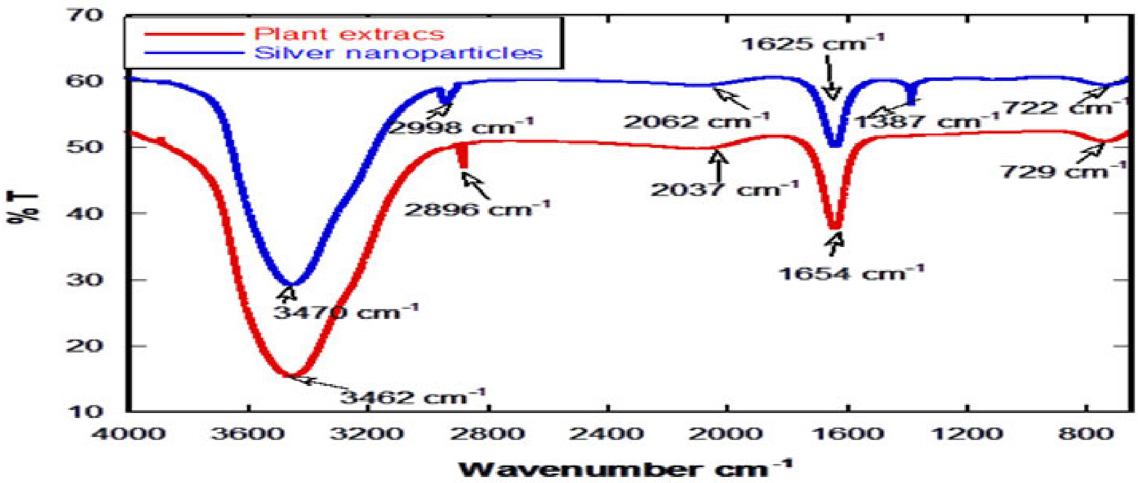

Fig. 4. FTIR spectrum of *Clerodendrum viscosum* extract and *Clerodendrum viscosum* silver nanoparticles

Table 1. Phytochemical test for aqueous leaf extract of *Clerodendrum viscosum*

Tested Phytochemicals	
Terpenoids	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Steroids	-

$D=0.94\lambda/ \beta \cos\theta$

Where, D is the average crystal size, λ is the X-ray wave length and β is the full width at half maximum and θ is the diffraction angle. The average size of synthesized NPs calculated from Debye-Scherrer formula was found to be 39 nm and 30 nm at 25°C and at 60°C, respectively.

Scanning Electron Microscopy analysis

Scanning electron microscopy is an important method for the morphological characterization of synthesized nanomaterials with a high degree of spatial resolution. It has been observed that the size differences, size distribution, and capacity for aggregation depend on experimental conditions, stabilities etc [28]. Fig. 6 (A) and (B) indicate the SEM image of the synthesized silver nanoparticles sample at 25°C and 60°C, respectively. SEM analysis shows that the nanoparticle formed was spherical in shape with average size 56 nm (36 nm -74 nm) at 25°C and 53 nm (45 nm-64 nm) at 60°C.

Transmission Electron Microscopy

The size, shape and morphology of the synthesized silver nanoparticles were determined with the help of transmission electron microscopy. Fig. 7 (A) and (B) show the TEM images of the AgNPs synthesized at 25°C and 60°C temperature, respectively. The AgNPs formed at 25°C are almost spheroidal in shape with a wide size distribution average size 28 nm (19 nm to 39 nm) and at 60°C average particle size 24 nm (14 nm to 38 nm). Most of them were spheroidal and decreased for the higher temperature (60°C) revealing that increasing the reaction temperature leads to AgNPs with narrow size distribution.

Dynamic Light Scattering

It is used to determine the size distribution, surface charge and quality of nanoparticles. It is also convenient to understand the Poly dispersity index (PDI) of the Ag-NPs. Dynamic Light Scattering measure hydrodynamic diameter, which is the diameter of the ions and particle or molecules that are attached to the surface and moves with the AgNPs in solution. PDI measures the homogeneous of nanoparticles. The smaller the PDI the more homogeneous nanoparticles

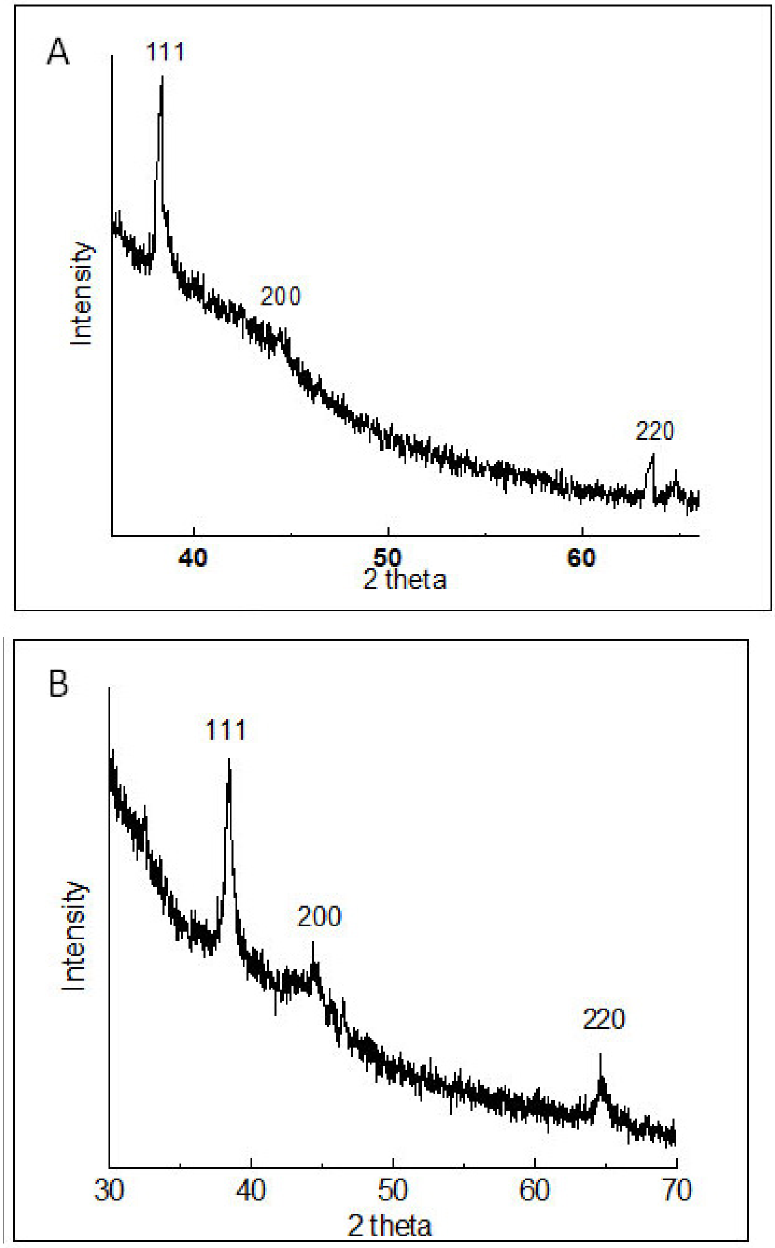

Fig. 5. XRD Spectra of Silver nanoparticles synthesis from *Clerodendrum viscosum* leaf extract at (A) 25ºC (B) 60ºC

produced. Nanoparticles with PDI value smaller than 0.5 is considered acceptable for drug delivery [29].It is an indicator of aggregation in the particles as the value is more it shows a polydisperse system. The hydrodynamic size and PDI value are shown in Table 2.

Antibacterial activity of AgNPs

Silver nanoparticles have been using in many industries such as the pharmaceuticals, water treatment, food storage and paint due to its antimicrobial activities [30]. It is apparent that diameter of inhibition zone was increased by increasing the concentration of AgNPs against tested organisms, which differed from 10 to 17 mm. Aqueous leaf extract showed some moderate activity with zone of inhibition in between 6 to 9 mm, and 0.01M $AgNO_3$ (40µg/disc) did not

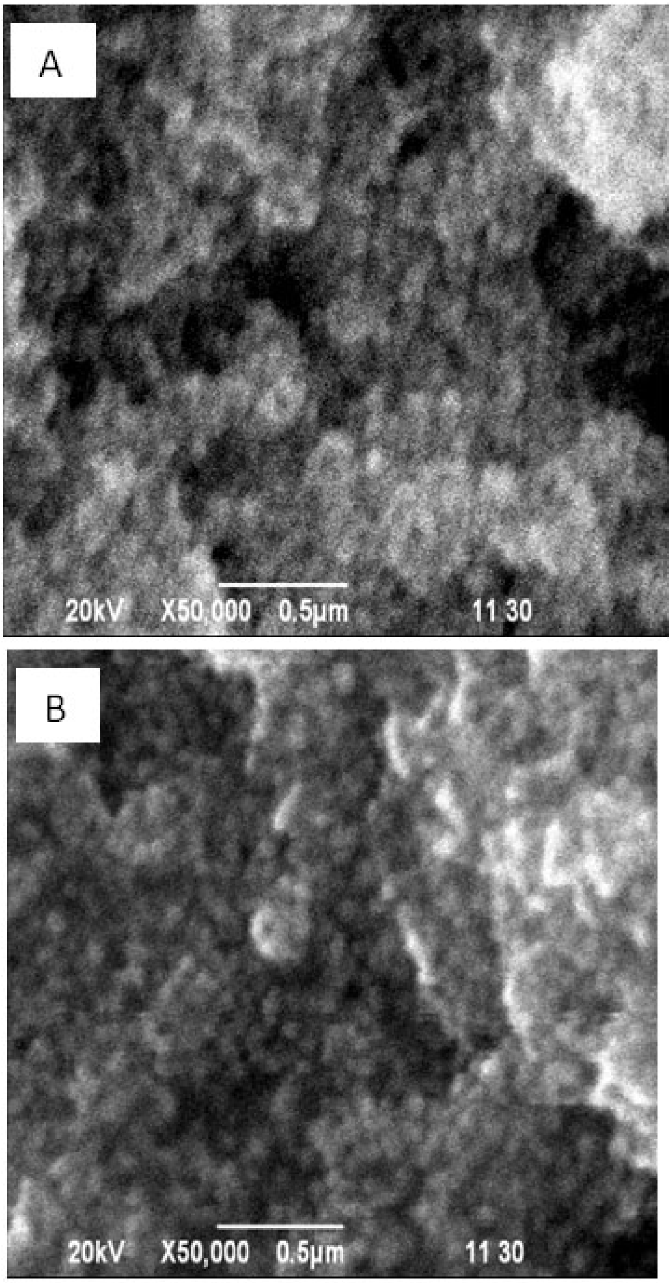

Fig. 6. SEM image showing surface morphology of the synthesized silver nanoparticles at (A) 25°C and (B) 60°C

show activity against any of the bacteria (Table 3). The antibiotic tetracycline was used as standard antibacterial agent against all microbes. From the results, it concludes that silver nanoparticles have significant activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* whereas less against *Bacillus subtilis*.

CONCLUSION

Clerodendrum viscosum leaf extract can be very effective for the biosynthesis of silver nanoparticles. Various parameters influencing the formation of silver nanoparticles like concentration of silver nitrate solution, concentration of plant extract, time, temperature and pH of the reaction mixture.

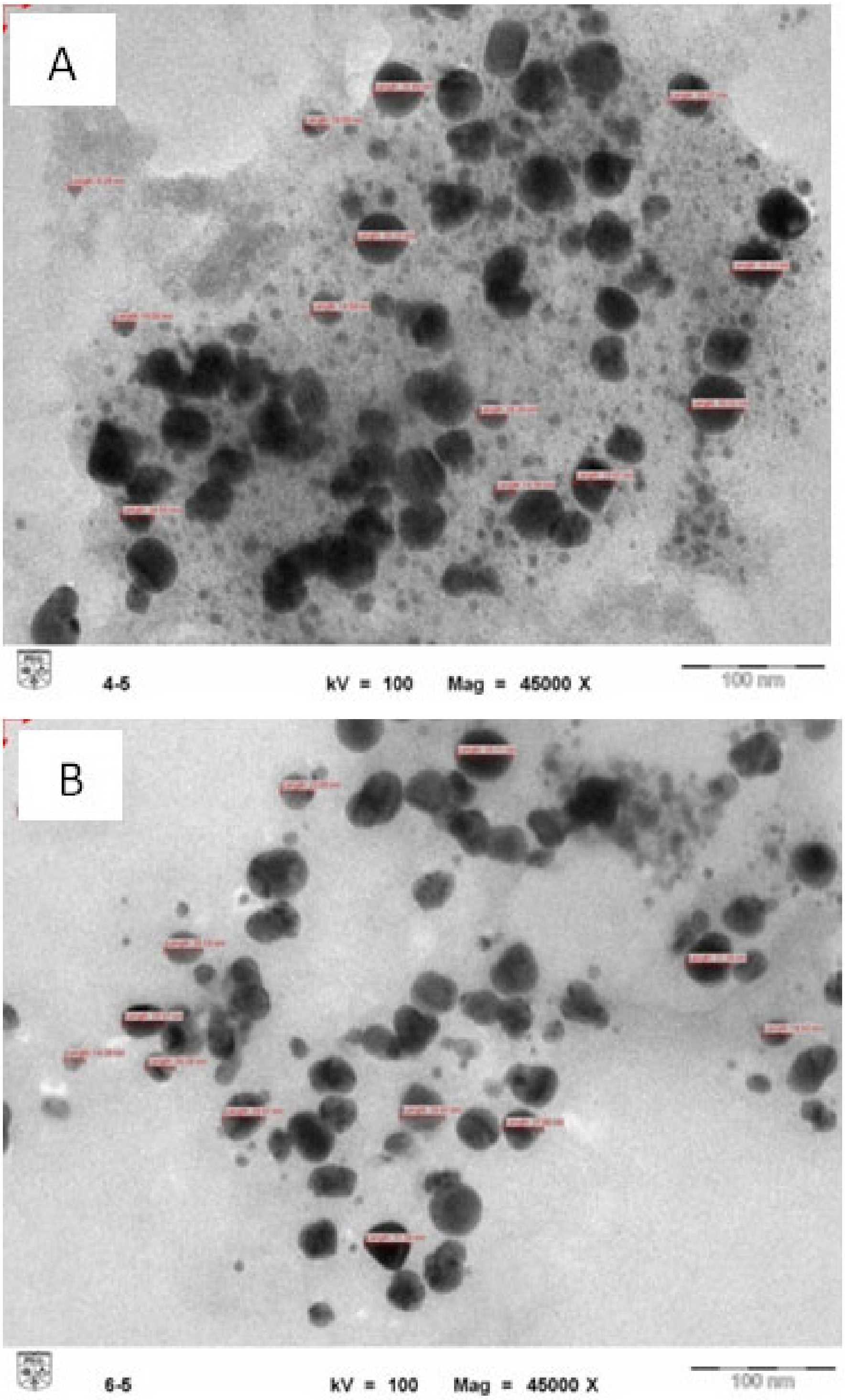

Fig. 7. TEM image for synthesized silver nanoparticles at (A) 25ºC and (B) 60ºC

Table 2. Size and PDI value for synthesized silver nanoparticle

Clerodendrum viscosum	Hydrodynamic Size	PDI
AgNPs at 25 ºC	173 nm	0.332
AgNPs at 60 ºC	166 nm	0.37

Table 3. Antibacterial activity of tested samples at different doses

Name of bacteria	Zone of inhibition (mm)					
	Plant extracts	$AgNO_3$	Antibiotic (tetracycline)	AgNPs		
	40µg /disc	40µg /disc	10µg /disc	30µg /disc	40µg /disc	50µg /disc
E. coli	6 ± 0.5	-	10 ± 1.2	11 ± 0.5	12 ± 1.1	13 ± 0.5
Pseudomonas aeruginosa	9 ± 1.1	-	12 ± 0.4	15 ± 1.2	17 ± 0.5	17 ± 0.7
Bacillus subtilis	7 ± 0.6	-	9 ± 0.5	10 ± 1.0	11 ± 0.6	12 ± 0.4
Staphylococcus aureus	7 ± 1.0	-	11 ± 1.1	13 ± 0.6	14 ± 1.0	16 ± 1.1

Values are given as mean ± S.D. (n = 3).

The nanoparticle was separated by centrifugation while characterization was done by UV-Vis spectra, XRD, SEM, TEM and DLS methods. It was found that maximum absorbance peaks of silver nanoparticles occur at 422 nm, indicating the formation of AgNPs. The FTIR results found various phytochemicals responsible for the rapid reduction of ions, leading to AgNPs synthesis. The synthesized AgNPs were also evaluated for antibacterial activity against some bacteria. It was found that the AgNPs showed moderate to very good bactericidal properties to tested bacteria. Finally, this study demonstrated an eco-friendly, cost-effective and rapid approach to the synthesis of AgNPs by using aqueous leaf extract of *Clerodendrum viscosum*.

ACKNOWLEDGEMENTS

This work was carried out with support of National Science and Technology Ministry, Bangladesh.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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