
Polysaccharide capped antibacterial silver nanoparticles synthesis using green chemistry

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Abstract: Advancement of an environment friendly, trustworthy, and speedy route for the production of Ag-NP using natural system is an essential urge in nanotechnology. Biological synthetic techniques are considered as a better alternative over other conventional methods. Silver is a safe inorganic element that is projected as 'next-gen' antimicrobial agent and had been extensively used against several bacterial strains from the ancient times. Here, we document a low-cost green synthesis approach for construction of Ag-NP using fruit extracted polysaccharide of *Bruguiera cylindrica*, a mangrove plant of Sundarban. During the investigation of GC-MS an adequate amount of glucose was found as major carbohydrate molecule in the extracted polysaccharide. Synthesised Ag-NP were also characteristically described by UV-Vis, DLS, TEM, EDAX, XRD and FTIR. The average diameter of the Ag-NP was 4.5 ± 1 nm. Additionally, antibacterial nature of Ag-NP was also found out against some of the pathogenic gram-positive and gram-negative bacteria.

Keywords: green synthesis; *Bruguiera cylindrica*; polysaccharide; silver nanoparticle; bacterial growth.

Reference to this paper should be made as follows: Sarkar, J., Maity, G.N., Khatua, S., Mondal, S. and Acharya, K. (2020) 'Polysaccharide capped antibacterial silver nanoparticles synthesis using green chemistry', *Int. J. Nano and Biomaterials*, Vol. 9, Nos. 1/2, pp.80–94.

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1 Introduction

In nanotechnology, when a particle performs the similar characteristic features and transports properties like the whole unit then it is indicated as a small object. Depending on the size, it is furthermore categorised into three classes such as fine particles, ultrafine particles and nanoparticles (Taylor et al., 2013). In respect of diameter, the fine particles are specified in between the scale of 100–2,500 nanometres, while for both ultrafine particles and nanoparticles; it is ranged in between 1–100 nanometres. But the nanoparticles probably be or not be revealed the same size-related characteristic features that diverged considerably from those detected in fine particles or bulk materials (Taylor et al., 2012, 2013; Hewakuruppu et al., 2013). Like the nanoparticles, when the distribution of size of any clustered material ranges in single dimension between 1–10 nanometres, it is specified as nanoclusters whereas the assembling of these nanoclusters is recognised as nano-powders. In a similar way, the single nano-sized crystals or ultrafine single-domain particles are recognized as nanocrystals (Fahlman, 2011). Over the recent past, the field of nanotechnology has introduced a new age of science which involves creation of materials near atomic scale with unique optical, chemical, physical and thermal features (Mohammadlou et al., 2016).

Silver is a safe inorganic element that has the capability to kill more than 650 variants of disease causing microorganisms (Jeong et al., 2005). The observed antimicrobial feature of Ag-NP is a growing interest in the arena of microbiology (Choi et al., 2008). This Ag-NP have been projected as ‘next-gen’ antimicrobial representatives (Rai et al., 2009b). As a consequence, metallic silver made a significant recovery in form of Ag-NP with powerful antimicrobial effects. Investigations have revealed that after exposure to bacteria, the nano-silver gets adhered to cell membrane, penetrates inside and attacks respiratory chain that finally causes to cell death. In addition, these particles discharge silver ions inside cells which hinder the ability of bacterial DNA synthesis and causes

deactivation of proteins containing thiol groups (Rai et al., 2009a; Ydollahi et al., 2016). This has unfolded novel strategies to use pure silver against many antibiotic resilient microorganisms and due to this, it has been adopted in many commercial products like topical ointments, toothpaste, soap, socks etc. (Ahmed et al., 2016; Zhao et al., 2016). In addition, Ag-NP has also emerged up as a promising agent for waste water disinfection system (Bora and Dutta, 2014). In a recent work, the use of antimicrobial silver compounds against coliform bacteria of waste water has been well established (Jain and Pradeep, 2005).

So far, a number of conventional physical and chemical techniques are available for Ag-NP synthesis; although majority of them are expensive, complicated, energy generative and involve use of hazardous chemicals (Ayyub et al., 2001; Backman, 2005; Kimling et al., 2006; Dong et al., 2010; Rajeshkumar, 2016). Conversely, the biological techniques are recognised as an advance and better replacement over other methods as it involves natural reagents like sugars, biodegradable polymers, plant extracts, microorganisms (Saha et al., 2010; Sarkar et al., 2011b, 2012, 2014, 2017; Acharya and Sarkar, 2014; Railean-Plugaru et al., 2016; Sarkar and Acharya, 2017). Previously, several attempts have been made to synthesise Ag-NP by using polysaccharide extracted from different plants (El-Rafie et al., 2013; Sathiyarayanan et al., 2013; Sanyasi et al., 2016; Vasquez et al., 2016; Maity et al., 2019). But we have first time made this attempt to synthesis Ag-NP by using the polysaccharide extracted from a mangrove plant. Polysaccharides from mangrove plants such as *Bruguiera cylindrica* (L.) Blume can be used as a bio-factory for synthesis of nanoparticles because these are extremely stable, benign, non-toxic and with known biological activities (Maity et al. 2019). In this context, the mangrove plant, *Bruguiera cylindrica* (L.) Blume, could be a better substitute as it was scientifically proved that it has numerous bioactive components. It has been traditionally used as remedial measures for hepatitis, diabetes, wounds, ulcers, boils, diarrhoea, angina and dysentery (Kathiresan and Ramanathan, 1997; Bandaranayake, 1998). Recent investigations have reported that *Bruguiera cylindrica* possesses antioxidant (Banerjee et al., 2008), antinociceptive (Uddin et al., 2005), antidiabetic (Nabeel et al., 2010) and antimicrobial properties (Sakagami et al., 1998; Ravikumar et al., 2010). Thus, the recent work was aimed to produce Ag-NP by a greener method using the fruit extracted polysaccharide of *Bruguiera cylindrica* (L.) Blume, characterisation of the particles and appraisal of antibacterial nature.

2 Materials and methods

2.1 Separation of polysaccharide from fruit extract of *B. cylindrica*

Fresh and healthy fruits were accumulated from the mangrove forest of Sundarban, West Bengal. To remove all noticeable unwanted dust particles, the fruits were carefully washed with tap water and distilled water respectively. After that, they were desiccated and sliced into small pieces. 750 gm of these finely sliced fruits were steeped into distilled water (volume 250 millilitres) and simmered for five hours. The subsequent suspension was retained at 4°C for nightlong and then filtration was achieved by nylon cloth. Precipitation of the polysaccharide was made by the inclusion of five volumes of ethanol (99% alcohol). The precipitate was retained nightlong at 4°C. After centrifugation at 8,000 rpm for ten minutes, the precipitate was again liquefied in distilled

water and dialysed through DEAE cellulose bag for two hours to remove low molecular weight polysaccharides. Further, the lyophilised extract was exposed to sephadex G-100 gel permeation column (50×1.5 cm) using water as eluent (flow rate 0.5 millilitres/min). The eluate was accumulated (2 millilitres/tube) and carbohydrates were unveiled by phenol-sulphuric acid method (Masuko et al., 2005; Mecozzi, 2005). Finally, polysaccharide was pooled from test tube number 15–30 and freeze-dried.

2.2 *Physical and chemical characterisation of fruit extracted polysaccharide*

The entire content of sugar was calculated by phenol sulphuric acid method. Glucose was provided as standard. The sum total of protein was estimated by Bradford using BSA as reference. Gallic acid was defined as a reference to enumerate the total amount of phenolic amalgam from the polysaccharide using Folin-Ciocalteu reagent. All the data were presented as gram of standard equivalents per 100 gram of dry polysaccharide (Saha et al., 2013). To establish the monosaccharide composition, 2 milligrams polysaccharide was hydrolysed with 2M TFA at 100°C for two hours in screw cap vial. TFA was eliminated by desiccation at 55°C by bringing down the pressure (Rotavapor R3, Butchi, Switzerland). To dissolve hydrolysate, 50% ethanol (1 millilitre) was included to the vial. The centrifugation was performed for five minutes at 12,000 rpm to remove non-hydrolysed polysaccharide and further scrutinised by GC-MS as conveyed in our earlier publication (Khatua and Acharya, 2016).

2.3 *Synthesis of Ag-NP*

AgNO₃ (> 99.9% pure) was procured from Merck, India. For Ag-NP synthesis, 20 millilitres of 0.5 milligrams/millilitre of isolated polysaccharide was mixed with 20 millilitres of 1 mM AgNO₃ solution and stirred with magnetic stirrer for 90 min at room temperature. The colour change from pale yellow to brown specified the generation of the Ag-NP due to reaction of polysaccharide with silver metal ion. Simultaneously, the polysaccharide solution without any addition of AgNO₃ (positive control) and only the AgNO₃ solution (negative control) were kept by following the similar environment of reaction solution.

2.4 *Characterisation of synthesized Ag-NP*

The generation of Ag-NP was established by UV-Vis spectrophotometer (Hitachi 330 spectrophotometer) with plasmon peaks at various regions of the spectral range 200–900 nm which corresponded to different signature marks for different nanoparticles respectively. The particle size was found out by using Zen 1600 Malvern nano-size particle analyser ranging between 0.6 nm and 6.0 μ m. For XRD measurement, the spectra were logged in a Panalytical X'Pert Pro X-ray Diffractometer (Cu K α radiation, λ 1.54) running at 40 mA and 45 kV. The diffracted intensities were documented from 35° to 90° 2 θ angles. EDX analysis of Ag-NP was performed by the Hitachi S 3400N instrument. For FTIR analysis, the dried Ag-NP were mixed with KBr at a ratio of 1:100. Furthermore, the prepared pellet was viewed by Shimadzu 8400S FTIR spectrophotometer. The spectral range was set down in between 4,000 cm⁻¹ and

400 cm^{-1} . In addition, the synthesised particles were examined and visualised by TEM using Tecnai G2 spirit Biotwin instrument (FP 5018/40), operating at around 80 kV accelerating voltage.

2.5 Analysis of effect of synthesised Ag-NP on some pathogenic bacteria

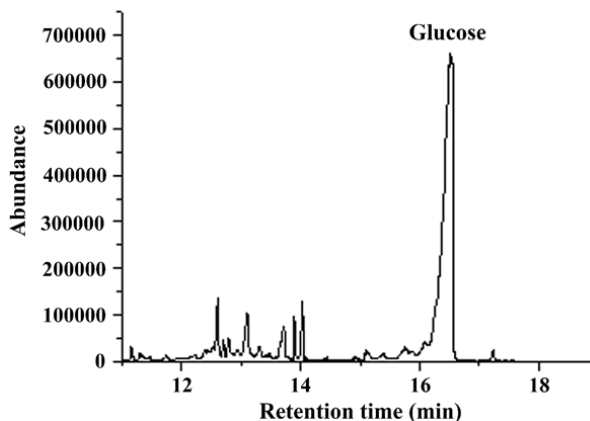
Staphylococcus aureus ATCC® 700699™, *Bacillus subtilis* ATCC® 6633™ (MTCC 736), *Listeria monocytogenes* ATCC® 19111™ (MTCC 657), *Escherichia coli* ATCC® 25922™ and *Salmonella typhimurium* ATCC® 23564™ (MTCC 98) were utilised for the experiment. The antibacterial effect was estimated by determining MIC values according to microdilution method (Stojković et al., 2013). The five investigating bacteria were cultured freshly and 1×10^5 CFU/millilitre concentrated dilutions were constructed separately. Reactions were performed in 96 well plates consisting of 200 μl of NB, 20 μl of inoculum and different dilutions of nanoparticles. Following incubation for one day at 37°C, 40 μl of INT dye (0.2 milligrams/millilitre) was mixed and incubated for a next round of 30 min. The concentration that inhibited 50% progression of bacteria growth as compared with positive control was calculated as MIC value. Streptomycin was cast-off as a standard drug.

3 Results and discussion

3.1 Physical and chemical characterisation of isolated polysaccharide

The extractive yield of polysaccharide from fruit extract of *B. cylindrica* was $0.026 \pm 0.006\%$ of dry matter. Total carbohydrate content was 51 ± 5 gm/100 gm of the dried polysaccharide whereas the total estimated protein content was 4 ± 0.06 gm/100 gm of the respective sample. Very negligible percent of phenol was detected (0.5 ± 0.05 gm/100 gm of dry polysaccharide). Further, the molecular contents were finalised by GC-MS where glucose was detected as the dominant monomer (Figure 1).

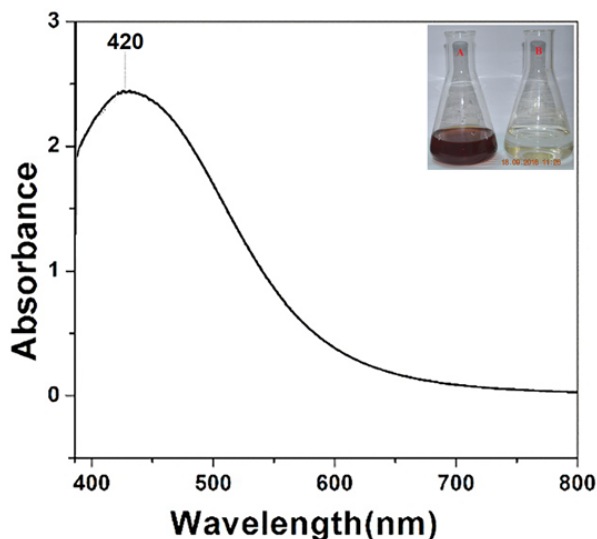
Figure 1 GC-MS of polysaccharide extracted from fruits of *Bruguiera cylindrica*



3.2 Synthesis of Ag-NP

The characteristic brown colour of reaction mixture, a signature mark for the generation of Ag-NP in the solution, arose due to the collective vibration of free electrons of Ag-NP in resonance with light wave. The SPR absorption band of reaction solution in the visible spectral region of electromagnetic radiation further confirmed the claim of the formation of Ag-NP (Sarkar et al., 2011a). The generation of Ag-NP by reduction of the metal ions during exposure of 20 millilitres of 0.5 milligrams/millilitre of the polysaccharide isolated from *Bruguiera cylindrica* into 20 millilitres of 1 mM AgNO₃ solution, was detected by brown colour formation (Figure 2 inset).

Figure 2 UV-Vis spectrum of the synthesised silver nanoparticles (see online version for colours)



Notes: Inset: represents (A) colour change of reaction mixture of the polysaccharide with AgNO₃ solution after 24 hours and (B) only AgNO₃ solution.

3.3 UV-Vis spectrophotometric analysis of biosynthesised Ag-NP

The evolution of silver from Ag⁺ ions to Ag⁰ state was categorised for spectral analysis. Broad and strong SPR band of reaction solution was obtained in the visible spectrum at 420 nm, which was specific for Ag-NP (Figure 2). Furthermore, this spectral analysis advocated that the Ag-NP were not in aggregated form. They scattered very well in the suspension (Sarkar et al., 2011a).

3.4 Analysis of size distribution of the Ag-NP by DLS analyser

The DLS measurement was performed to get the knowledge of size of the Ag-NP [Figure 3(a)]. Observations revealed the heterogeneous nature of the dispersed Ag-NP, with a size distribution between 1–10 nm.

3.5 Analysis of the crystallinity of Ag-NP by X-ray diffraction

The XRD measurement often proves to be a useful investigative device for newly formed compounds and their phases. Crystallinity of Ag-NP was established by this analysis. The XRD spectra of Ag-NP displayed four identical appearing at $2\theta = 38^\circ$, 44° , 64° and 78° conforming to the (111), (200), (220) and (311) facets of silver, respectively (JCPDS card file no. 04-0783) (Saha et al., 2010) [Figure 3(b)].

Figure 3 (a) Particle size distribution of bioreduced silver nanoparticles (b) representative XRD pattern of silver nanoparticles (see online version for colours)

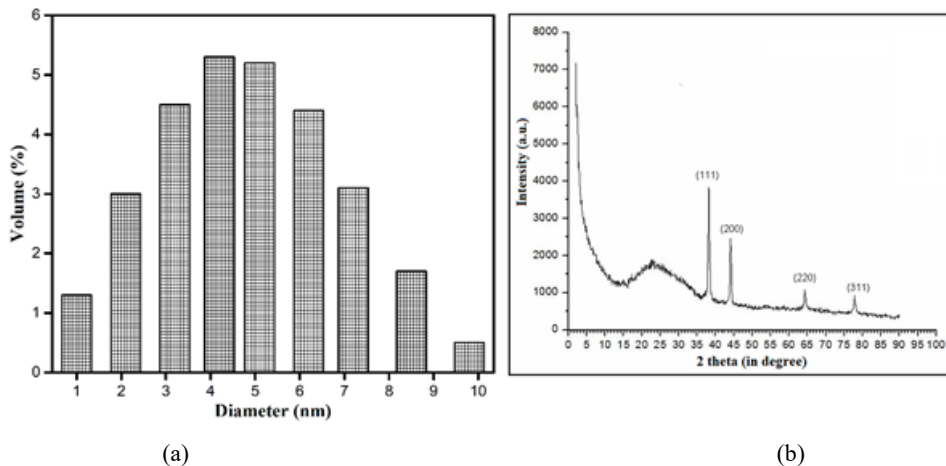
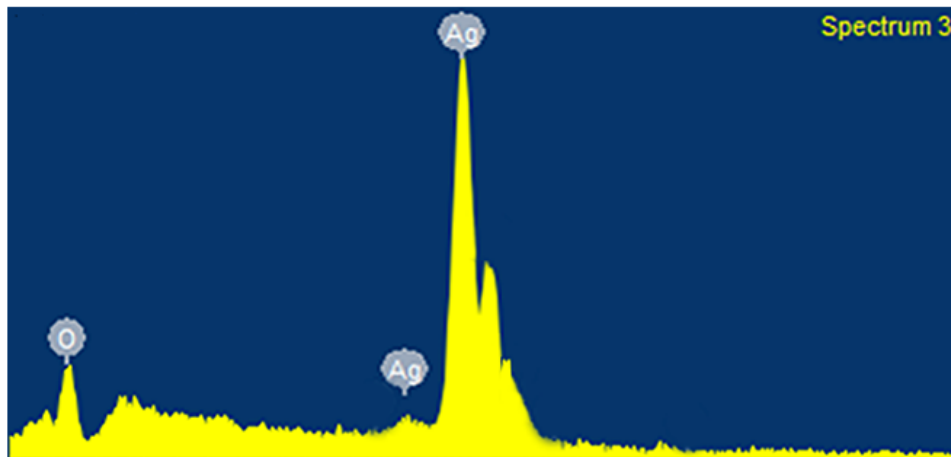


Figure 4 EDX spectrum of silver nanoparticles (see online version for colours)



3.6 Investigation of elemental framework of the produced Ag-NP by EDX

In Figure 4, a sharp wide peak characteristic to silver was observed around 3–4 keV (Magudapathy and Gangopadhyay, 2001; Durán et al., 2005; Jaidev and Narasimha,

2010; Banu et al., 2011). The existence of a sturdy signal from Ag atoms (85.29%) finalised that the produced nanoparticles were solely made by silver. Other EDX peaks like the peaks of oxygen also advocated that they were mixed precipitates of the polysaccharide and silver salt. XRD analysis also agreed with the generation of nano silver.

3.7 FTIR study of isolated polysaccharide and bio-synthesised Ag-NP

Figure 5 shows the FTIR absorption spectra for the synthesised Ag-NP [Figure 5(a)] and untreated polysaccharide [Figure 5(b)] respectively. Both the spectra exhibited the existence of bands around $3,430\text{ cm}^{-1}$ and $1,620\text{ cm}^{-1}$ signifying the O-H stretching and C=C group respectively (Jin and Bai, 2002; Socrates, 2004; Sanghi and Verma, 2009; Sathyavathi et al., 2010). The intensity of these peaks was revealed the chief role of O_2 -containing functional groups in the reduction of Ag^+ ions in contrast to untreated polysaccharide [Figure 5(a)] where the peak of the $-\text{COOH}$ (carboxyl) group was shortened due to interaction with the surface of the Ag-NP (Ebrahiminezhad et al., 2016b). In the 5B spectrum, bands observed at around $2,920$, $1,450$ and $1,250\text{ cm}^{-1}$ indicated the aldehydic C-H stretching, $-\text{COO}$ stretching and C-C stretching vibration respectively (Barth, 2007; Sathyavathi et al. 2010). Due to diminution of Ag^+ to Ag^0 , the peaks at around $2,920$, $1,450$, $1,250$ and $1,070\text{ cm}^{-1}$ were eliminated from the 5A spectrum (Ebrahiminezhad et al., 2016b). A new peak visible only at $1,380\text{ cm}^{-1}$ in Figure 5(a) showed the existence of $-\text{NO}_3$ which was derived from AgNO_3 (Sarkar et al., 2011a; Ebrahiminezhad et al., 2016b). The bands noticed in the 500 to 750 cm^{-1} spectral region indicated the existence of R-CH group (Singh et al., 2010). The characteristic peaks for C=O stretching vibration appeared at $1,745\text{ cm}^{-1}$ was shifted to $1,760\text{ cm}^{-1}$ in the spectrum of Ag-NP which framed that the carbohydrates performed a major role both as a diminishing as well as capping agent of the Ag-NP (Dasgupta et al., 2017). Majority of the carbohydrates have variety of O_2 -containing functional groups, like carbonyl, phenolic, hydroxyl and carboxylic groups. Silver ions displays a powerful attraction to these functional moieties via coordination or electrostatic interactions. These interactions allow electrons to pass to the Ag^+ ions resulting in nucleation and growth of Ag-NP (Ebrahiminezhad et al., 2016a).



In lieu of the above explanation it can be itemised that the carbohydrates present over the Ag-NP surface may also acts as capping agent for their stabilisation. The result of this spectroscopic analysis established that the carbohydrates extracted from the plant, *Bruguiera cylindrica*, has the capability to perform the function regarding the stability of the Ag-NP over long period.

3.8 TEM analysis of the Ag-NP

Figure 6(a) displays the TEM image of the Ag-NP with a diverse range of their sizes which were produced after the bioreduction of the AgNO_3 solution by the

polysaccharides. These findings inferred that the produced Ag-NP were homogeneous and polydisperse in nature and majority of them were spherical in shape. The diameters of these Ag-NP were in the range of 1–10 nm. The mean diameter was observed to be 4.5 ± 1 nm. The bright circular spots in the SAED pattern further confirmed the single crystalline nature of the Ag-NP [Figure 6(b)].

Figure 5 FTIR absorption spectra of, (a) synthesised silver nanoparticles (b) untreated polysaccharide

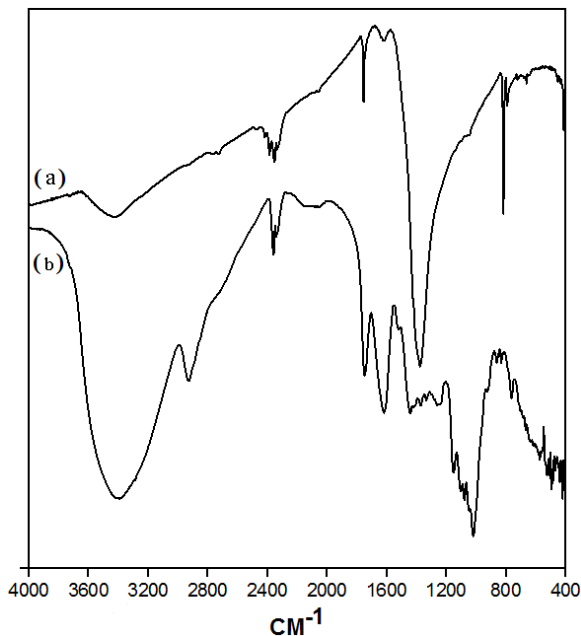


Figure 6 (a) Transmission electron microscopic image of silver nanoparticles (b) SAED patterns of crystalline silver nanoparticle (see online version for colours)

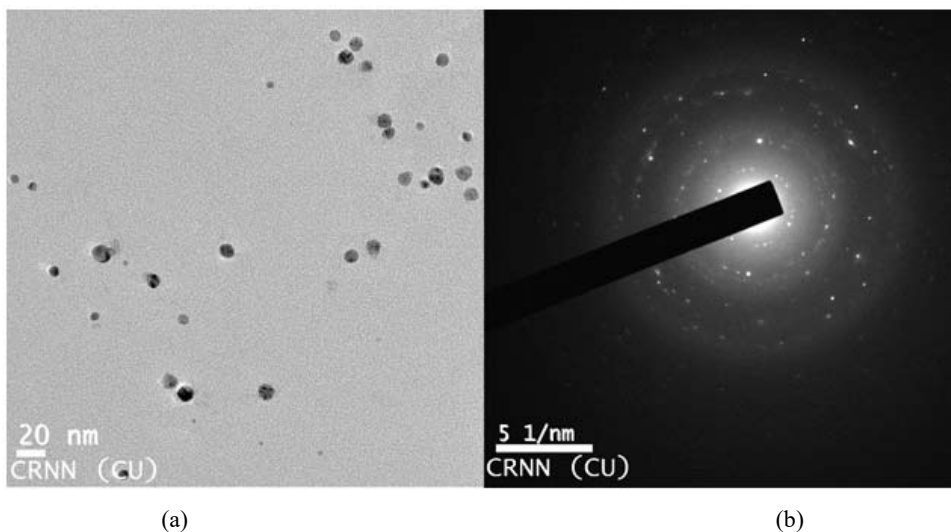


Table 1 Antibacterial activity of synthesised silver nanoparticles as determined by minimum inhibitory concentration (MIC) value ($\mu\text{g/ml}$) (mean \pm SD; n = 3)

Type of bacteria	Name of bacteria	Minimum inhibitory concentration by	
		Silver nanoparticles	Streptomycin
Gram-positive	<i>Listeria monocytogenes</i>	28.31 \pm 2.4	4.68 \pm 0.17
	<i>Bacillus subtilis</i>	30.14 \pm 5.43	5.61 \pm 0.01
Gram-negative	<i>Escherichia coli</i>	32.72 \pm 9.53	5.41 \pm 0.11
	<i>Salmonella typhimurium</i>	42.83 \pm 0.08	5.09 \pm 0.03
	<i>Klebsiella pneumoniae</i>	39.69 \pm 1.67	5.294 \pm 0.143

Note: Streptomycin was considered as a positive control.

3.9 Analysis of effect of polysaccharide based Ag-NP on some pathogenic bacteria

The antibacterial nature of Ag-NP was studied against both gram-positive and gram-negative bacteria using microdilution method. As unveiled in Table 1, the growth of all experimental strains was affected in appearance of the Ag-NP in contrast to negative control. In case of *B. subtilis*, a gram-positive bacterium, introduction of 25 μg /millilitre of polysaccharide capped Ag-NP caused 50.74 \pm 3.56% of reduction of bacterial density. Interestingly, growth of all examined gram-negative bacteria were also inhibited in presence of similar doses of the nanomaterials. The treatment of 50 μg /millilitre of synthesised Ag-NP showed maximum inhibition 62.32 \pm 2.86 and 65.98 \pm 6.57% with reference to *E. coli* and *K. pneumoniae* respectively. The antibacterial activity was not represented by inhibitory zone, rather by determination of minimum inhibitory concentration (MIC). Thus, the value was higher in case of experimental sample which means superior concentration was required for the sample to inhibit bacterial growth in comparison with the standard drug. These findings indicated the strong antibacterial potentiality of synthesised Ag-nano.

4 Conclusions

This study described biosynthesis of stable Ag-NP using polysaccharide from fruit extract of *B. cylindrica* plant. The generation of biosynthesised nanomaterials was established by UV-Vis, XRD, FTIR. The green synthesised Ag-NP presented a dynamic antibacterial nature antagonistic towards some pathogenic gram-positive (*L. cytomonogenes*, *B. subtilis* and *S. aureus*) and gram-negative (*S. typhimurium* and *E. coli*) bacteria. Thus, the green approach for the production of Ag-NP using plant polysaccharide was an environment friendly and low cost method in contrast to the conventional physical and chemical synthesis techniques.

Acknowledgements

Joy Sarkar and Gajendra Nath Maity have equal contribution.

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Abbreviations

Ag	Silver
Ag-NP	Silver nanoparticles
AgNO ₃	Silver nitrate
BSA	Bovine serum albumin
DLS	Dynamic light scattering
EDX	Energy dispersive X-ray
FTIR	Fourier transform infrared,
GC-MS	Gas chromatography-mass spectrometry
MIC	Minimum inhibitory concentration
KBr	Potassium bromide
SAED	Selected area electron diffraction
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
XRD	X-ray diffraction
UV-Vis	UV-Visible