An Early Year History of Biological Preparation of Silver Nanoparticles in West Bengal and their Antibacterial Activity: A Review

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ABSTRACT

Biologically prepared silver nanoparticles are in trend to be used as antibacterial agents throughout the globe. Silver nanoparticles prepared from different biological sources have been tested against Staphylococcus aureus, Escherichia coli, and other clinical bacteria in West-Bengal also. The size, shape and activity of the biogenic silver nanoparticles will vary depending upon the biological sources and its concentration used for nanoparticle preparation. UV-Vis spectrophometry, Dynamic light scattering, FESEM, HRTEM are the techniques which can be used for characterizing silver nanoparticles of different size and shape. From the history of last decade of research upon silver nanoparticles' green synthesis and its antibacterial, antifungal, antilarval as well as anticancer agents, researchers used plant parts, fungus and bacteria as biological sources for the reduction of silver ions to silver nanoparticles. Which showed promising activity against different bacterial strains, either procured from ATCC (American type culture committee) or from any clinical sources. When it comes to analyse the activity of the prepared silver nanoparticles against multidrug-resistant (MDR) clinical bacterial strains, there are lesser evidences from West-Bengal. This review will work as a reservoir for biologically prepared silver nanoparticles in West-Bengal in the last decade and will also help researchers to characterize biogenic silver nanoparticles.

Keywords: Biogenic Silver Nanoparticles, Characterizing Silver Nanoparticles, Staphylococcus Aureus, West-Bengal.

INTRODUCTION

From ancient time silver and its components had been used as bactericidal agents against gram positive and gram negative bacteria.[1,2,3,4] The first concept of nanoparticles and nanoparticle based drug targeting was born from one of the eminent scientist Paul Ehrlich from an opera; he had visited.[5,6] Nanoparticles can be defined as any particulate matter of size less than 100nm at any dimension.[7] Multiple reports confirm the highly toxic nature of silver ions and silver based products against various microorganisms, including 16 species of bacteria.[4,8,9,10] AgNPs (silver nanoparticles) along with other noble metal nanoparticles are widely applied in cosmetics, shampoo, toothpastes and other biomedical products which directly come in contact with human body.[11] Chemically reduced AgNPs have severe side effects on human health. So, biologically synthesized nanoparticles are widely suggested as possible ecofriendly alternatives to chemically or physically synthesized nanoparticles.[12] There are evidences of photosynthesis of silver and gold nanoparticles from coriander leaves.[13] Sundried *Cinnemomum camphora* leaves[14], phylanthin extract[15], henna leaves[16], tulsi leaves[17], papaya fruit extract[18], are also able to be used for biogenic synthesis of silver nanoparticles. Other than the above mentioned extracts, a lot more plants are available to be used to produce silver nanoparticles efficiently, such as *Azadirachta indica*[19], *Catharanthus roseus*[20], *Datura metel*[21], *Nelumbo nucifera* (lotus)[22], *Medicago sativa*[23], *Alternanthera denate*[24], *Cymbopogon citrates*[25], *Argyreia nervosa*[26], *phlomis*[27], *Aloe vera*[28], *Moringa oleifera*[29], *Ziziphora tenuior*[30], *Centells asiatica*[31], *Vitex negundo*[32], *Swietenia mahagoni*[33], *Boerhavia diffusa*[34], *Cocos nucifera*[35], *Brassica rapa*[36], *Melia dubia*[37], *Pogostemon benghalensis*[38], *Garcinia mangostana*[39], *Psoralea corylifolia*[40], etc.

With the increasing trend of using silver nanoparticles (AgNPs) as antibacterial agents, we are trying to see the scenario of biogenic preparation of AgNPs at a glance in West Bengal, India. This review includes the biological methods used by researchers to prepare AgNPs, their characterization and their antibacterial effect upon clinical isolates taken from different bacterial infection sites of patients of various hospitals and pathological laboratories of West Bengal or procured from ATCC.

Article availability

Google search with keyword "silver nanoparticles synthesis west Bengal" gave a lot of search results. From them only 31 relevant articles were selected, among which 22 papers were published from west Bengal between 2010-2019, upon green synthesis of silver nanoparticles. Articles selected for the study were published in nature, springer, wiley and other renowned and well established journals.

How far West Bengal is preparing silver nanoparticles from biological sources in recent decade (table 1)

Characterization methods: From table[41-59], we can say that a lot of ways and techniques are available to characterize silver nanoparticles. The first characterization will be done by observing change in colour after reduction of silver ions to AgNP, due to SPR (surface Plasmon resonance) property

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Biogenic synthesis of silver nanoparticles and its properties						
Plant/Organism used	**Plant parts used**	**Characterization methods used**	**Size of NPs**	**Year**	**Antibacterial activity upon different organism**	**Reference**
P. florida (Mushroom)	NA	UV-Vis spectrophotometer, HR-TEM, XRD, FTIR	2.445 ± 1.08 nm	2013	*K. pneumoniae* YSI6A (MDR)	Sen, I. K. et al.[41]
Musa balbisiana (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi)	Leaf	UV-Vis spectrophotometer, DLS, SEM, TEM, EDS, FTIR	200 nm with different shapes	2014	*E. coli* & Bacillus sp.	Banerjee, P. et al.[42]
P. aeruginosa	NA	UV-Vis spectrophotometer, FTIR, SEM	50-85nm	2014	*E. Ccoli, V. cholerae, S. flexneri, B. subtilis, S. aureus & M. luteus*	Paul, D. et al. [43]
Andrographis paniculata (green chireta)	Leaf	UV-Vis, SEM, FTIR	40-60nm	2015	*E. Ccoli, P. aeruginosa, V. cholerae, S. flexneri, B. subtilis, S. aureus & M. luteus*	Sinha, S. N. et al.[44]
Amaranthus gangeticus (chineese red spinach)	Leaf	UV-Vis, HR-TEM, SAED, FTIR	11-15nm	2015	*S. flexneri & B. subtilis*	Kolya, H. et al.[45]
S. asoca (Asoka tree)	Bark	UV-Vis, DLS, AFM, Zeta potential, FTIR	3-10nm	2015	*E. coli, B. subtilis, S. aureus & P. aeruginosa*	Banerjee, P. et al.[46]
Passiflora vitifolia, Albizia lebbeck, Acacia mangium and A. auriculiformis	Leaf	UV-Vis, PSA & FTIR	41-77nm	2015	NA	Ghosh, D. et al. [47]
Pongamia pinnata	Seed	UV-Vis, Zeta potentail, FE-SEM, TEM, EDX, FTIR	5-30nm	2016	*E. coli*	Beg, M. et al.[48]
Croton bonplandianum	Leaf	UV-Vis, XRD, TEM, EDX	15-40nm	2016	*E. coli, S. aureus & P. aeruginosa*	Khanra, K. et al.[49]
Cassia fistula (pudding-pipe tree)	Leaf	UV-Vis, DLS, FTIR, FE-SEM	10-65nm	2016	*E. coli, B. subtilis, S. aureus & P. aeruginosa*	Mohanta, Y. K. et al[50]
Butea monosperma (palash)	Bark	UV-Vis, HR-TEM, SAED, EDX, DLS, FTIR & XRD	18-50nm	2017	*E. coli & B. subtilis*	Pattanayak, S. et al. [51]
Polianthus tuberosa (Indian Kudzu)	Bud	UV-Vis, XRD, TEM & FTIR	50 ± 2nm	2017	NA	Rawani, A. [52]
Mentha arvensis (mint)	Leaf	UV-Vis, DLS, XRD, FTIR, EDX, AFM, TEM	NA	2017	NA	Banerjee, P. P. et al. [53]
Elaeocarpus floribundus (Indian olive)	Fruit	UV-Vis	NA	2017	*B. cereus, S. aureus, A. baumanni, E. coli, K. pneumonia, P. vulgaris & P. aeruginosa*	Sircar, B. et al. [54]
Ganoderma sessiliforme (mushroom)	NA	UV-Vis, DLS, XRD, FTIR, HR-TEM & FE-SEM	45nm	2018	*E. coli, B. subtilis, S. faecalis, L. innocua & M. luteus*	Mohanta, Y. K. et al. [55]
Alstonia scholaris (Scholar tree/chattim)	Leaf	UV-Vis	NA	2018	*B. subtilis, E. coli, S. aureus & P. vulgaris*	Bandopadhyay, S. et al. [56]

Continue...

Biogenic synthesis of silver nanoparticles and its properties						
Plant/Organism used	**Plant parts used**	**Characterization methods used**	**Size of NPs**	**Year**	**Antibacterial activity upon different organism**	**Reference**
Colocasia esculenta (taro)	Stem	UV-Vis, FTIR, SEM, TEM, EDX, XRD & Zeta potential	13-50nm	2019	NA	Mondal, A. et al.[57]
Adiantum lunulatum (fern)	Whole plant	UV-Vis, DLS, Zeta Potential, FTIR, EDX, XRD, and TEM	30-98nm	2019	*B. subtilis, E. coli, S. aureus & L. monocytogenes, K. pneumonia & S. typhimurium*	Chatterjee, A. et al.[58]
Ceriops decandra (Mangrove plant)	Fruit	UV-Vis, DLS, TEM, EDAX & XRD	28nm	2019	*L. cytomonogenes, B. Subtilis, and S. aureus, S. typhimurium & E. coli*	Maity, G. N. et al.[59]

Table-1: Biogenic synthesis of silver nanoparticles.

of AgNP.[60,61] We are going to discuss some major and prominent AgNPs characterization methods.

UV-Vis Spectrophotometer: UV-Vis spectroscopy can be the preliminary step to identify and characterize silver nanoparticles. With this technique we can say the polydispersity of the prepared nanoparticles.[62] Presence of AgNPs can be confirmed by observing the peak of absorption spectra from 350-500nm wavelength.[63] Some can find it at 350nm[64], 413nm[61] and can also find at 470nm[62], it will vary depending upon the type of extract used and also upon the size of the prepared nanoparticles.[65,66] Most of the publications suggest scanning between 200-800nm wavelengths.

DLS: Dynamic light scattering method can be used to analyse the average size of the prepared AgNPs.[67] DLS can illustrate AgNPs PDI (polydispersity index) and hydrodynamic diameter, which can be used to assess the stability of nanoparticles produced.[68] It requires the viscosity and the refractive index of the medium, in which the AgNP is present/solubilised.

XRD: X-ray diffraction technique is used to identify the phase, orientation and size of nanoparticles.[60] With the help of this method one can confirm the crystalline nature of the synthesised nanoparticles. Bragg's diffraction peaks can be observed at (2θ) 33.38°, 44.48°, 64.66°, 77.56° and 81.66° corresponding to 111, 200, 220, 311 and 222, planes respectively.[69,68] Which represents the face centered cubic (FCC) structure of the nanoparticles. Joint committee on powder diffraction standards (JCPDS) – file number: 89-3722 can also be used to compare experimented data with the standards.[70] Some unassigned and unpredicted peaks may also appear due to organic compounds present in the plant parts extracts[71] or due to unreduced $AgNO_3$ present in the solution.[72] Average crystalline size of the nanoparticles can be estimated from the diffractogram by using Debye-Scherrer formula: $D=0.9\lambda/\beta Cos\theta$, where D stands for the size, λ is wavelength of the X-rays used, and β is full width at half maximum (FWHM) of the peak observed.[72,70]

FTIR: Fourier-transform infrared spectroscopy analysis is to investigate the present functional groups in the plant extract.[70] This might be responsible for the reduction of silver ions to silver nanoparticles.[68,73] FTIR data shows a shifting of peaks observed in AgNPs prepared from Plant extract. The absorption peak at different position indicates the presence of different functional groups of the biomolecules. Peak at 1639cm^{-1} shows a strong stretching of carbonyl group of α,β-unsaturated compounds, whereas peaks at 3421cm^{-1} & 1056cm^{-1} indicates the presence of OH and C-O stretching.[74,75] O-H group in polyphenols or proteins or polysaccharide can also be confirmed by observing the peak at 3186cm^{-1} and 3341cm^{-1}.[76,77] Presence of silver nanoparticles can be assessed by observing peaks at 1138cm^{-1}, 821cm^{-1}, 764cm^{-1}, 595cm^{-1}.[69] Shifting of carbonyl stretching frequency from 1639cm^{-1} to 1630cm^{-1} may also be taken as evidence of reduction of Ag^{+} to Ag^{0}.[68]

Zeta Potential: This measurement will be done to analyse the net surface charge of the nanoparticles, which will provide stability so that the nanoparticles will not agglomerate when present in the solution.[78] Zeta potential value range from >+30mV to <-30mV confirms the best stability of nanoparticles.[79,80]

FE-SEM/SEM/TEM/HR-TEM: Field emission scanning electron microscopy/scanning electron microscopy/ transmission electron microscopy/ high resolution transmission electron microscopy can provide us a detailed and prominent image showing the shape and size and morphology of the prepared nanoparticles.[60,63,62]

EDX/EDAX: Energy dispersive R-ray spectroscopy reveals the elemental composition details of the nanoparticles examined by providing EDS (energy dispersive spectrum).[81] According to researchers, metallic silver nanoparticles show a strong signal peak at 3keV.[82,83] Whereas, it can range between 2-4keV.[84]

How far West-Bengal is using biogenic silver nanoparticles as antibacterial agents:

Reviewing a decade history of preparation of biogenic silver nanoparticles and its antibacterial effects, we can see a total of 19 publications relevant to our aim of this review.

Sen, I. K. et al, (2013) prepared AgNP-glucan conjugates from mushroom using glucan, collected from mushroom (*Pleurotus florida*).[41] They have extracted the crude polysaccharide by boiling *P. Florida* blue variant in 4% NaOH followed by centrifugation and precipitation in alcohol. For the establishment and characterization of extracted polysaccharide, they have used 2D-NMR technique.[85] They found a better stability and small size (2.45nm) of their prepared AgNP than AgNPs prepared by chitosan or starch. Upon analyzing their AgNP-glucan conjugates as antibacterial agents, they revealed MIC value 40µg/ml and LD_{50} value 15µg/ml. They have also proved a huge reduction in cell count from 4.9×10^8 CFU to 1.0×10^5 CFU at MIC level 40µg/ml. The strain they have used was MAR (multiple antibiotic-resistant) bacterium *K. pneumonia* YS16A, which may be resistant to two or more antibiotics.[86] MAR bacteria can also be said as MDR (multi-drug resistant) bacteria, which is a global threat.[87] Banerjee, P. et al. (2014) synthesized AgNP from leaf extract of banana, neem and black tulsi.[42] The shape morphology of the prepared AgNPs was spherical, triangular and cuboidal as per SEM analysis they have done with various sizes upto 200nm range. The microorganisms they used as test organisms were gram-positive *Bacillus* and gram-negative *E. coli* procured from IMTEch Chandigarh. Whether the organisms were drug resistant or not, has not been revealed by the authors. Authors have done DAD (disc agar diffusion) method to assess the antibacterial activity of the AgNPs with proper protocol.[88] They have concluded that the AgNPs prepared from banana leaf extract had shown maximum inhibition for gram-positive *Bacillus*, as these particles had smallest diameter than those prepared from neem and tulsi leaf extracts. Apart from the above conclusion author said that crude leaf extract had no bactericidal effect, as compared to $AgNO_3$ & AgNPs. Another researcher Paul, D. and his team has done extracellular synthesis of spherical AgNPs (50-85nm size) from bacteria *Pseudomonas aeruginosa* (KUPSB12)[43], a phosphate solubilizing bacterial strain from a jute mill effluent exposed area of river Ganga at Bansberia, West-Bengal. Identification of the particular strain was done on the basis of proper protocol, including 16s rRNA sequencing.[89,90] Test organisms were used *E. coli*, *Vibrio cholerae*, *Shigella flexneri*, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*, collected from MTCC (Microbial type culture collection and gene bank). Antibiotics resistance profiles of the tested organisms were not revealed by the authors. They showed highest antibacterial activity against *E. coli* and least against *Staphylococcus aureus*. There is another green method of preparing AgNPs by photosynthesis using *Andrographis paniculata* leaf extract by Sinha, S. N. and his team (2015).[44] The plant was collected from their University campus, West Bengal and was identified by one of the botanist of the same University. SEM analysis revealed that the AgNPs they prepared were 40-60nm size and were also showed antibacterial activity against *P. aeruginosa*, *B. subtillis*, *M. luteus*, and *E. coli* with descending order of zone of inhibition (ZOI) 21.33mm, 18.66mm, 17.66mm & 16.66mm respectively as per well diffusion method. *V. cholerae*, *S. flexneri* and *S. aureus* were found to have ZOI 15.4, 15.2 and 13.7 millimetre respectively. As per broth microdilution method, author revealed highest MIC value 50µg/ml against *S. aureus* and least MIC value 3.125µg/ml against *Pseudomonas*. Another leaf mediated synthesis of AgNPs (size 11-15nm) was done by Kolya, H. et al. (2015) by using *Amaranthus gangeticus* Linn. leaf extract.[45] Using standard agar-well diffusion method[91,92], authors tested AgNPs against *Bacillus* and *Shigella* procured from ATCC (American type culture collection) taking levofloxacin as standard or control. They have seen greater ZOI in case of gram-negative bacteria (5.5mm) than gram-positive bacteria (4mm). AgNPs prepared by this method have showed catalytic activity towards degradation of Congo red dye, a non-biodegradable dye. Next study in the same year 2015 revealed the synthesis of AgNPs using bark extract of *Saraca asoca* as reducing agent.[46] They have prepared AgNPs of 4.5nm average size and showed antibacterial activity against *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa* with increase in diameter of ZOI with increasing concentration of nanoparticles using standard disc diffusion method.[93] Ghosh, D. et al. (2015), synthesized AgNPs from leaf extracts of *P. vitifolia*, *A. Lebbeck*, *A. Mangium* and *A. auriculiformis* with 40.93nm, 54.97nm, 67.78nm and 76.46nm size respectively.[47] Beg, et al. (2016), biogenically synthesized AgNPs with average size 16.4nm using seed extract of *Pongamia pinnata* and tested its antibacterial activity against *E. coli* procured from ATCC.[48] They found an MIC value 2.0nM and LD_{50} value 1.0nM against the tested strain. They have also found a similar kind of activity of prepared AgNPs when tested synergistically with ampicillin against the same strain with basic protocol.[94] Again, *Croton bonplandianum* Baill. leaves were used by Khanra, K. et al, (2016) to synthesize AgNPs of 32nm average size, and they have also tested its antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* with basic protocol.[95] They revealed that the effect of AgNPs on gram-negative bacteria were more than on gram-positive bacteria, may be due to difference in cell wall thickness among both kinds of organisms.[49] *Cassia fistula* (Linn.) leaf extract was used by Mohanta, Y. K. and team (2016), to prepare silver nanoparticles and its bactericidal activity was tested as per agar cup method[96] against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E coli*, procured from MTCC.[50] Unlike other investigation results discussed earlier, they have found greater ZOI against gram-positive strains than gram-negative. Same kind of results was obtained by the author in case of MIC test. *Butea monosperma* bark extract was used by Pattanayak and his team (2017) to produce spherical AgNPs of average size 35nm as per HRTEM analysis.[51] Gram-positive *Bacillus subtilis* and gram-negative *E. coli* were used as test organisms, and *E. coli* results larger ZOI than *B. subtillis*. Along with the antibacterial property, prepared AgNPs showed anticancerous

activity as well. Banerjee, et al. In 2017 also showed the anticancerous effect of green synthesized AgNPs from Mentha arvensis (Linn.).[53] Researchers also found promising larvicidal activity of AgNPs prepared from the extract of bud of *P. tuberosa* of size 50nm approximately[52] and also from *Colocasia esculenta* (L.) stem of size 13-50nm.[57] Sircar, et al. in 2017 synthesized AgNPs using olive fruit parts extract and tested its antibacterial activity against gram-positive and gram-negative bacteria. They have concluded with the higher efficacy of AgNPs prepared from mesocarp-epicarp than seed.[54] Mohanta, Y. K. et al. in 2018 prepared AgNPs from wild mushroom and found potent antimicrobial activity against food borne pathogens.[55] In this study they have evaluated the potential of AgNPs prepared against *E. coli*, *B. subtilis*, *S. faecalis*, *L. innocua* and *M. luteus*. Among which *L. innocua* (22mm) had greater ZOI than others and least ZOI was found against *E. coli* (11mm). Another study on green synthesis of AgNPs was conducted by Bandyopadhyay, S. et al. in 2018[56] using leaf extract of *Alstonia scholaris* and antimicrobial activity was also tested against *B. subtilis*, *S. aureus*, *E. coli* and *P. vulgaris* as per cup-plate method. They have found better activity of AgNPs against gram-negative than gram-positive strains. Chatterjee, A. et al. (2019) prepared AgNPs (30-100nm) by using fern for the first time and tested its antibacterial activity against various gram-positive and gram-negative bacteria procured from MTCC. They found maximum MIC value against *B. subtilis* and minimum against *E. coli*.[58] Now coming to the last article of this review, Maity and his team prepared AgNPs (20-58nm) from mangrove fruit polysaccharide and tested against gram-positive and gram-negative bacteria. Highest MIC value was recorded against *S. aureus* (179.24mg/L) and minimum against *L. monocytogenes* (62.77mg/L).[59]

CONCLUSION

The present study deals with the characterization processes used for characterizing AgNPs which are biologically prepared and its effect upon different bacteria of both kinds (gram positive and gram negative). Silver nanoparticles seem easier and efficient to be produced by various biological sources, whether it is bacteria, fungus or a plant itself. Almost every plant part, like leaf, root, bark, etc. can be used to get reduced AgNPs from ionic silver. When it comes to characterization, first colour change can be observed and then will provide a peak of absorbance between 350-500nm wavelengths when studied under UV-Vis spectrophotometer. For imaging, FE-SEM and TEM/HR-TEM will be the best options to study small size nanoparticles. For polydispersity of the prepared nanoparticles and particle size analysis, DLS method can be used. Researchers must focus on the MDR bacteria, while testing AgNPs as antibacterial agents. MDR clinical isolates have other features that might not be the same in case of normal clinical isolates.

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