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SHORT COMMUNICATION

Synthesis, Characterization and Antimicrobial Evaluation of *Piyar* Gum-Induced Silver Nanoparticles

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Abstract *Buchanania lanzan* gum commonly known as *char*, *achar*, *piyar* and *chironji* is a naturally occurring water-soluble, edible polysaccharide derived from the bark of *Buchanania lanzan* Spreng. Silver nanoparticles have been synthesized using *Buchanania lanzan* gum exudates adopting green synthesis methodology and characterized by UV–Vis, Fourier transform infrared spectroscopy, zeta potential and particle size analysis, scanning electron microscopy, transmission electron microscopy and atomic force microscopy confirming the synthesis of AgNPs. The synthesized *piyar* gum-induced AgNPs are spherical in shape, small enough in size (14.74–19.86 nm) and noncytotoxic. Evaluation of bactericidal activity of synthesized *piyar* gum-induced AgNPs against 17 strains of 14 Gram-negative and Gram-positive bacterial pathogens, using agar well diffusion method, exhibited strong bactericidal activity against Gram-negative bacterial pathogens. The minimum inhibitory concentration against two Gram-negative bacterial strains, i.e., *Escherichia coli* and *Avibacterium avium*, was found to be 0.52 µg/mL and 0.53 µg/mL, respectively, which was at par with that of positive control (0.5 mM silver nitrate solution).

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Introduction

Plant exudate gums/polysaccharides constitute an important component of life matter and have generated tremendous interest because of their diverse pharmaceutical applications such as diluents, binders, disintegrants, bioadhesives, thickeners, emulsifiers, stabilizers, gelling agents and their use in cosmetics, textiles, paints and papermaking, etc. Nanoparticle synthesis with natural polysaccharides has several advantages over conventional synthetic chemical agents. The renewable, nontoxic components of this natural phyto-exudate play dual role of reducing and stabilizing agents for the silver ions. Sufficient availability of the gum makes this green method amenable to large-scale production of silver nanoparticles (AgNPs). Over last few years, there has been an upsurge in the study of plant gum-induced AgNPs on account of their immense importance due to inherent antimicrobial efficacy. These are also being seen as future-generation therapeutic agents against several drug-resistant microbes. Further, the green method of synthesis of nanoparticles is easier, more efficient, eco-friendly and incurs low cost in comparison with the chemical-mediated or microbe-mediated synthesis [1–3].

During the last decade, a number of researchers have reported synthesis of silver nanoparticles using natural plant gum exudates adopting green synthetic methodology, such as gum *acacia* [4], cashew gum [5], gum *kondagogu* [6], gum *olibanum* [7], gum *karaya* [8], gum *tragacanth* [9], gum *ghatti* [10], *neem* gum [11], carboxymethyl *neem*

gum [12], gum *arabic* [13], etc., as a stabilizing agent to improve their stability and biocompatibility.

Buchanania lanzan (*B. lanzan*) gum commonly known as *char*, *achar*, *piyar* and *chironji* is naturally occurring water soluble, edible polysaccharide derived from the bark of *Buchanania lanzan* Spreng belonging to family Anacardiaceae. It is traditionally used for various medicinal purposes. Siddiqui et al. [14] have reported significant qualitative and quantitative intraspecific variations in *B. lanzan* gum exudates, collected from different places, for their phytochemicals, physicochemical properties and antioxidant activity. Because of the *B. lanzan* gum's traditional multipurpose uses and this being a natural polysaccharide, easily available with no research having been conducted on the nanoparticles' synthesis, the present study was taken up. In this paper, we have reported the facile synthesis of AgNPs using silver nitrate and *B. lanzan* gum in benign solvent water under sterile environment. This natural polysaccharide not only facilitates the synthesis process but also encapsulates and stabilizes the synthesized AgNPs. The synthesized piyar gum-based AgNPs were characterized by UV–Vis, Fourier transform infrared spectroscopy (FTIR), particle size analysis (Z-average diameter) and zeta potential, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The synthesized nanoparticles have demonstrated bactericidal efficacy against Gram-negative bacterial pathogens.

Silver nitrate was purchased from Merck India Ltd., Mumbai, India. Double-sterilized Milli-Q water was used throughout the experiments. The test strains including *Escherichia coli* (3), *Avibacterium avium*, *Staphylococcus intermedius*, *Paenibacillus macerans*, *Serratia rubidaea*, *Erwinia mallatovora*, *Enterococcus faecalis*, *Staphylococcus haemolyticus*, *Proteus mirabilis*, *Staphylococcus epidermidis* (2), *Staphylococcus chromogenes*, *Enterobacter agglomerans*, *Staphylococcus capitis* ssp. *capitis, Staphylococcus capitis* ssp. *urealyticus* were used to evaluate the antibacterial potential of the synthesized *B. lanzan* gum-induced AgNPs. All the isolates used in the study were revived from glycerol stocks available in Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly (Uttar Pradesh), through inoculation of contents of stock in 10 mL trypticase soy broth (TSB, BBL, Difco, USA) and incubating overnight at 37 °C. Then, they were streaked on to blood agar (blood agar base with 5% defibrinated sheep blood) plates to determine their purity, and pure cultures were restocked in glycerol broth to store at − 20 °C and also on nutrient agar slants for use in the study [15].

Buchanania lanzan gum exudates, collected from Bilaspur (Chhattisgarh), after manual cleaning and sorting were converted into fine powder and passed through 25 µm mesh sieve and packed in airtight container for further analysis. A homogenous 2.0% (w/v) *piyar* gum solution was prepared by adding the fine powder of sieved gum to brown bottle containing Milli-Q water with constant stirring on a magnetic stirrer at room temperature. The solution was centrifuged to remove the insoluble material, and the supernatant was used for the experiments. Two dilutions, i.e., 1.0% and 2.0%, of *piyar* gum solution and 0.5 mM silver nitrate ($AgNO_3$) solution were used for the synthesis of AgNPs. Reactions were carried out by taking equal quantity (1:1 ratio) of both the reactants adopting autoclaving methodology at 121 °C and 15 psi for 30 min [6, 10]. The synthesis of AgNPs was monitored by UV–Vis spectra at 250–700 nm. The solutions of the synthesized nanoparticles were lyophilized and recovered in amorphous form using SCANVAC freeze dryer and stored in vacuum desiccator.

In order to study the formation of *piyar* gum-induced AgNPs, the UV–Vis absorption spectra of the synthesized AgNPs were recorded using CECIL CE 7200, UK Spectrophotometer from 250 to 700 nm along with gum and $AgNO_3$ solutions. The FTIR spectra of lyophilized AgNPs along with *piyar* gum were recorded in Shimadzu, IR Prestige-21 Shimadzu, Japan, in the range of 4000–500 cm^{-1} in KBr. The size and shape of the AgNPs were obtained with JEOL JEM-1011 Electron Microscope. Samples were prepared by depositing a drop of colloidal solution on a carbon-coated Ni + Pd grid of 400 nm size and dried in vacuum desiccators. For particle size analysis of the synthesized AgNPs, solutions were diluted with Milli-Q water and the particle size was determined using laser diffraction particle size analyzer (LS 13,320) and zeta potential with sub-micron particle size analyzer (Model, Nano ZS; Make, Malvern Instruments, UK). The surface morphology of the AgNPs was examined in a scanning electron microscope (SEM) (JEOL JSM-6390 LV). For 3D visualization of the synthesized AgNPs, atomic force microscopy (AFM) was carried out using atomic force microscope (Model, Solver Pro 47; Make, NTMDT, Russia).

For determining minimum inhibitory concentration (MIC) of the synthesized AgNPs and controls ($AgNO_3$ and *piyar* gum solution), agar well diffusion method was used [15]. Briefly, nine wells, each of 6 mm diameter, were cut in Mueller Hinton Agar (MHA, BBL, Difco) plates under sterile environment, and bottoms of wells were sealed with the same medium. Six-hour-old actively growing culture of test microbe in TSB was swab inoculated, and wells were filled with 50 µL of serially diluted test preparations in sterile phosphate buffer (pH, 7.2) so that well number one to eight contained 8.50, 4.25, 2.13, 1.06, 0.53, 0.27, 0.13 and 0.07 µg/mL of the test preparations, respectively. Central well contained sterile phosphate buffer only. Plates

were incubated under appropriate growth conditions for 2 h without inversion to get the preparation absorbed in the medium and then overnight after inversion in appropriate environment required for the optimum growth of the microbe. Measurable zone of growth inhibition around the well containing the highest dilution of the preparation was marked as MIC value of the preparation for the microbe. Tests were conducted in triplicate for confirmation.

In vitro cytotoxicity of the synthesized *piyar* gum-induced AgNPs was measured against Vero Cell Line (African green monkey kidney normal cell line) using MTT assay along with controls in 96-well microtiter plate in triplicates with concentrations ranging from 10 µM, 100 µM and 250 µM following standard protocol [16]. The assay was conducted at Division of Biochemistry, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly (Uttar Pradesh), India.

On recording UV–Vis spectra in the range of 250–700 nm, a broad peak at 415–440 nm was detected corresponding to the typical surface plasmon resonance (SPR) of AgNPs formed by reduction of aqueous Ag ions when 1% *piyar* gum solution was taken but intensity decreased at 2% gum concentration. Further, no absorption peak corresponding to SPR in respect to controls (gum and silver nitrate solutions) was observed. However, at 250–300 nm, a small peak was observed in all the spectrum excepting 0.5 mM $AgNO_3$ might be of tannin present in the gum (Fig. 1). During autoclaving at 121 °C and 15 psi pressure, this biopolymer appears to be more accessible for the silver ions to interact with the available functional groups on the gum due to abundance of arabinose and galactose moieties in it. The autoclaving methodology was adopted as a synthetic route for the synthesis of silver nanoparticles, completely free from bacteria, viruses and spores, which could then be safely used for antibacterial applications. Not only the presence of large numbers of hydroxyl, carbonyl,

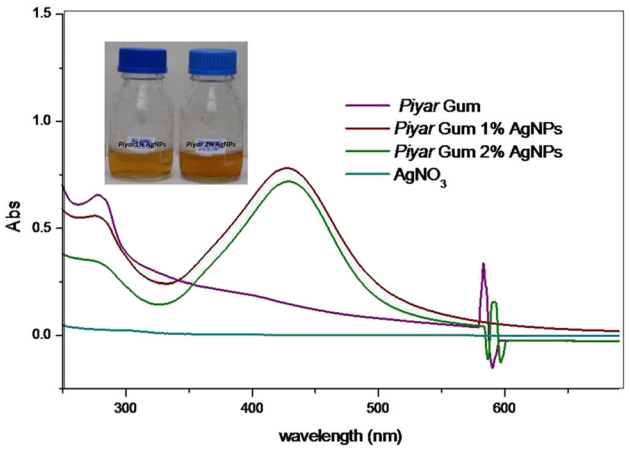

Fig. 1 UV–Vis absorption spectra of synthesized *piyar* gum-induced AgNPs along with controls

carboxylic and amino groups facilitates the formation of metal nanoparticles through the reduction of metal ions but also proteins present in the gum subsequently encapsulate and stabilize these particles along with polysaccharide molecules [8]:

$$Ag^{+}NO_3^{-} + \text{Plant Polysaccharides}(-OH,\ >C=O,\ -NH_2, \text{RCOOH groups}) \rightarrow Ag^{0}\text{nanoparticles}$$

The results obtained, by equimolar reaction of 0.5 mM $AgNO_3$ and 1% *piyar* gum solution, are in close proximity to the results reported in the cases of other exudate gums like *tragacanth, neem*, etc.[9, 11]. The major biomolecules responsible for the green synthesis of AgNPs present in the aqueous extract of *B. lanzan* gum were identified by FTIR. In FTIR spectra, the flattening of broad band at 2144 cm^{-1} (due to various carbonyl groups of gum) confirms that the reduction of the silver ions is coupled to the oxidation of the hydroxyl and carbonyl groups of the gum. Further, the change in the intensities of absorbance peaks 3300 cm^{-1} (due to stretching vibrations of O–H groups) and 1600 cm^{-1} (due to characteristic asymmetrical stretch of carboxylate group) is suggestive of the binding of silver ions with hydroxyl and carboxylate groups, respectively [8–10]. The zeta potential and Z-average diameter values of the synthesized AgNPs were found to be − 19.8 mV and 176.0 (d nm), respectively. Polydispersity index (PDI) of the synthesized AgNPs was found to be 0.288, indicating that the particles are in monodispersed phase with very low chances of aggregation. Analysis of *piyar* gum and its synthesized AgNPs by laser diffraction particle size analyzer revealed the d_{50} values to be 1.185 µm and 0.055 µm, respectively. Surface morphological image of the synthesized AgNPs taken at X500 magnification and 50 µm scale showed quite distinct interwoven net-like structures. TEM image of the synthesized *piyar* gum-induced AgNPs showed particles size ranging from 14.74 to 19.86 nm at 1,50,000X magnification (Fig. 2a). TEM results confirmed that AgNPs are not agglomerated, well dispersed and almost spherical in shape with particles size in nano-range. Results obtained from dynamic light scattering and TEM were further confirmed by AFM (Fig. 2b).

Evaluation of antibacterial activity of the synthesized *piyar* gum-induced AgNPs against 17 strains of 14 Gram-negative and Gram-positive bacterial pathogens, using agar well diffusion method, revealed that silver nitrate (0.5 mM solution) used as control inhibited growth of all the bacteria tested and the MIC was 0.53 µg/mL, while *piyar* gum did not inhibit any of the bacterial strains tested (MIC ≥ 2.5 mg/mL). The MIC of synthesized AgNPs was the maximum (≥ 8.5 µg/mL) for Gram-positive bacterial pathogen *S. intermedius*, whereas MIC against two Gram-negative

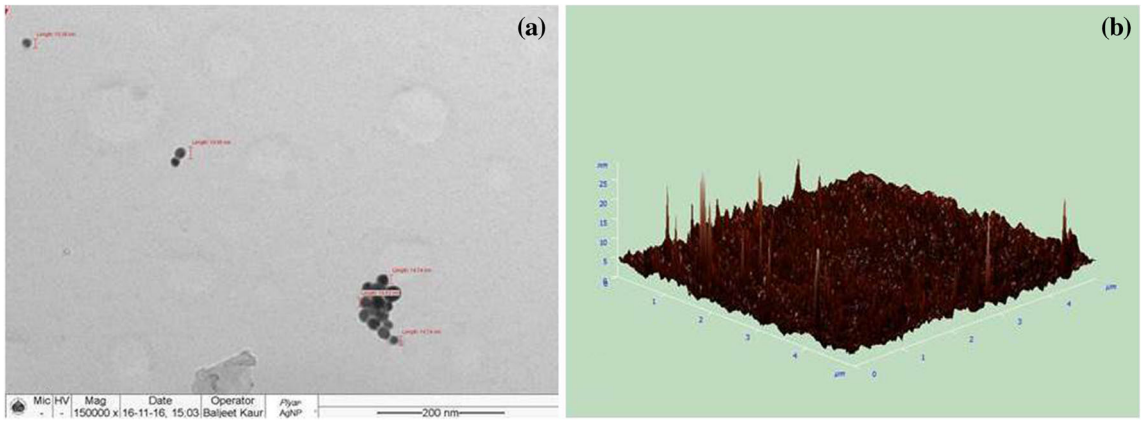

Fig. 2 **a** TEM image and **b** AFM image of synthesized *piyar* gum-induced AgNPs

bacterial strains, i.e., *E. coli* (0.52 μg/mL) and *Avibacterium avium* (0.53 μg/mL), was at par with that of positive control (0.5 mM silver nitrate solution) (Table 1). This could be due to the presence of thicker peptidoglycan layer in Gram-positive than Gram-negative bacteria, preventing the entry of AgNPs and its antibacterial activity. Similarly, a number of researchers have reported that the Gram-negative bacterium *E. coli* showed a greater inhibition zone compared to that of the Gram-positive bacteria, which was probably due to their thick cell walls in Gram-positive bacteria than in Gram-negative bacteria. Generally, Gram-positive bacteria are composed of a three-dimensional thick peptidoglycan (20–80 nm) layer compared to that of Gram-negative bacteria (8–10 nm). The peptidoglycan layer possessing linear polysaccharide chain is cross-linked by more short peptides, thus forming a complex structure leading to difficult penetration of AgNPs into Gram-positive bacteria compared to that of Gram-negative bacteria [17, 18]. It has also been found that the higher concentrations of *piyar* gum (viz. 2%) used in the synthesis of AgNPs exhibited adverse effect on MIC. Bactericidal images of *piyar* gum, 0.5 mM silver nitrate solution and synthesized *piyar* gum-induced AgNPs against *E. coli* are given in Fig. 3a–c.

The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay measures the cell proliferation

Table 1 Determination of minimum inhibitory concentration (MIC) of *piyar* gum-induced AgNPs using agar well diffusion method

Bacteria/number of isolates	Minimum inhibitory concentration (MIC)		
	0.5 mM $AgNO_3$ solution (μg/mL)	1.0% *Piyar* gum solution (mg/mL)	*Piyar* gum-AgNPs (μg/mL)
Gram negative			
Escherichia coli (3)	0.53	≥ 2.5	**0.52**–4.25
Erwinia mallatovora (1)	0.53	≥ 2.5	4.25
Proteus mirabilis (1)	0.53	≥ 2.5	1.06
Serratia rubidaea (1)	0.53	≥ 2.5	2.12
Avibacterium avium (1)	0.53	≥ 2.5	**0.53**
Enterobacter agglomerans (1)	0.53	≥ 2.5	4.25
Gram positive			
Enterococcus faecalis (1)	0.53	≥ 2.5	1.06
Paenibacillus macerans (1)	0.53	≥ 2.5	1.06
Staphylococcus epidermidis (2)	0.53–4.25	≥ 2.5–1.25	1.06–4.25
Staphylococcus haemolyticus (1)	0.53	≥ 2.5	1.06
Staphylococcus intermedius (1)	≥ 8.5	≥ 2.5	≥ 8.5
Staphylococcus chromogenes (1)	0.53	≥ 2.5	1.06
Staphylococcus capitis ssp. *capitis* (1)	0.53	≥ 2.5	1.06
Staphylococcus capitis ssp. *urealyticus* (1)	0.53	≥ 2.5	1.06

The bold values are to reflect their equalness with the positive control used during the experiment

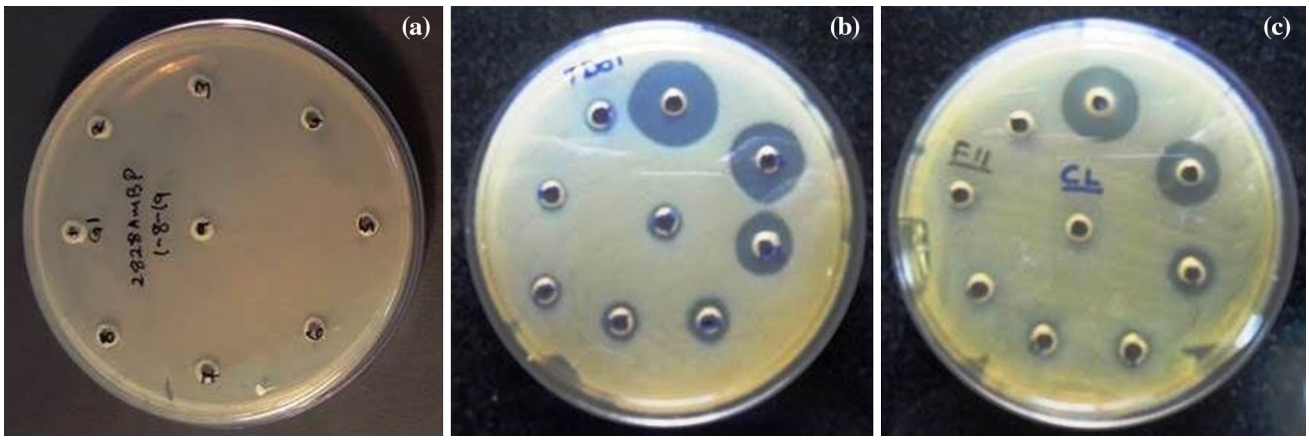

Fig. 3 Bactericidal image of **a** *piyar* gum, **b** 0.5 mM silver nitrate solution and **c** synthesized *piyar* gum-induced AgNPs against *E. coli*

rate and conversely the reduction in cell viability when metabolic events lead to apoptosis or necrosis. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble blue formazan compound, depending on the viability of the cells [19]. In vitro cytotoxic activity of the synthesized *piyar* gum-induced AgNPs against Vero Cell Line showed that the synthesized *piyar* gum-induced AgNPs are noncytotoxic with increased cells viability percentage. % survival of Vero cells against the synthesized AgNPs was found to be on quite higher side (128.074) as compared to $AgNO_3$ (14.579) at 100 μM concentrations.

Undoubtedly, there was a boom of plant gum exudates-induced synthesis of AgNPs during past decade but it never means that this is the full stop and there is nothing to be done. Plant gum exudates-induced synthesis of metal nanoparticles is an immensely promising field of research, and the continuous advancements in such fields with new commodities will further explore and upstream the approach in all, the commodity, yield and the applications, etc. The present study demonstrates a greener approach to synthesize *piyar* gum-induced silver nanoparticles. The synthesized *piyar* gum-induced AgNPs are in nano-range and noncytotoxic. They have exhibited potential bactericidal activity against Gram-negative pathogens and could be used as potent antimicrobial agents in various therapeutical applications.

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