Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6581

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (2):** 1632-1639 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Biological Synthesis and Antibacterial Activity of Silver Oxide Nanoparticle Prepared from *Carica papaya* Root Extract

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Received: 10.03.2018 | Revised: 16.04.2018 | Accepted: 21.04.2018

ABSTRACT

Higher research and development of silver nanoparticles (AgNPs) is great due to their use full applicationsin present time silver nanoparticles were successfully synthesized by using carica papaya root extract. In the present time the aim of the biosynthesis of silver oxide nanoparticle to making a new drug. Temperature and extract concentration were found to control the size and shape of the biosynthesized silver nanoparticles The synthesized AgNPs were characterized by UV–visible spectroscopy, scanning electron microscopy (SEM), Morphological images of prepared nanomaterials exposed that the particles are in spherical shape and size ranging between 10 and 100 nm. The mode of action of AgNPs on the bacteria was studied against isolated bacterial pathogens, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa using confocal and electron microscopy, Crystalline nature of nanoparticles in face centered cubic (fcc) structure was ensured by diffraction pattern peaks corresponding to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes spectroscopy analyses indicates that nanoparticles

Key words: *Biosynthesis of silver oxide nanoparticle , UV- VIS, SEM. Anti bacterial activity, Carica papaya root*

INTRODUCTION

Traditional methods for metal nanoparticle synthesis simply require the use of highly toxic chemicals, solvents, capping agents and also acute controlled conditions that increase cost and cause environmental pollution. The biogenesis of nanoparticles by bio chemical approach could reduce the cost and would be in nature biodegradable. Present time nanoparticles attracts worldwide due to its unique thought physical and chemical properties and significant use in many applications such as medicine, biology, biotechnology, chemistry, physics, catalysis, electronics, material sciences, optoelectronics, optics and sensors[1–5].

Cite this article: Yadav, M., Lawrence, R.S., Jalees, M., Lawrence, K., Tiwari, A. and Shani, M., Biological Synthesis and Antibacterial Activity of Silver Oxide Nanoparticle Prepared from *Carica papaya* Root Extract, *Int. J. Pure App. Biosci.* **6(2):** 1632-1639 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6581

In particular, The metal nanoparticles (MNPs) have been synthesized by using various methods, including chemical, physical, electrochemical, irradiative, photochemical, and biological techniques[6–12]. Although most of the methods are successful in producing pure and well defined nanoparticles AgNPs are better disinfectants that can significantly reduce many bacterial infections for longe time compared to usage of common drug, penicillin, and tetracycline. AgNPs are very effective since they are potent biocides against various microorganisms such as bacteria, fungi, and viruses. Certain bacteria are resistant to antibiotics, and some have developed resistance. Silver nanoparticles have become the center of broad research due to its good antimicrobial value against multi drug resistant bacteria, viruses and other eukaryotic microorganisms. Previous studies showed that Ag NPs are capable of the stage effective antibacterial property against *Bacillus cereus P. vulgaris Pseudomonas aeruginosa, clostridium* However, the synthesis of nanoparticles using the chemical methods available is often costly uses toxic chemicals, and is time utlize and cost prohibitive. Therefore, there is a need for eco-friendly, rapid, lowcost and less time-saving techniques to produce nanoparticles. Many new studies have indicated the easy synthesis of such materials without focusing on optimizing production. In our study, we used *Cacica papya* root extract to produce silver nanoparticles and optimized the production process. *Cacica papya* is commonly known as the papaya in Ayurveda treatment. The *Cacica papya* species contain more than 80 varieties and are hole of world tropics. This tree is found to Bangladesh, India, and Sri Lanka. Extracts of various plant parts of *Cacica papaya* have been known for their anathematic, analgesic, anti-inflammatory, antioxidant, antidiabetic, immuno-modulatory, and antimicrobial activities since ancient times, and the root has been used for anti bactrial. The stem, leaf, bark, fruit, and sap extracts of *Cacica papaya* have been used as an ayurvedic medicines to cure various diseases like dysentery, teeth disorders, skin disorders like sores, piles, diarrhea, and rheumatism, and to boost the immune system .These two bacteria are normally present in low numbers in the plaque of affected individuals. When salivary flow decreases, the pH of the plaque drops, leading to selection of aciduric (acid-tolerant) bacteria such as *Bacillus cereus Pseudomonas, clostridium* we evaluated the potential of the green synthesis of noble metal silver nanoparticles and production optimization and characterized their antibacterial activity against the *Pseudomonas clostridium* using *Cacica papya* root extract.

Synthesis of silver oxide nanoparticles

For the synthesis of silver oxide nanoparticles (Ag_2O NPs), FBPRE 10 mL was added to 90 mL of 1 mM silver nitrate solution in 250-mL Erlenmeyer flasks to form a reaction mixture, and the reaction was performed under ambient conditions. Initial confirmation of silver nanoparticle production is by change in color from light yellow to dark brown in the reaction mixture. The SPR of nanoparticles was measured using a UV–vis spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) within a working wavelength range of 300–800 nm using a dual beam operated at 1 nm resolution.

Production and optimization of silver oxide *Cacica papaya* root extract nanoparticles

To optimize the nanoparticle production, parameters such as pH 3.0–10.0; silver nitrate 44.5:0.5–40:5.0 mL; Ag^+ at 0.1–1.0 mM concentrations; and a time frame of 0–270.0 min were tested. Once silver nanoparticle production was complete, the obtained product was centrifuged for 15 min at 12,000 rpm, followed by several washes with copious amounts of nanopure water and ethanol to ensure separation of free entities from the silver nanoparticles. The obtained material was freeze-dried to make a powder and used for further characterization and antibacterial studies.

Characterization

The reduction of pure Ag+ ions was monitored by measuring UV–Vis spectrum of reaction solution at various time intervals as a function of temperature and concentration of extract UV–visible spectral analysis has been achieved by using a Shimadzu UV-3101PC spectro photometer. The silver nanoparticles dispersed in water were placed on a carbon coated copper grid, excess was blotted after 5 min and grid was dried. Morphological images of the nanomaterials were analyzed using a transmission electron microscopy (HITACHI-JP/H7600) functioning at an accelerating voltage of 100 Kv.

Bactericidal activity by disk diffusion method

The antibacterial activity of biosynthesized silver nanoparticles was tested by the disk diffusion method. Solid nutrient agar plates were spread with 100 ll suspensions of *S. typhimurium* (KCCM 11862) bacterial culture. Whatman filter paper (6 mm) disks were prepared and sterilized. The disks were loaded with 50, 100, 150 and 200 lg/disk concentrations of synthesized silver nanoparticles in solution and dried under sterile conditions. The dried disks were placed on inoculated plates and plates were incubated at 37 _C for 24 h. The diameters of inhibition zones were measured in millimeters. The tests were performed in triplicates.

RESULTS AND DISCUSSION

Silver nanoparticle biosynthesis

The biosynthesis of silver nanoparticles was performed using 45 ml of silver nitrate solution and 5 ml of extract. Change in color of solution from colorless to dark yellow or brown was obsaverd for the formation of silver nanoparticles in the solution. The color change was indicate at 4th hour of incubation, indicating the formation of silver nano particles. The nanoparticles biosynthesized by the reduction of silver nitrate aqueous solution were analyzed by the UV– Vis spectroscopy to ensure formation and stability of silver nanoparticles in the solution. The peaks were found at 422,414,424, 436 nm for silver nano particles synthesized at 20, 30, 35 respectively. For 5, 10, 15, 20 and 25 ml of extract used to synthesize silver nanoparticles, the maximum absorption peaks were obtained at 414, 422 436, 432 and 432 nm, respectively. The synthesis of silver nanoparticles occurred at 2rd–4th hour interval of addition of extract and completed within 24 h. As the time increased, the color intensity of solution increased with simultaneous increase in absorbance as represented in the Figs. 1 and 2.The change in color from light yellow to brown was noticed at 4th hour of addition of plant extract to the silver nitrate solution. The peaks obtained within the 420–440 confirmed the biosynthesis of silver nanoparticles. The intensity of peaks increases with increase in the time, which indicated the generation of more and more nanoparticles and stability of the bio synthesized nanoparticles. The increase in absorbance was higher during time intervals 4–8 h and 8–12 h and later after 24 h increase in absorbance was observed but the rate was slower as compared with the time interval 4–24 h. The increase in intensity could be attributed by reduction of silver nitrate to silver nanoparticles in the solution. This indicates that maximum production of nanoparticles at different temperatures was between 4 and 24 h. The maximum absorption peaks varied for samples incubated at various temperatures as well as for samples with varying concentrations of extract of *Cacica papaya* but the maximum absorption peak for nanoparticles synthesized at different temperatures did not vary much it was 422 nm for nanoparticles synthesized at 20 _C, 432 nm for nanoparticles biosynthesized at 35, 50 and 65 _C. As the temperature was increased, wavelength of maximum absorbance was also increased; which indicated the size dependence of nanoparticles with temperature. The intensity was observed to be higher for nanoparticles synthesized at lower temperature of 20 _C followed by 50, 65 and 35 _C. Silver nanoparticles biosynthesized using various concentrations of aqueous extract revealed their effects on nanoparticle synthesis. Nanoparticles were successfully synthesized at all the five concentrations 5%, 10%, 15%, 20% and 25% of extract tested. But increased concentration of extract after particular concentration decreased the production of the nanoparticles, which could be noticed from intensity of the peaks obtained by UV–Visible spectroscopy as shown in Fig. 2. Increase in the concentration of extract from 5 to 10 and 15% increased the intensity of peaks indicating more production of silver nanoparticles, but further increase to 20% and 25% decreased the yield of synthesized nanoparticles and it was also relatively slow, which could be attributed by decreased concentration of silver nitrate in the solution. Increased production occurred with 10% and 15% extract is because of the availability of more amount of reducing agents from the aqueous extract of *T. nucifera*. The red shift of absorbance maximum was observed, when 10 ml of extract was used and blue shift occurred at the concentrations of 15, 20 and 25 ml but it was found to be lower for nanoparticles synthesizedusing 5 ml of the aqueous extract. According to Heet as the size of nanoparticles increases their UV–Vis absorption peak would shift to red. But in our study much difference in the maximum absorbance was not observed with 10%, 15%, 20% and 25% of used extract for silver nanoparticle synthesis. As per the results obtained by Bio-SEM the minimum size of nanoparticles obtained was 10 nm with all concentrations of extract.

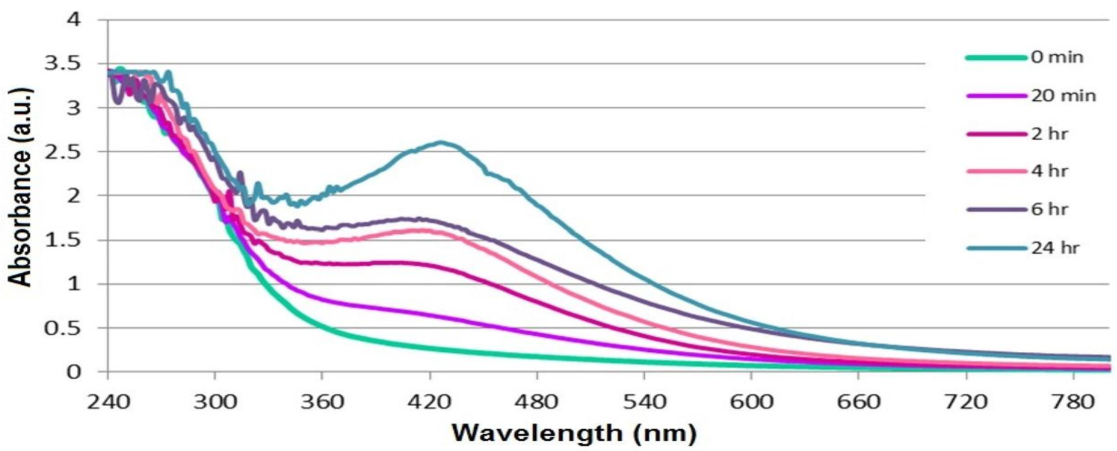

Fig. 1: uv –vis spectrophotometer

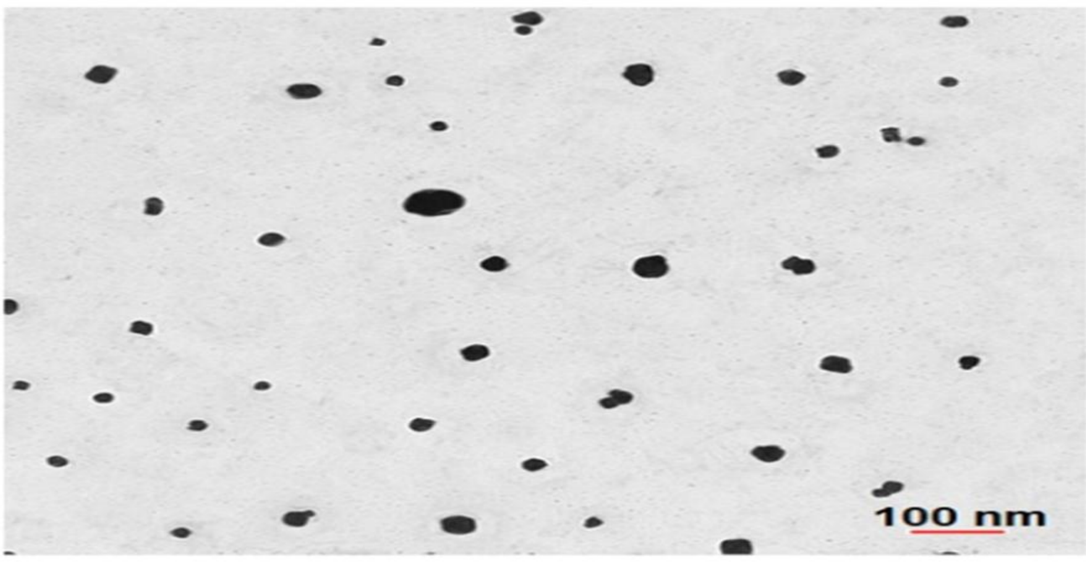

Fig. 2: Sem Emage

Antibacterial activity of silver oxide nanoparticles

AgNPs have currently received a great deal of attention and concern due to their antibacterial activity[45]. In the curent study, the biologically synthesized AgNPs showed an unique antimicrobial activity against test microorganisms. Table shows the result of antimicrobial activity for biosynthesized AgNPs, aqueous solution of AgNO3 and aqueous extract of *C. papaya* by disc diffusion method. Clear zone around the disc was found as the inhibitory effect. As it is shown in figure, AgNPs had respectable inhibition zone against all strains. The zone of inhibition of AgNPs for *E. coli Bacillus cereus Pseudomonas, clostridium* was 15.1 • } 0.2, 14.3 • } 0.3, 13.0 • } 0.1 and 14.3 • } 0.2 mm, respectively. In general, AgNPs showed more anti-bactericidal activity compared with the AgNO3 solution, the inhibition zone diameter of AgNO3 solution was 8.1 • } 0.1, 7.6 • } 0.4, 8.3 • } 0.2 and 8.0 • } 0.1 mm for *E. coli, Bacillus cereus Pseudomonas ,clostridium* respectively. It should be pointed out that the *C. nobile* extract showed no activity towards any of the organisms. Moreover, the antimicrobial properties of synthesized silver nanoparticles were determined by means of minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) MIC was recorded as the lowest concentration.

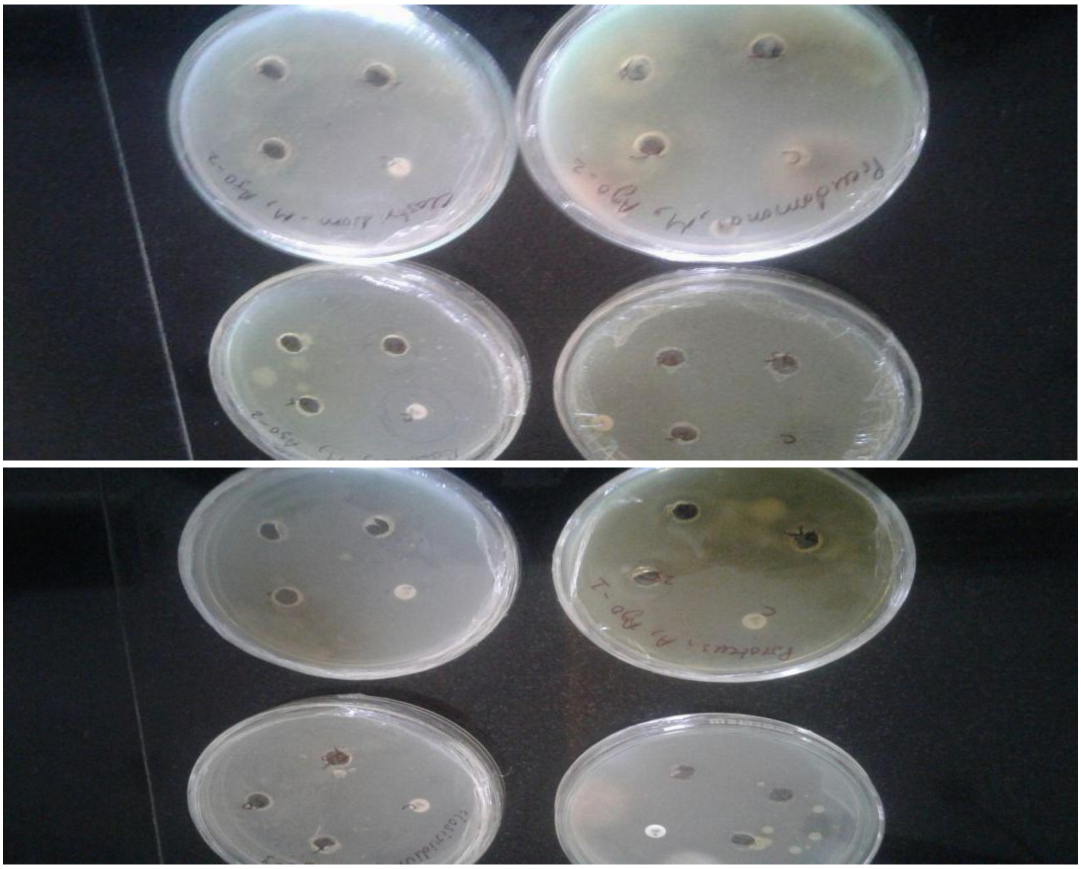

Figure Anti microbial activity against *Bacillus cereus Pseudomonas ,clostridium* A E.colai

Table 3: Minimum inhibition concentrations (MIC) of AgNPs

	AgNPs		$AgNO_3$		*C. nobile* aqueous extract	
Bacteria	MIC (μg ml^{-1})	MBC (μg ml^{-1})	MIC (μg ml^{-1})	MBC (μg ml^{-1})	MIC (μg ml^{-1})	MBC (μg ml^{-1})
E. coli	7.8	15.6	15.6	15.6	NA[a]	NA
	7.8	15.6	15.6	31.2	NA	NA
S	31.2	62.5	62.5	125	NA	NA
	15.6	31.2	31.2	62.5	NA	NA

No Antibacterial activity was found with the concentrations used in this work.

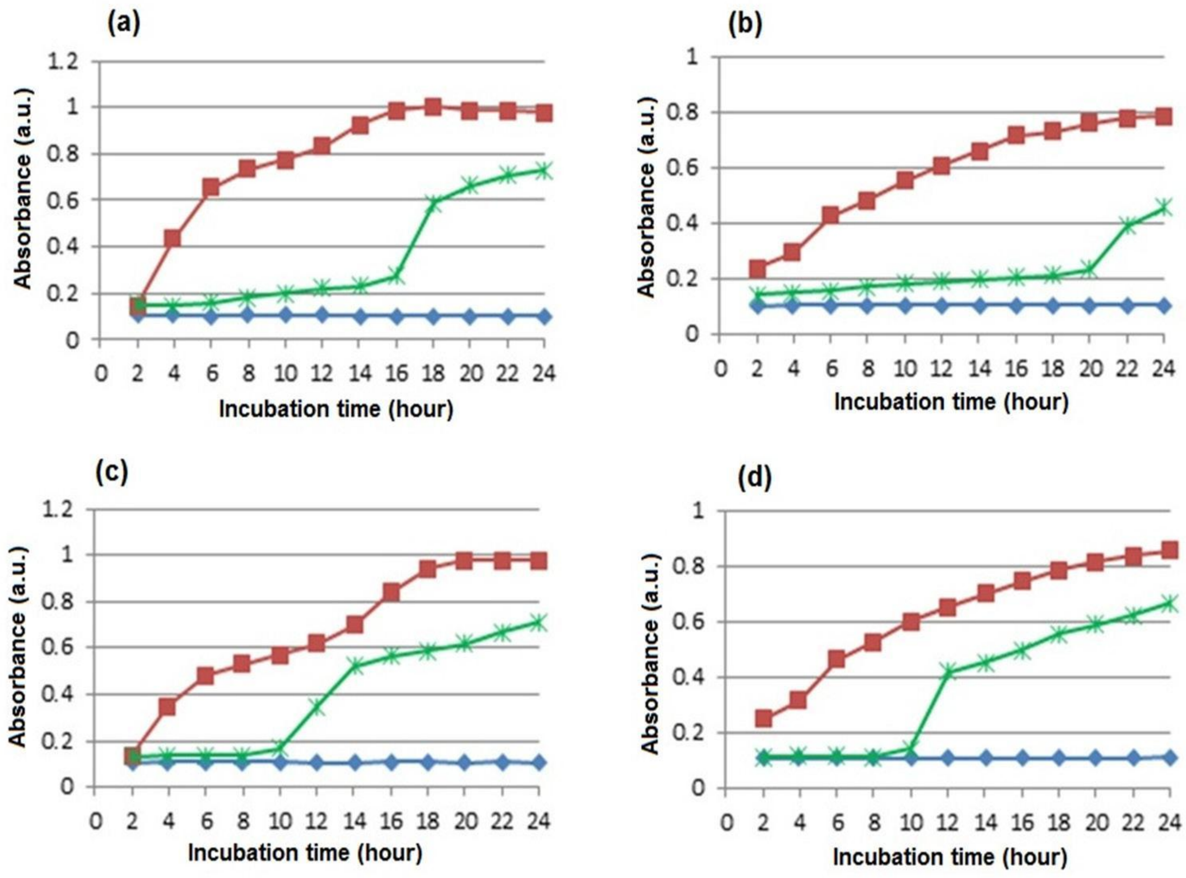

figure Growth curves of different bacterial strains exposed to Ag-NPs synthesized by cacica papaya root extract during 24 h: (a) E. coli,(b). *Bacillus cereus Pseudomonas ,clostridium* ✳(green star symbol): AgNPs with concentration of (a) 7.8 µg ml^{-1} for E. coli,(b) 7.8 µg ml^{-1} for pesudomonas (c) 31.2 µg ml^{-1} forclosstridium and (d) 15.6 µg ml^{-1} for B. subtilis. ♦ (blue diamond symbol): negative control, ■ (red square symbol): positive control.

CONCLUSIONS

The synthesis of silver nanoparticles by phytoreduction of silver nitrate yielded spherical, rod, triangular and irregular hexagonal shaped silver nanoparticles. Temperature was found to play a vital role in determining the size where as concentration of extract was involved in determining the shape of nanoparticles. The increase in concentration of extract reduced the yield of silver nanoparticles. The biosynthesized nanoparticles were found to be stabilized by proteins; which contain no hazardous chemicals and are ecofriendly. The biosynthesized silver nano particles exhibited superior antibacterial activity against Bacillus cereus Pseudomonas ,clostridium which validates its use as antibacterial agents in purification of water and can also be used as coating on food packaging to avoid contamination of *Bacillus cereus Pseudomonas, Clostridium*.

Acknowledgement

This research was supported by the Allahabad agriculture university Lucknow.

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