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ORIGINAL ARTICLE

Green synthesis and characterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties

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X-ray diffraction spectroscopy (XRD);
Bactericidal efficiency

Abstract The evolution of nanotechnology and the production of nanomedicine from various sources had proven to be of intense value in the field of biomedicine. The smaller size of nanoparticles is gaining importance in research for the treatment of various diseases. Moreover the production of nanoparticles is eco-friendly and cost effective. In the present study silver nanoparticles were synthesized from *Carica papaya* leaf extract (CPL) and characterized for their size and shape using scanning electron microscopy and transmission electron microscopy, respectively. Fourier transform infrared spectroscopy (FTIR), Energy dispersive X-ray spectroscopy (EDS/EDX) and X-ray diffraction spectroscopy (XRD) were conducted to determine the concentration of metal ions, the shape of molecules. The bactericidal activity was evaluated using Luria Bertani broth cultures and the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were estimated using turbidimetry. The data analysis showed size of 50–250 nm spherical shaped nanoparticles. The turbidimetry analysis showed MIC and MBC was >25 μg/mL against both Gram positive and Gram negative bacteria in Luria Bertani broth cultures. In summary the synthesized silver nanoparticles from CPL showed acceptable size and shape of nanoparticles and effective bactericidal activity.

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1. Introduction

The synthesis of nanoparticles and applications are gaining intense importance in biomedicine, the smaller size of nanoparticles (1–100 nm), high surface area and reactivity provide them the ability for therapeutic purpose in different dosage forms and dosing routes. Nanoparticles could be derived from

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various sources of gas, liquid or solid phases. They can be synthesized using different synthetic methods like physical, chemical, and biological synthesis (Iravani et al., 2014).

Carica papaya belongs to family Caricaceae and commonly known as *Papaya*, Paw Paw, Kates, and Papaw. The *C. papaya* is one of the medicinal plants. The *papaya* fruits, bark, leaves are being used as medicine to treat various diseases such as warts, corns, constipation, amenorrhoea, general debility, sinuses, eczema, cutaneous tubercles, glandular tumours, blood pressure, dyspepsia, cancer cell growth, diabetes, malaria, expel worms and stimulate reproductive organs, syphilis and gonorrhoea (Aravind et al., 2013; Sinhalagoda et al., 2013). The literature suggests that *C. papaya* fruit and leaf extracts are being used to treat dengue fever (Nisar et al., 2011) to increase RBC and platelet counts (Sinhalagoda et al., 2013). It is also reported that the *C. papaya* leaf extract works against sickling of RBC (Imaga et al., 2009). The Schistosomicidal and leishmanicidal activities of *C. papaya* stem extract (Rashed et al., 2013) are also reported. Recent research reports on *C. papaya* fruit extract exerting antioxidant and immunostimulant properties against acrylamide toxicity in rats (Kadry, 2012). The extract of *C. papaya* leaves and fruit is rich in vitamins, phenols, proteolytic enzymes which acts as a good antioxidant and an excellent antimicrobial agent (Zuhair et al., 2013a,b; Maisarah et al., 2013; Ozkan et al., 2001).

The biosynthesis of nanoparticles was done using microbial strains, enzymes and metabolites (Ali et al., 2011), plant extracts (Harekrishna et al., 2009; Nagati et al., 2012, 2013), and biodegradable products (Avnesh et al., 2010). Biosynthesis of nanoparticles by using *C. papaya* fruit and leaf extract had been previously reported to be having antimicrobial properties (Jain et al., 2009; Ratika and Vedpriya, 2013). In the present study *C. papaya* silver nanoparticles (CPL-AgNPs) were biosynthesized using the biological approach. CPL-AgNPs were synthesized by mixing $AgNO_3$ solution with extract of *C. papaya* leaves. The chemical reaction involved in the formation of nanoparticles is the reduction of silver ions by the aqueous extract. The obtained nanoparticles were characterized by using UV–visible spectrophotometer, electron microscopy (SEM and TEM) EDX, FTIR, X-ray diffraction and evaluated for antibactericidal properties using bacterial strains.

2. Materials and methods

2.1. Materials

C. papaya leaves were taken from the local fields of Nalgonda, Telangana, India. The reagents such as Luria Bertani Broth (Cat. No.: M1245, Himedia, Mumbai), Nutrient Agar (Cat. No.: M001, Himedia, Mumbai) and ampicillin sodium salt (Cat. No.: TC021, Himedia, Mumbai), were procured from Himedia laboratories, Mumbai, India. Silver nitrate (Cat. No.: 209139, Sigma Aldrich, India), Bacterial cultures (*Staphylococcus auerus* MCC 2408; *Bacillus subtilis* MCC 2511; *Micrococcus luteus* MCC 2155; *Escherichia coli* MCC 2412; *Klebsiella pneumoniae*MCC 2451) were procured from MCC-NCCS, Pune, India. Clinical sample of *Pseudomonas putida* was obtained from Department of Microbiology, Osmania University, Hyderabad, India.

2.2. Methods

2.2.1. Preparation of *C. papaya* leaf extract and 1 mM $AgNO_3$

Fresh leaves of *C. papaya* (25 g) were diced into fine pieces and transferred to sterile 250 mL conical flask. MilliQ WATER 200 mL was added to the flask and heated at 60 °C for 5–10 min and incubated on sand bath for 30 min to facilitate the formation of aqueous extract. The extract was filtered using Whatman No. 1 filter paper and the filtrate was stored at 4 °C for further use. Silver nitrate ($AgNO_3$, Sigma Aldrich, USA), 0.0421 gm was added to 100 mL of double distilled water and dissolved thoroughly. The solution obtained was transferred to an amber coloured bottle to prevent autoxidation of silver.

2.2.2. Determination and synthesis of silver nanoparticles

The aqueous leaf extract of *C. Papaya* and 1 mM $AgNO_3$ were mixed in the ratio of 1:4 and heated on a sand bath at 60 °C for 30 min until change in colour was observed. The colour change indicated the formation of silver nanoparticles by *C. papaya* leaf extract (CPL-AgNPs) (Fig. 1).

2.2.3. Characterization of *C. papaya* leaf silver nanoparticles (CPL-Ag nanoparticles)

2.2.3.1. UV–visible spectrometric analysis of silver nanoparticles. An ELICO SL-159 UV–visible spectrophotometer (Andhra Pradesh, INDIA) was employed for the spectrometric analysis of biosynthesized silver nanoparticles. The reduction of silver was measured periodically at 200–800 nm. A spectrum of silver nanoparticles was plotted with wave length on *x*-axis and absorbance on *y*-axis. The absorbance peaks can be observed in Fig. 2.

2.2.3.2. Fourier transform infrared (FTIR) analysis of silver nanoparticles. For removing the biochemical compounds or uncapping ligands of the nanoparticles, the 200 mL residual solution of silver nanoparticles was centrifuged at 10,000 rpm for 30 min and the precipitate was resuspended in 10 mL ethanol and then in sterile distilled water. The centrifugation and resuspension processes were repeated for 3–4 times. The purified suspension was dried in an oven to obtain the powder and analysed by Fourier transform infrared spectrum (FTIR), Nicolet Avatar 660 (Nicolet, USA).

2.2.3.3. Scanning electron microscopy–energy dispersive X-ray spectrometry (SEM–EDX) analysis of silver nanoparticles. Scanning electron microscope (SEM) analysis was carried out using Zeiss EVO 18-EDX special edition machine compatible with EDX machine. The silver nanoparticles were centrifuged at 10,000 rpm for 30 min and the pellet was redispersed in 10 mL ethanol and washed 3 times with sterile distilled water to obtain the pellet. The pellet was dried in an oven and thin films of dried samples (10 mg/mL) were prepared on carbon coated copper grid and analysed for size determination. The particle size and texture of nanoparticles can be analysed by using image magnification software compatible with SEM and helps in determining the presence and formation of silver nanoparticles.

2.2.3.4. Transmission electron microscope. Transmission electron microscopy (TEM) technique was used to visualize the

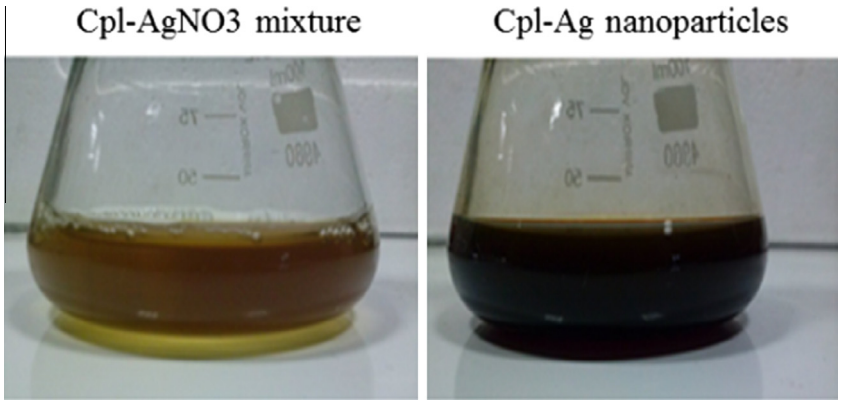

Figure 1 Digital picture of *C. papaya* leaf extract with 1 mM $AgNO_3$ solution before and after the CPL-AgNPs.

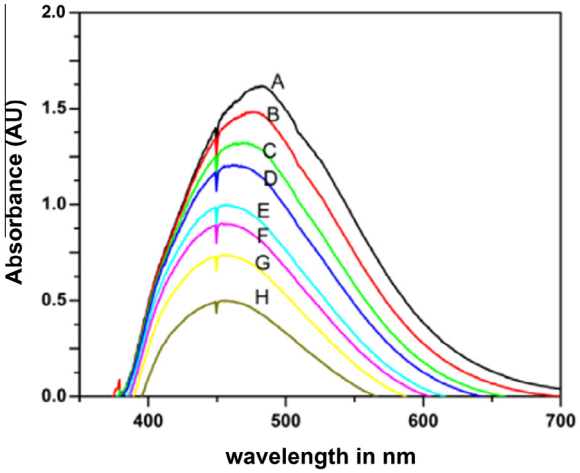

Figure 2 UV–visible spectra of *C. papaya* leaf-silver nanoparticles (CPL-AgNPs) and absorbance peak noted at 470 nm.

morphology of the CPL-AgNPs. The make of Transmission electron microscope (TEM; Philips model CM 200) technique was to visualize the morphology of nanoparticles. The instrument was operated at an accelerating voltage of 200 kV with ultra-high-resolution of 0.2 nm and magnification of 2,000,000 X. TEM's grid size is 3 mm diameter which was prepared placing a 5 µL of the silver nanoparticles solutions on carbon-coated copper grids and drying under mercury lamp and then analysed. The size of the silver nanoparticles can be determined by using the image magnifying software and this software will be able to magnify the particles with size less than 10 nm and give clear morphological data.

2.2.3.5. X-ray diffraction analysis of silver nanoparticles. X-ray diffraction measurements of biologically reduced silver-nitrate solution drop coated onto glass slides were determined by an X'Pert Pro P Analytical X-ray diffractometer instrument with Xan'Pde high score plus software operating at a voltage of 40 kV and a current of 30 mA with Cu K α radiation. The scanning of biosynthesized silver nanoparticles is exercised in 2θ region and the data are analysed. The Debye–Scherrer equation was employed to calculate the average particle size of the CPL-AgNPs.

$$D = k\lambda/\beta 1/2\cos\theta$$

2.2.4. Assessment of antibactericidal activity using disc diffusion method

The antibacterial assays were done on human pathogenic strains like *Staphylococcus aureus, B. subtilis, M. luteus, P. putida, K. pneumoniae and E. coli* by the standard disc diffusion method. Briefly, Luria Bertani (LB) broth/agar medium was used to cultivate bacteria strains. Fresh overnight inoculum (100 µL) of each culture was spread on to Luria Bertani agar plates. Sterile Whatman No. 1 paper discs of 5 mm diameter containing 10 µL of *C. papaya* leaf extract (5 µg), 10 µL of CPL-AgNPs (5 µg and 10 µg/mL), and 5 µL of ampicillin (10 µg) were placed in each plate in a serial order. After incubation overnight at 37 °C, zone of inhibition was measured (diameter in mm). The bactericidal activity is evaluated by the size of clear zone and greater the zone of inhibition greater the bactericidal activity.

2.2.5. Minimum inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) studies

The MIC and MBC studies were performed to determine the concentration of biosynthesized silver nanoparticles showing growth inhibition of bacterial strains. The serial dilutions of silver nanoparticle lyophilized powder in 1:2 ratio were used and the concentrations were ranging between 3.25 and 1600 µg/mL. To the serially diluted tubes 10 µL/mL of overnight inoculum (10^{-3} to 10^{-5} CFU) was added and incubated for 24 h at 37 °C. After 24 h incubation a 100 µL (10 times diluted sample) of sample from each culture tube for individual bacterial strain was plated on to sterile Luria Bertani agar plates and incubated overnight.

3. Results

The UV absorption spectrometric analysis of CPL-AgNPs showed absorbance spectra at 470 nm suggesting bioreduction of silver nitrate into silver nanoparticles (Fig. 2). The SEM and TEM analyses showed the particle size between 50 and 200 nm and average size of silver nanoparticles between 5 and 40 nm with a spherical morphology (Figs. 4–6Fig. 3). The quantitative analysis using EDX showed high silver content of 41%. The spectrum also showed the presence of oxygen and silicon of 46.11% and 7.11%, respectively (Fig. 5). The FTIR analysis spectrum showed sharp absorbance between 500 and 4000 cms^{-1}. There are other peaks in the spectrum at 633,

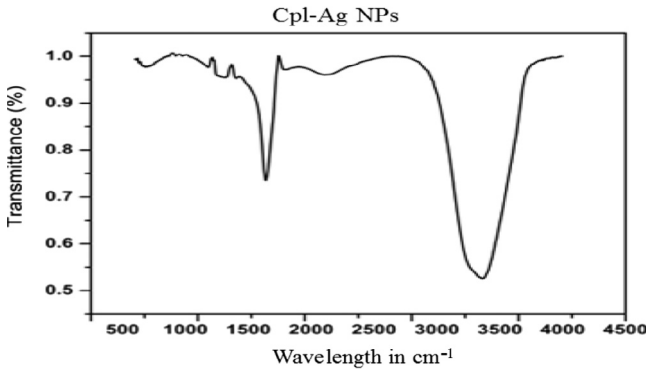

Figure 3 Fourier transform infrared (FTIR) spectrum of CPL-AgNPs.

869, 925, 976, 1181, 1315, 1406, 1640, 1782, 2103 and 3359 which could be the esters, ethers, carbonyl (polyol's) or aromatic compounds (). XRD data show diffraction peaks at 2θ = 38.2°, 44.4°, 64.6°, 77.5°, and can be indexed to (111), (200), (220), (311), and (222) planes of pure silver ions indicating the biosynthesis of silver nanoparticles (Fig. 7).

The results obtained from the disc diffusion method showed the effect of CPL-AgNPs, CPL extract and ampicillin on both Gram positive and Gram negative bacterial strains. The zone of inhibitions measured for different bacterial strains were 7 mm and 9 mm for *M. luteus* at 5 μg/mL and 10 μg/mL of CPL-AgNPs, and in case with CPL extract and ampicillin alone it was 5 mm and 16 mm, respectively. *S. aureus* showed 8 mm and 11 mm upon treatment with 5 μg/mL and 10 μg/mL of CPL-AgNPs, and in case with CPL extract and ampicillin alone it was 6 mm and 14 mm, respectively. For *B. subtilis* it was observed to be 7 mm and 11 mm for CPL-AgNPs, and for CPL extract and ampicillin alone it was 5 mm and 14 mm. *E. coli* showed zone of inhibition of 7 mm and 13 mm when treated with 5 μg/mL and 10 μg/mL of CPL-AgNPs and with CPL extract and ampicillin it gave 5 mm and 13 mm. *P. putida* showed 6 mm and 10 mm with 5 μg/mL and 10 μg/mL of CPL-AgNPs and with CPL extract and ampicillin it was observed to be 4 mm and 15 mm. *K. pneumoniae* showed zone of inhibition of 7 mm and 9 mm when treated with 5 μg/mL and 10 μg/mL of CPL-AgNPs and with CPL extract and ampicillin it was 4 mm and 14 mm. The concentration of CPL extract (5 μg/mL) and ampicillin (10 μg/mL) was kept constant throughout the experiment (Fig. 8); Table 1. The turbidimetry analysis of CPL-AgNPs showed good antibacterial activity with MIC and MBC of 100 μg/mL for *S. aureus* and *E. coli; B. subtilis* and *K. pneunominae* showed MIC and MBC of 50 μg/mL; *P. putida* and *M. luteus* were more sensitive to the extract with MIC and MBC of 25 μg/mL.

4. Discussion

Nanomaterials had proven to be the efficient mode of drug delivery in modern science (Arokiyaraj et al., 2014). The

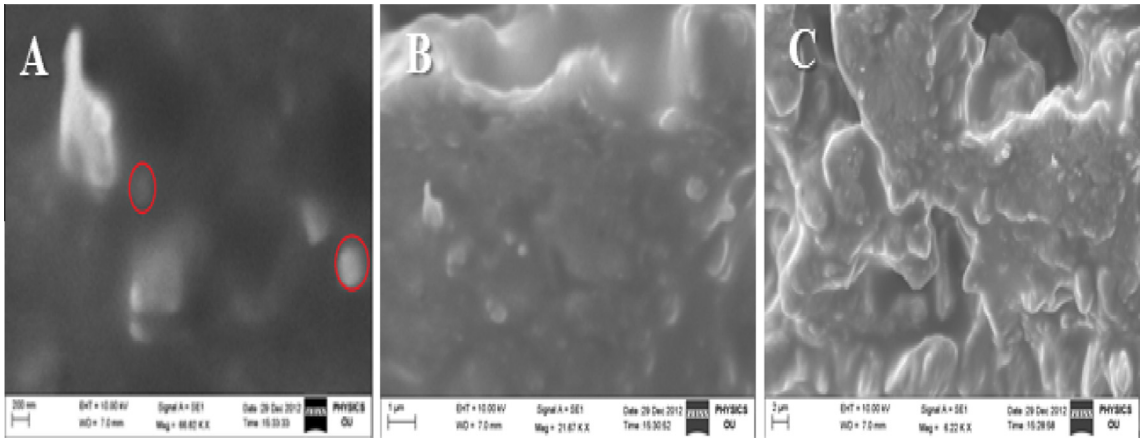

Figure 4 Scanning electron microscopy (SEM) micrographs of synthesized *C. papaya* leaf silver nanoparticles (CPL-AgNPs), (A) show the morphology of nanoparticles on 200 nm scale, (B) showing nanoparticles on 1 μm scale and (C) showing nanoparticles on 2 μm scale.

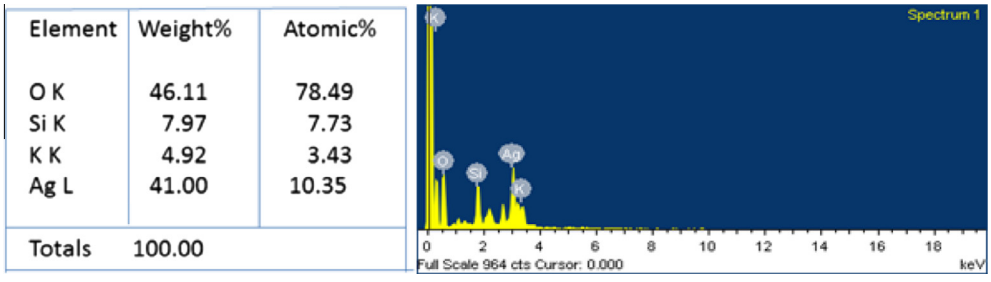

Figure 5 EDX characterization spectrum obtained for CPL-AgNPs powder. Visible peaks confirm the presence of silver, oxygen and silicon dioxide substances in the tested sample.

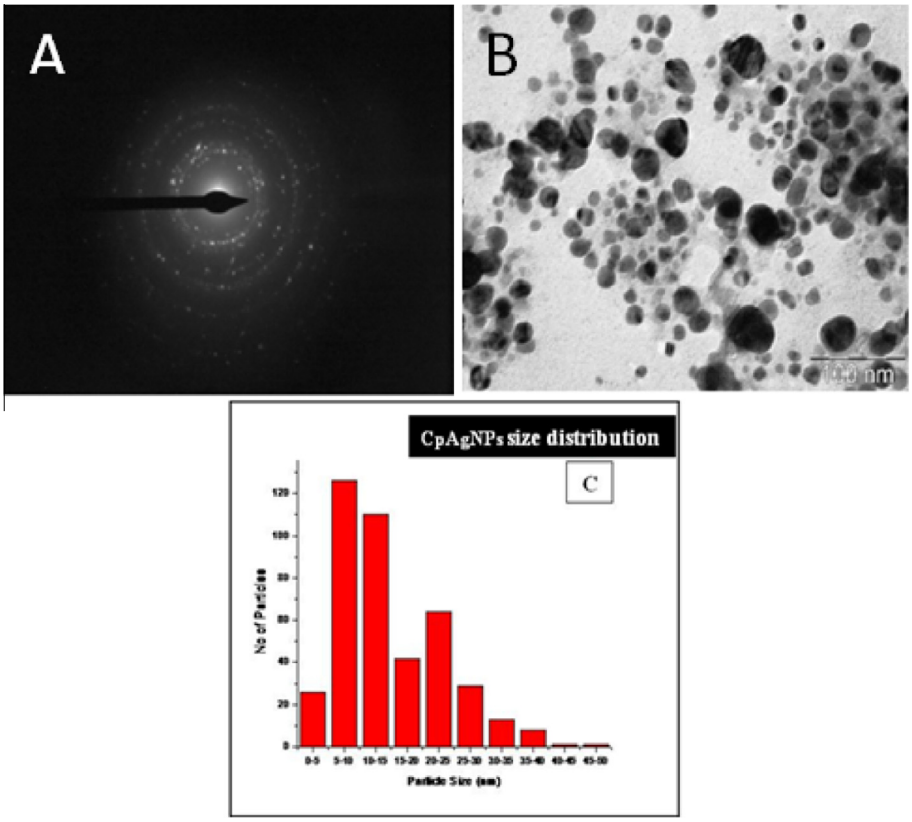

Figure 6 Transmission electron microscopy (TEM) micrographs of green synthesized CPL-AgNPs dispersed on the copper grid (A and B) and histogram showing particle size distribution (C).

utilization of medicinal plant materials, microbes, enzymes for synthesis of nanoparticles has been revolutionized in recent years and could serve as alternative for antibiotics (Palanisamy et al., 2014). In the present study, the CPL-AgNPs showed particle size ranging in between 5 and 200 nm, which may confer the ability to penetrate the cells/microbes and execute the bactericidal property. The mechanism of action of silver nanoparticles is ambiguous in microorganism (Kim et al. 2007,2011).

The characterization of nanoparticles was done using various techniques such as UV–visible spectroscopy, electron microscopy, X-ray diffraction, Energy dispersion X-ray diffraction and Fourier transmission infrared. The results obtained from the techniques and antibacterial studies have coincided with the literature in the field of nanoparticle research (Ali et al., 2011; Ashok, 2012) and certainly gives proof about the nanoparticle synthesis and its efficiency as antibacterial agent (Palanisamy et al., 2014). The

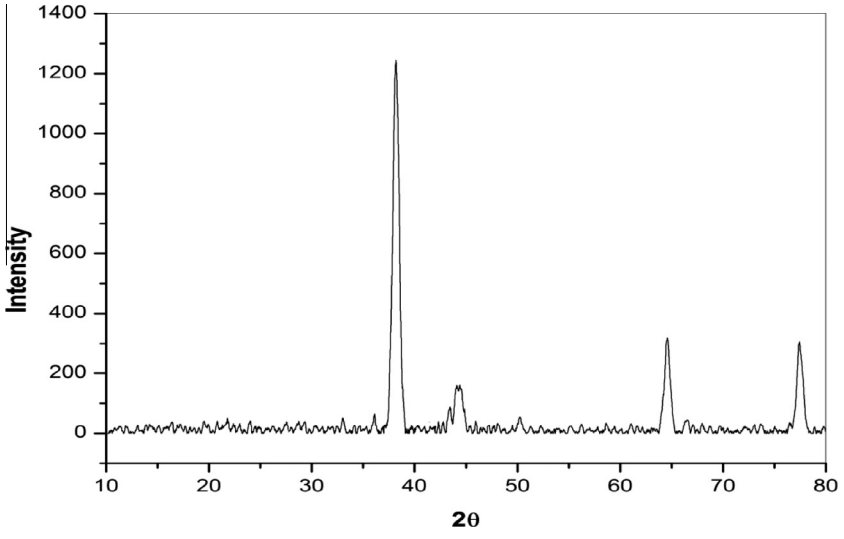

Figure 7 X-ray diffraction pattern of synthesized silver nanoparticles from *C. papaya* leaf extract.

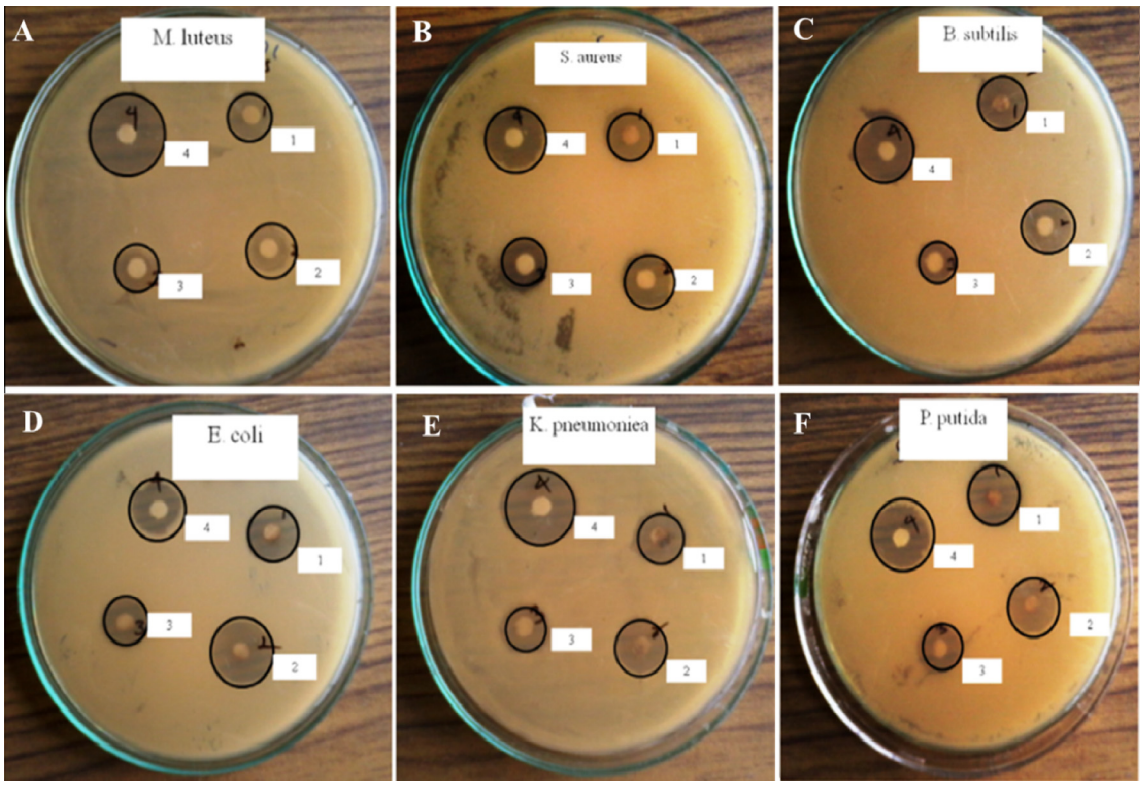

Figure 8 The antibacterial efficiency of *C. papaya*-silver nanoparticles (CPL-AgNPs), *C. papaya* leaf (CPL) extract and ampicillin against Gram positive (*M. luteus, S. aureus, B. subtilis*) and gram negative bacteria (*E. coli, K. pneumoniae and p. putida*). *Note*: Disc No. 1 (5 μg/mL CPL-AgNPs, Disc No. 2 (10 μg/mL CPL-AgNPs, Disc No. 3 (5 μg/mL CPL extract), Disc No. 4(10 μg/mL ampicillin).

Table 1 Antibacterial capacity of *C. papaya* leaf (CPL) extract, *C. papaya*- silver nanoparticles (CPL-AgNPs) and ampicillin against gram-positive and gram-negative bacteria.

Microorganism	Zone of inhibition in mm			
	Disc No. 1 CPL-AgNPs (5 μg/mL)	Disc No. 2 CPL-AgNPs (10 μg/mL)	Disc No. 3 CPL Extract (5 μg/mL)	Disc No. 4 Ampicillin (10 μg/mL)
Gram positive strains				
M. luteus (MCC 2155)	7	9	5	16
S. aureus (MCC 2408)	8	11	6	14
B. subtilis (MCC 2511)	7	9	5	14
Gram negative strains				
E. coli (MCC 2412)	7	13	5	13
P. putida (clinical sample)	6	10	4	15
K. pneumoniae (MCC 2451)	7	9	4	14

bio-compounds in leaf extract having the carbonyl groups are believed to be reducing the silver ions and converting to unsaturated carbonyl groups by auto-oxidizing (Ashok, 2012). Surface plasmon resonance of metal UV–visible spectrometry showed a sharp peak at 470 nm confirming the formation of silver nanoparticles (Baia and Simon, 2007; Ayman et al., 2014). The FTIR analysis of CPL-AgNps showed two sharp absorption peaks at 1640 cm^{-1} and 3359 cm^{-1} indicating the possible interaction between proteins and silver nanoparticles. The absorption peak at 1640 cm^{-1} could be due to the amide bond coming from the carbonyl group of a protein (Macdonald and smith, 1996) and the peak at 3359 cm^{-1} may be because of OH groups present in alcohols and phenolics (Jilie and Shaoning, 2007; Ali et al., 2011; Theivasanthi and Alagar, 2012).

The diffraction peaks obtained in XRD corresponded to face centred cubic structure of metallic silver ions in its purest form and the size of particle ranging between 5 and 50 nm (Theivasanthi and Alagar, 2012; Arokiyaraj et al., 2014). The characterization studies by scanning electron microscopy has provided more information on the synthesized silver nanoparticles in the presence of *papaya* leaf extract such as size and morphology of nanostructures at scale of 200 nm, 1 μm and 2 μm (Fig. 4) (Ali et al., 2011). The transmission electron microscopy studies have given further inputs on the morphology and size of biosynthesized silver nanoparticles ranging

between 5 and 50 nm with a scale of 100 nm and histogram showing sizes of particles with spherical morphology (Fig. 6) (Ruparelia et al., 2008; Agnihotri et al., 2014). EDX analysis shows the presence of pure silver and other elements confirming the biosynthesis of silver nanoparticles (Ali et al., 2011).

Literature suggests that there are several mechanisms by which silver nanoparticles could be killing the microorganisms; (i) destructuring the cell wall and ceasing the cell permeability (ii) formation of free radicals, (iii) inactivating important enzymes by interacting with thiols, (iv) interaction of silver nanoparticles with DNA and interruption of DNA replication and translation and by dephosphorylating the tyrosine residues on peptides inhibiting the signal transduction and growth in bacteria (Kim et al. 2007,2011). The antibacterial studies with CPL-AgNPs showed profound antibacterial effect against both Gram positive and Gram negative strains. The disc diffusion studies demonstrated larger inhibition zones in comparison to *papaya* leaf extract and similar to that of ampicillin (Positive control) which are analogous to the results reported by Nagati et al. (2012). Based on the varying sizes (in mm) of zone of inhibitions, we could confirm the bactericidal efficiency against individual bacterial strains (Fig. 8 Table 1) (Ashwani et al., 2014; Ruparelia et al., 2008). The MIC and MBC studies showed varied concentrations of CPL-AgNPs against both Gram positive and Gram negative strains. The strains like *S. aureus* and *E. coli* showed MIC and MBC of 100 μg/mL, *B. subtilis and K. pneumoniae* showed a MIC and MBC of 50 μg/mL and MIC and MBC against *M. luteus* and *P. putida* is 25 μg/mL (Ashwani et al., 2014). The variation in effective concentrations of CPL-AgNPs could be due to the existence of different modes of action on individual microorganisms (Arokiyaraj et al., 2014).

5. Conclusion

The green synthesis and characterization of CPL-AgNPs was done and confirmed by UV–visible spectrophotometer, FTIR, SEM, TEM, XRD and EDX techniques. The nanoparticles appeared to be spherical in shape with smooth surface and the size of the particles varied from 5 to 50 nm, but amongst them most of the particles obtained were sized in between 5 and 15 nm. The MIC and MBC of the CPL-AgNPs had exhibited inhibitory value >25 μg/mL against both Gram positive and negative bacterial species. In summary, the CPL extract mediated synthesis of silver nanoparticles was efficient and provides additional property such as bactericidal efficiency and might act as long searched alternative and could be the answer to antibiotic resistance.

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