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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.

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 re-dispersed 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