



Antimicrobial Efficacy of Biogenic Silver Nanocomposite against Methicillin Resistant *Staphylococcus aureus*


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ABSTRACT

The present study was conducted during 2019–2020 to characterize the *Carica papaya* leaf synthesized silver nanoparticles and to know the MIC of *Carica papaya* leaf extract along with silver nanoparticles synthesized using *Carica papaya* leaf extract against MRSA by broth dilution method. Aqueous extract and silver nanoparticles of *Carica papaya* leaves was prepared using freshly collected disease free leaves. Characterization of *C. papaya* aqueous leaf extract synthesized silver nanoparticles (CPAgNP) was done by UV-VIS spectra analysis, SEM analysis, Fourier transform infrared (FTIR) analysis and Zeta potential and Particle Size Analysis. A single, strong and broad SPR peak in UV-visible spectrum of the green synthesized silver nanoparticles using the *Carica papaya* extract was observed at 400 nm. FTIR spectrum revealed the band set at 3465.80 cm⁻¹, 3136.95 cm⁻¹, 2917.61 cm⁻¹, 1625.08 cm⁻¹, 1511.51 cm⁻¹ and 1384.81 cm⁻¹ respectively. Electron microscopy showed that *Carica papaya* aqueous leaf extract crystalline nanoparticle had definite particle size. The MIC of AECPL against MRSA was found to be 31.25 mg ml⁻¹ while MIC of CPAgNPs against MRSA was found to be 62.5 µg ml⁻¹. MIC of CPAgNPs was reported quite lower as compared to that of AECPL, thus it could be assumed that antibacterial activity of AECPL might potentiate the antibacterial property of Ag-NPs. Therefore, CPAgNPs could be employed as therapeutic agent against bacterial infection.

KEYWORDS: Antimicrobial, extract, microscopy, nanoparticles, papaya, silver, spectrum, therapeutic

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1. INTRODUCTION

Carica papaya is herbaceous plants belonging to the member of the *Caricaceae* family (Arumuganathan and Earle, 2018). Papaya plant (*Carica papaya*) is widely found in India. Almost all parts of the plant can be utilized for food and medicinal purposes. *C. papaya* plants have medicinal value due to the presence of natural metabolites found in leaf, bark, and twigs that possesses both anti-tumor and pesticidal properties (Adachukwu et al., 2013, Basalingappa et al., 2018). Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5,7- dimethoxycoumarin, caffeic acid, kaempferol, quercetin, chlorogenic acid (Kaur et al., 2019). These compounds have antimicrobial activity and have been proven to be able to inhibit the growth of bacteria. The metallic nanoparticles (NPs) are the most encouraging as they show great antibacterial properties because of their extensive surface range to volume proportion, which is coming up as the ebb and flow enthusiasm for research (Nath and Dutta, 2016, Khandel et al., 2018). The leaves are commonly used in the treatment of varied forms and stages of medical complications (arthritis, digestive disorders hypertension, malaria, and ringworms) (Shubham et al., 2019) and are of particular importance is in the treatment of dengue virus infection (Joy et al., 2019). *C. papaya* leaf extracts help to increase platelet levels and have demonstrated definitive beneficial effects in patients with dengue infection (Koul et al., 2022).

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 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). Metallic nanoparticles (MNPs) produced from inorganic sources was found to be more effective as compared to organic nanomaterials due to their unique physicochemical properties (Nqakala et al., 2021). Green synthesized silver nanoparticle drug delivery systems hold a high level of promise in the ever-evolving drug design and delivery systems (Sujitha et al., 2015). Various nanoparticles synthesized nowadays by the use of nanotechnology have a great potential to serve as an alternative to antibiotics and to control microbial infections. Silver (Ag) has been known to have a toxicity effect over an extensive variety of small organisms; hence silver-based combinations have been widely exploited for its antibacterial applications. Silver is used in the form of silver nitrate (AgNO_3) because of its antimicrobial and wound healing effects; when silver

is used at the nanoscale, it shows enhanced action against microbes because of its increased surface area (Rybka et al., 2023). Biosynthesis of nanoparticles by using *C. papaya* fruit and leaf extract had been previously reported to be having antimicrobial properties (Sinhlagoda et al., 2013, Ratika and Vedpriya, 2013). The silver nanoparticles synthesized using the peel of papaya exhibited good antibacterial activity against pathogenic *Escherichia coli* and *Staphylococcus aureus* (Balavijayalakshmi and Ramalakshmi, 2017). The pharmaceutical companies and the researchers are in search of novel antibacterial agents to solve the problem of development of antibiotic resistance against pathogenic bacteria and to induce the diabetic wound healing process. Hence, the present study was undertaken to know the antibacterial activity and characteristics of biogenic silver nanocomposite against methicillin resistant *Staphylococcus aureus*.

2. MATERIALS AND METHODS

The present study was carried out at College of Veterinary Science & AH, located in Durg district of Chhattisgarh, India lies between 20°54' and 21°32' north latitude & 81°10' and 81°36' east longitude, during August, 2019 –March, 2021

2.1. Synthesis and characterization of silver nanoparticles

Aqueous extract of *C. Papaya* leaves was prepared using freshly collected disease free leaves (50 g). 250 ml of Milli-Q water was added in flask and heated at 80°C for 30 min and incubated on sand bath for 1 h, filtered through normal filter paper and stored at 4°C for further use.

For synthesis of silver nanoparticles, 1mM aqueous solution of silver nitrate was prepared freshly for synthesis of silver nanoparticles. Aqueous leaf extract and Silver nitrate solution were mixed in the ratio 1:4 in a conical flask and heated in sand bath at 70°C for 30 m. To remove unreacted Ag ions, solution of biosynthesized AgNPs was treated with 1% solution of NaCl. AgNPs precipitate was centrifuged at 8,000 rpm for 10 m and washed thrice with ethanol. This AgNP pellet was dried in oven to obtain powder. Different concentrations of CPAgNPs were obtained by dissolving CPAgNP powder in deionized sterile water.

The characterization of *C. papaya* leaf synthesized silver nanoparticles (CPAgNP) was done by UV-VIS spectra analysis, SEM analysis (SAIF, AIIMS, New Delhi) and Fourier transform infrared (FTIR) analysis

2.2. Determination of minimum inhibitory concentration (MIC)

In the present study, methicillin resistant *Staphylococcus aureus* (MRSA) isolate no. SA2018_3 maintained at Department of Veterinary Microbiology, College of Veterinary Science



& A.H., Anjora, Durg was used. Antimicrobial activity of silver nanoparticles (CPAgNPs) and *C. papaya* leaf extract (AECPL) against MRSA was determined by MIC using broth dilution method. The lowest concentration of test compound showing complete inhibition of bacterial growth *i.e.* lowest concentration showing OD value equal to that of negative control was considered as MIC.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of Silver nano-particle

The formation of silver nanoparticles was primarily monitored by using the UV-Vis Spectrophotometer at a wavelength range of 300–800 nm. The reduction of particles was primarily characterized by UV-visible spectrophotometry, and the SPR peak was identified to be at 414 nm. The colour change from light yellow to reddish brown signifies the reduction of AgNO_3 by aqueous extract of *Carica papaya* leaf (AECPL) as an effective bioreducing agent.

The FTIR spectrum of the CPAgNPs is shown by Figure 1 with broad spectrum ranged from band at 4000 cm^{-1} to 400 cm^{-1} . FTIR spectrum revealed band set at 3465.80 cm^{-1} , 3136.95 cm^{-1} , 2917.61 cm^{-1} , 1625.08 cm^{-1} , 1511.51 cm^{-1} and 1384.81 cm^{-1} . The FTIR spectrum shows the interaction of AgNPs with leaf biomolecules of *C. papaya*. It shows the broad band at 3465.80 cm^{-1} is due to the stretching vibrations of -O-H group and the peak for NH stretching was obtained at 3136.95 cm^{-1} . The absorbance peaks at 3432 cm^{-1} can be assigned to N-H stretching vibration (Zia et al., 2016). Bands observed at 2917.61 cm^{-1} and 2849.77 cm^{-1} region arising from C-H stretching of aromatic compound (Karthik et al., 2013). Band observed at 1625.08 cm^{-1} region arising for the carbonyl group. The carbonyl groups witnessed the presence of flavanoids on the surface of nano-sized silver particles interact with the carbonyl groups. The carbonyl group had stronger ability to bind with metal nanoparticles or act as capping and stabilizing agents.

In the present study the band observed at 1631 cm^{-1} can be attributed to the C=C stretching vibration as reported by Annamalai et al. (2016). The peak at 1384.81 cm^{-1} corresponding to C-H sym. deformation vibration and at 1033 cm^{-1} can be allocated to C-O stretching vibration (Kumari et al., 2016). The two absorptions recorded at 1214.22 cm^{-1} and 1038.00 cm^{-1} could be due to the presence of (aliphatic amine) structure vibration.

The present FTIR results confirm the presence of -NH, -OH, C=C and CH groups, indicating the presence of hydroxyl and amine groups substituted flavonoids in plant extracts. The two weak bands at 1038.00 cm^{-1} and 617.47 cm^{-1} are banding vibrations of -O-H and C-H groups,

respectively. FTIR spectral analysis represents the presence of hydroxyl and carboxyl groups act as reducing and stabilizing agent and phenolic group acts as capping agent. The FTIR results confirmed the presence of C=C, -OH, CH and -NH, groups, depicting the presence of hydroxyl and amine groups substituted flavonoids in plant extracts.

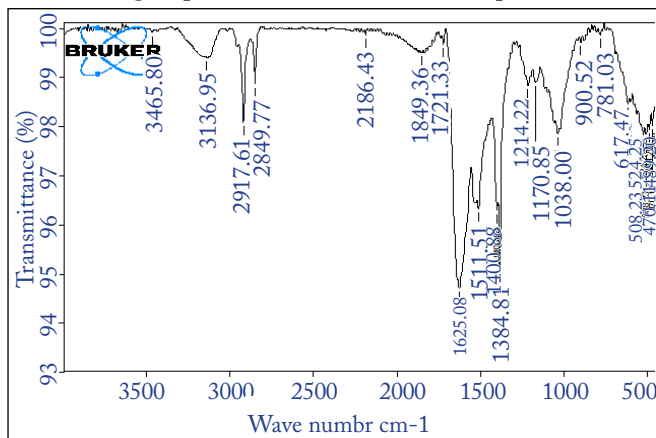


Figure 1: FTIR spectrum of the biosynthesized metal nanocomposites using *C. papaya* leaf extract

Electron microscopy (high-resolution microscopy) is the most accepted procedure to determine the morphology of the nanoparticles. Scanning electron microscopy provides more information regarding morphology and size of the silver nanoparticles. The green synthesized spherical shaped AgNPs with the size from 12–96 nm was observed using the aqueous extract of carica papaya leaves (Figure 2). The bactericidal properties of the nanoparticles are reported to be dependent on size as the nanoparticles that present a direct interaction with the bacteria specifically have a diameter of ~1–10 nm (Morones et al., 2005).

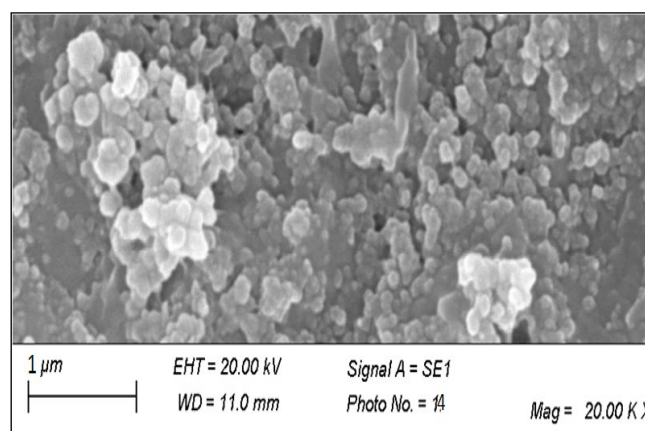


Figure 2: SEM image of the biosynthesized silver nanoparticles

3.2. Antimicrobial activity against MRSA

3.2.1. Efficacy of *C. papaya* leaf extract (AECPL) against MRSA

Present study determined MIC of *C. papaya* leaf extract

against MRSA by broth dilution method. MIC of AECPL against MRSA was 31.25 mg ml^{-1} (Figure 3a). Inhibition of MRSA growth was initiated at 4 h following incubation at 37°C i.e. during mid log phase ($0.13 \text{ OD}_{600\text{nm}}$) of bacterial growth (Figure 3b). Complete inhibition of bacterial growth was observed after 12 h of incubation. Present study reported lower MIC of AECPL than those reported by Anibijuwon et al. (2009) who observed MIC value in the range of $50\text{--}200 \text{ mg ml}^{-1}$ against *Staphylococcus aureus* which suggested comparatively better antimicrobial efficacy of present AECPL against MRSA. Dakal et al. (2016) found that *C. papaya* had most activity with MIC ranged from $25\text{--}50 \text{ mg ml}^{-1}$.

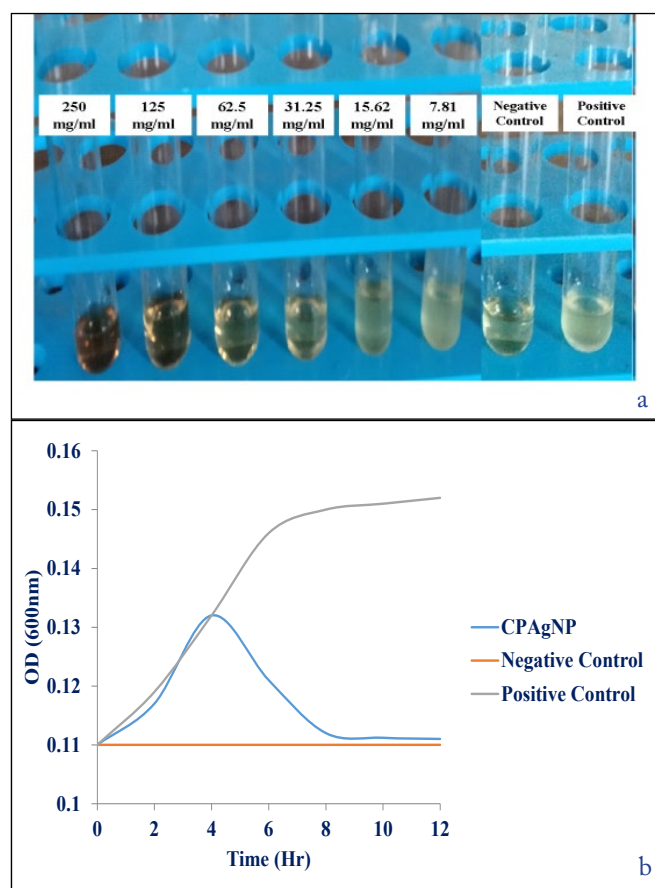


Figure 3: MIC of AECPL against MRSA (a) broth dilution method (b) Inhibition of MRSA growth Positive Control: MRSA culture in nutrient broth; Negative Control: Nutrient broth without MRSA; AECPL: MRSA culture treated with 31.25 mg ml^{-1} AECPL

The phytochemical analysis of present study revealed that *C. papaya* leaf extract contains active ingredients like; alkaloid, flavonoid, tannin, and phenols. These compounds are known to have antibacterial, anti-inflammatory, and antifungal properties (Ahmada et al., 2013). Thus, bioactive compounds from the AECPL with antibacterial activity can

be transformed into possible medication.

3.2.2. Efficacy of silver nanoparticles (CPAgNPs) against MRSA

MIC of CPAgNPs against MRSA was $62.5 \text{ } \mu\text{g ml}^{-1}$. Inhibition of MRSA growth was initiated at 4 h following incubation at 37°C i.e. during mid-log phase ($0.13 \text{ OD}_{600\text{nm}}$) of bacterial growth (Figure 4a and 4b). Complete inhibition of bacterial growth was observed at 8 h after incubation. MIC of CPAgNPs was reported quite lower as compared to that of AECPL. CPAgNPs were synthesized using *C. papaya* leaf extract and thus it could be assumed that antibacterial activity of AECPL might potentiate the antibacterial action of Ag-NPs. Present findings support the observation of Wypij et al. (2018) who reported broad spectrum activity of Ag-NPs against bacterial pathogens. In line with present observation, antimicrobial potential of CPAgNPs could be employed in therapeutics against bacterial infection.

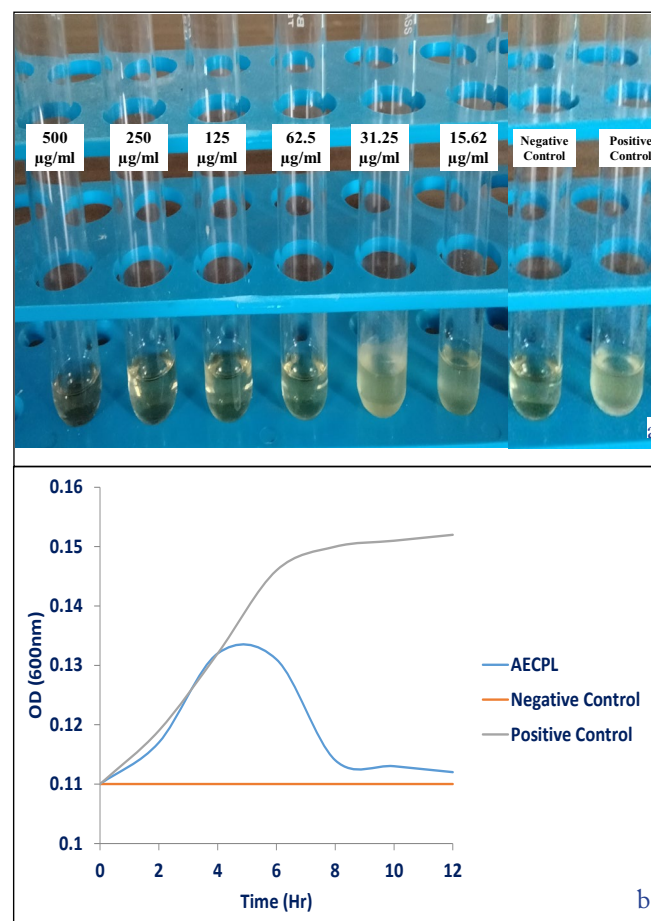


Figure 4: MIC of CPAgNP against MRSA (a) broth dilution method (b) Inhibition of MRSA growth Positive Control: MRSA culture in nutrient broth; Negative Control: Nutrient broth without MRSA; CPAgNP: MRSA culture treated with $62.5 \text{ } \mu\text{g ml}^{-1}$ CPAgNP

The literature regarding antibacterial mechanisms of AgNPs still remain unknown, however some researchers proposed that the action of AgNPs on bacteria might be due to its ability to penetrate the cell and further inactivation of cellular proteins by silver ions and the production of reactive oxygen species (ROS) (Dakal et al., 2016).

4. CONCLUSION

Carica papaya aqueous leaf extract had the potential for synthesis of silver nanoparticles. Phytosynthesized silver nanoparticles have antimicrobial efficacy against methicillin resistant *Staphylococcus aureus* with MIC of 62.5 µg ml⁻¹. In conclusion, the AgNPs synthesized using *C. papaya* leaf extract was proven to be efficient for antimicrobial effect against MRSA. This research indicates that papaya leaves have potential natural antibacterial compounds and can be channelized to meet therapeutic requirement.

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