COMPARATIVE STUDY OF SYNTHESIZED SILVER AND COPPER NANOPARTICLES USING CARICA PAPAYA EXTRACT AND THEIR ANTIBACTERIAL EFFICACY

Harshala Baddi*, Dhanshree Vaychal and Sandeep Waghulde

Konkan Gyanpeeth Rahul Dharkar College of Pharmacy & Research Institute, Karjat, University of Mumbai, Maharashtra, India

ABSTRACT

In the present scenario, nanotechnology is an important enabling technology which deals with the synthesis and control of matter at the molecular level, in scales smaller than 1 micrometer, normally 1 to 100 nanometers. Metal nanoparticles are one of the most attractive aspects of nanomaterials in recent years. Synthesis of nanoparticles can be carried out by using various chemical and physical methods. But use of such methods is harmful in one or the other way as the chemicals often used are toxic, flammable. The purpose of this research work is to provide information on the recently discovered green synthesis process over conventional. This research opens an innovative design to improve antimicrobial activities. We did comparative study of synthesized silver and copper nanoparticles using unripe fruit of carica papaya extract. The papaya extract mediated synthesis of silver and copper nanoparticles was efficient and provides additional property such as bactericidal efficiency and might act as long searched alternative and could be answer to antibiotic resistance. The antibacterial activity of extracted biological synthesized silver and copper nanoparticle were observed against *B. Substilis and E. Coli* using standard disc diffusion method. The green synthesis and characterization of silver and copper nanoparticles were done and confirmed by spectroscopic method and FTIR technique.

KEYWORD

Carica papaya ,silver nanoparticles, copper nanoparticles , green synthesis, FTIR technique

Introduction

In the present scenario, nanotechnology is an important enabling technology which deals with the synthesis and control of matter at the molecular level, in scales normally 1 to 100 nanometers. The purpose of this research work is to provide information on the recently discovered green synthesis process over conventional. This research opens an innovative design to improve antimicrobial activities. The smaller size of nanoparticles is gaining importance in research for the treatment of various diseases. Moreover the production of nanoparticles is ecofriendly and cost effective

Method of Preparations

Preparation of C. papaya fruit extract and 1 mM AgNO3

Fresh fruite of C. papaya (10 g) were diced into fine pieces and transferred to sterile 250 mL conical flask. Distilled water 200 mL was added to the flask and heated at 60℃ for 5–10 min and incubated at RT 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℃ for further use. Silver nitrate (AgNO3, 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.

The aqueous extract of C. Papaya and 1 mM AgNO3 were mixed in the ratio of 1:3 and heated on a water bath for 45-60 min until change in colour was observed. The colour change indicated the formation of silver nanoparticles by *C. papaya* extract.

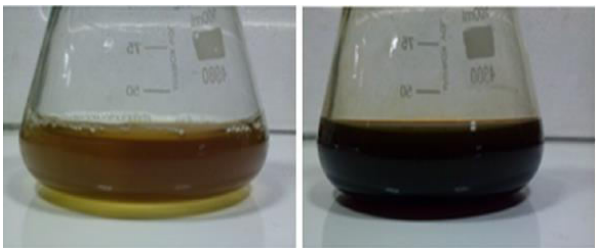

Fig.1 *C. papaya* extract with 1 mM $AgNO_3$ solution before and after the formation of silver nanoparticles with papaya extract.

Synthesis of Copper Nanoparticles

Copper salts were used as basic precursors, papaya fruit extract as stabilizer, Hydrazine Hydrate as reducing agent, L-Ascorbic acid as an anti-oxidant agent. NaOH was used as a catalyst and also to adjust the pH to 12. Copper chloride solution was prepared separately. L-Ascorbic acid was dissolved in Millipore water. Papaya extract and the solution of L-Ascorbic acid were added to copper chloride solution by heating to a temperature 50-60˚C under rapid stirring. Then the

solutions of Hydrazine Hydrate and NaOH were added to the mixed copper salt solution under stirring. The initial blue color of the reaction mixture eventually turned to brown-black color. Stirring was continued for another 1 hr to complete the reaction. The precipitate was washed twice with methanol after filtration and then dried and then the powder was obtained.

a) b) c)

Fig.2 a) *C.Papaya* Extract, b) Copper sulphate solution and c) Copper Nanoparticles with papaya extract

Characterization of synthesized silver and copper nanoparticles

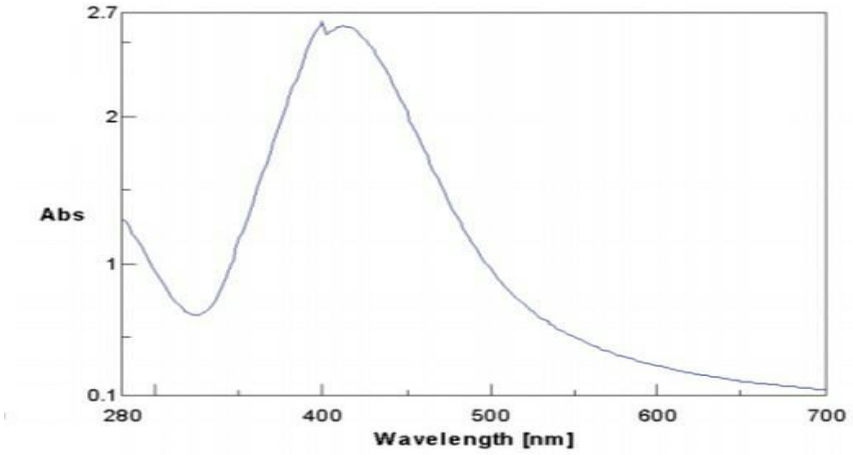

Fig.3 UV spectra papaya extract

The spectral responses of synthesized silver and copper nanoparticles were studied using UV-Visible spectrophotometer (Thermo Scientific–Evolution 201). Fourier-Transform Infrared Spectroscopy (FTIR) results were obtained from Jasco 6300 spectrometer (ATR mode) in the range of 400 - 4000 cm^{-1}.

Assessment of antibacterial activity using disc diffusion method

The antibacterial assays were done on human pathogenic strains like *B. subtilis* 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 microliter) of each culture was spread on to Luria Bertani agar plates. Sterile Whatman No. 1 paper discs of 5 mm diameter containing 10 microliter of C. papaya leaf extract (5microg/liter), 10 microliter of copper and silver nanoparticles (5microg/L and 10microg/L), and 5 microg/L of ampicillin (10microg/liter) were placed in each plate in a serial order. After incubation overnight at 37℃, 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 bacterial activity.

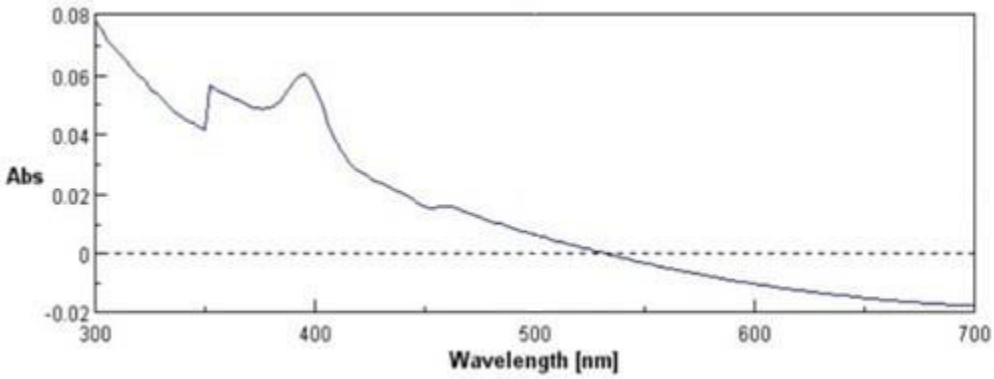

Fig. 4 UV Spectra of unripe Papaya Fruit

Minimum inhibitor concentration (MIC) and minimum antibacterial concentration (MBC) studies

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

Result and discussion

Spectroscopic Characterization

The UV absorption spectrometric analysis of silver and copper nanoparticle showed absorbance spectra at 470 nm suggesting bioreduction of silver nitrate into silver nanoparticles.

UV Spectra of Silver Nanoparticles

UV-Vis absorption spectra of the copper nanoparticles shows the copper nanoparticles prepared using different copper salts and papaya extract stabilizer display an absorption peak at around 560 nm.

FTIR analysis

The FTIR analysis of silver nanoparticles shows 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.

For copper nanoparticles, The IR spectrum of Cu nanoparticles shows band at 3373 cm^{-1}, 1635 cm^{-1}, 1516 cm^{-1}, 1376 cm^{-1}, 1198 cm^{-1} corresponds to O-H Stretching H-bonded alcohols and phenols, carbonyl stretching, N-H bend primary amines, corresponds to C-N stretching of the aromatic amino group and C-O stretching alcohols, ethers respectively. FTIR spectrum of Cu nanoparticles suggested that Cu nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acid. FTIR measurement was carried out to identify the possible molecules responsible for capping and reducing agent for the copper nanoparticles synthesized using papaya extract stabilizer.

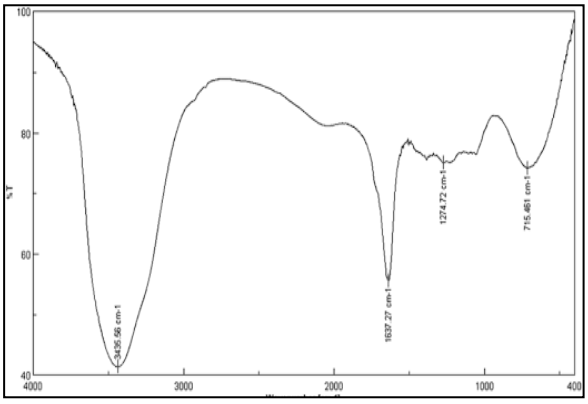

Fig. 5 FTIR spectra of Silver nanoparticles

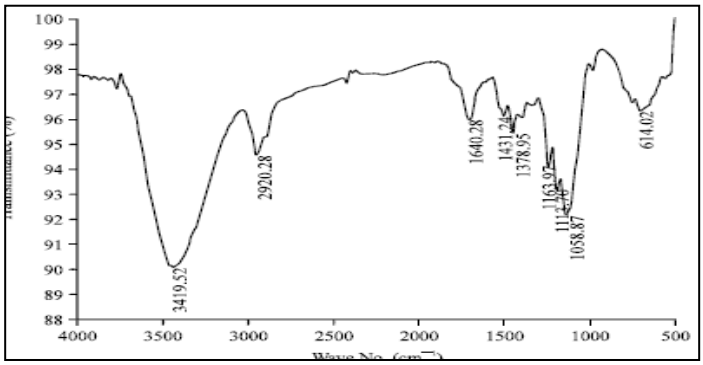

Fig. 6 FTIR spectra of copper nanoparticles

Discussion

To use silver nanoparticles against microbes in various fields, it is important and necessary to prepare silver nanoparticles in a green environment. In this study, we report a green method for the preparation of silver nanoparticles which is environmentally benign and cost-effective. For the assessment of the antimicrobial effects of silver nanoparticles and copper naoparticles, *B. subtilis* and *E. coli* were used in our study. The effect was investigated by growing *E. coli* on agar plates and in liquid LB medium, supplemented with copper and silver nanoparticles. The bacterial growth was completely inhibited in the presence of silver nanoparticles on the LB agar plate. The inhibition solely depended upon the silver nanoparticles concentration. It showed a clear zone of inhibition at and above the concentration 20µg/ml.

Freshly prepared copper nanoparticles sample (50µg/ml) was added into the wells. The samples were initially incubated for 15 min at 4 °C (to allow diffusion) and later on at 37 °C for 24 or 48 h for the bacterial cultures. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period.

The silver nanoparticles displayed antibacterial activity toward the tested pathogenic strains of *Bacillus subtilis* (21±1.612) and *E. coli* (26±0.985), respectively. *Bacillus subtilis* depicted the highest sensitivity to nanoparticles compared to the other strains and was more adversely affected by the silver nanoparticles that were observed between the inhibition zone observed in disk diffusion test and MIC/MBC determined based on liquid cultures with the various strains.

The copper nanoparticles displayed antibacterial activity toward the tested pathogenic strains of *Bacillus subtilis* (15±0.689) and *E. coli* (5±1.136), respectively. *Bacillus subtilis* depicted the highest sensitivity to nanoparticles compared to the other strains and was more adversely affected by the copper nanoparticles that were observed between the inhibition zone observed in disk diffusion test and MIC/MBC determined based on liquid cultures with the various strains.

Conclusion

The green synthesis and characterization silver and copper nanoparticles were done and confirmed by UV-Visible spectrophotometer, FTIR techniques. The MIC and MBC of the silver Nanoparticles (20 µg/ml) and Copper Nanoparticles (50µg/ml) against both Gram positive and negative bacterial species. In summary, the papaya 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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