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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM UNRIPE *CARICA PAPAYA* FRUIT EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT
The development of rapid and reliable process for the synthesis of nanosized materials is of great importance in the field of nanotechnology. Metal nanoparticles are one of the most attractive aspects of nanomateriales in recent years. There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, chemistry, energy and medicine. In this work desirable a cost effective and environmental friendly technique for green synthesis of silver nanoparticles from 1mM silver nitrate solution through the extract of *Carica papaya* unripe fruit. Nanoparticles characterized using UV –Vis absorption spectroscopy, FTIR and SEM. The silver nanoparticles synthesized via green route are highly toxic to multidrug resistant pathogens hence it has a great potential in biomedical application . Further these biologically synthesized nanoparticles exhibited for Antibacterial activity.

KEY WORDS: Silver nanoparticle, raw *Carica papaya* fruit extract, UV-Vis, FTIR, SEM and Antibacterial activity.

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at nanoscale level (Albrecht *et al.*, 2006). Silver nanoparticles are currently being studied extensively because of several properties that can be used in science and technology . Nanocrystalline silver particles have found tremendous application in the field of high sensitivity bimolecular detection and diagnostics, antimicrobial and therapeutics (M Rai and A Yadav, 2009). A number of approaches are available for the synthesis of silver nanoparticles such as sol-gel process, chemical precipitation and biological method. This work has done through using only green environmental friendly method to develop silver nanoparticle. Green synthesis of silver nanoparticles using ascorbic acid and citrate as reducing agent has recently been reported (Medina Ramierz *et al*., 2009). New application of nanoparticles and nanomaterial's are emerging rapidly (C.J Murphy. *et al*., 2008). Silver has long been recognized as having inhibitory effects on microbes present in medicinal and industrial process (Cobley, C.M., 2009). The most important application of silver and silver nanoparticles in medicinal industry such as topical ointments to prevent infection against burn and open wound (C. Lok *et al*., 2007). After discovering that silver nanoparticles are capable of blocking the entry of Human Immunodeficiency Virus (HIV) into the organism, a group of researchers from the University of Texas, in collaboration with Humberto Lara Villegas, specialist in nanoparticles and virology from the University of Monterrey, Mexico (UDEM), create a vaginal cream to control the transmission of the virus. Recently studies show that specially formulated silver nanoparticles have good antimicrobial activity (Ahmad *et al*., 2007). Nanosilver is highly toxic to several strains of bacteria including so called gram positive bacteria such as *Staphylococcus aureus* and *Staphylococcus pneumonia* and gram negative bacteria including *E. coli* and *pseudomonas* aeruginosa which is responsible for infections that resist treatment by conventional antibiotics. A recent study on *E. coli* has shown that silver nanoparticles react with cell wall and cytoplasmic membranes resulting in pits in cell wall of bacteria and finally kill them (Chamakura *et al.*, 2011). The aim of the present study is to synthesize silver nanoparticles by using aqueous of *carica papaya* unripe fruit extract and characterization of these silver nanoparticles and to see its antibacterial activity.

MATERIALS & METHODS

1. Plant material and preparation of the extract

Green unripe papaya (*Carica papaya*) fruit were used to make the aqueous extract. Unripe papaya fruit weighing 25g were thoroughly washed in distilled water, whipped slightly with tissue paper, cut into small pieces and crushed into 100 ml distilled water with the help of mortar and pestle. Then the mixture filtered through Watman No1 filter paper. The filter was further filtered through another Watman No1 filter paper .

2. Synthesis of silver nanoparticle

1 mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles 1:9 part of papaya fruit extract and aqueous solution of 1mM silver nitrate added for bio redaction into silver ions and kept at room temperature for 5 hours.

(i) U V- vis spectra analysis

The reduction of pure silver ions was monitored by measuring the U V –vis spectrum of reaction mixture at 5 hours. U V– vis spectral analysis was done by using U V –vis spectrophotometer between 300nm-700nm and the distilled water was used to adjust the base line.

(ii)F T I R analysis

The chemical composition of the synthesized silver nanoparticles was studied using FTIR spectrometer (Perkin – Elmer L5 55 – Luminescence spectrometer) The solution for FTIR analysis was prepared by mixing plant extract with 1mM silver nitrate. The samples were scanned using infrared in range of 1950-600 cm^{-1} using FTIR. The spectrum obtained was compared with reference chart.

(iii) S E M analysis of silver nanoparticles

The morphological features of synthesized silver nanoparticles from plant extract were studied by scanning electron microscope. SEM analysis done after drying the extract and dried powder used for SEM Images.

3. Antibacterial assays

i**. Nutrient Agar Medium (**1 L**)**

The medium was prepared by dissolving 28g of the commercially available Nutrient Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

ii**. Nutrient broth** (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

iii. **Gentamycin** (standard antibacterial agent, concentration: 20mg / ml)

PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains such as, *E coli*. Wells of approximately 10mm was bored using a well cutter and sample of 50, and100 µl conc: were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Gentamycin was used as a positive control.

RESULTS & DISCUSSION

Visual observation of silver nanoparticles confirmed through the development of white aqueous suspension to yellowish initially then after few hours turning into brown colour due to the reduction of silver ions (Fig-1) This is the indication of formation of silver nanoparticles.

FIGURE1: Aqueous *Carica papaya* Fruit extract as control and bio reduction of silver nanoparticle as yellowish brown Colour.

The synthesized aqueous solution of samples measured through UV-Vis Spectrophotometer. Fig-2(a) showing control and fig-2(b) showing the absorption spectra of which measured after 5 hours and the absorbance peak observed at 430.50nm, broadening of peak indicated that the particles are polydispersed.

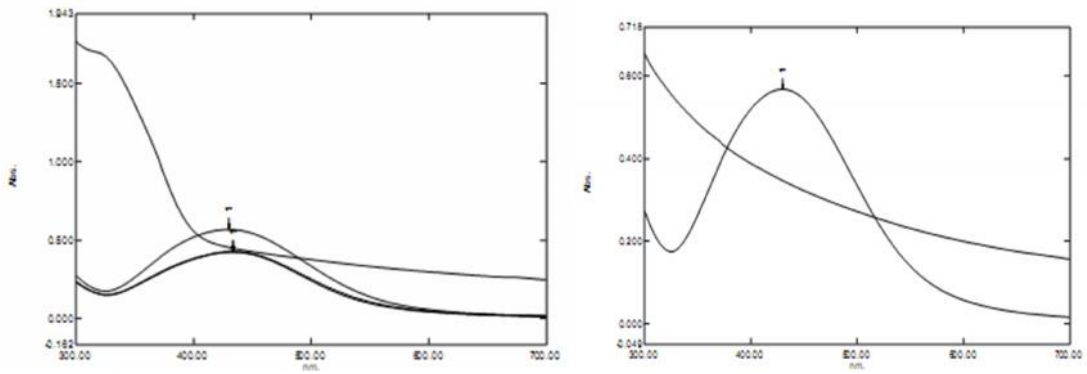

FIGURE: 2 UV-vis Analysis (a) control *carica papaya* fruit extract, (b) Silver nano particle

For the characterization of the *carica papaya* fruit extract and its reaction with silver nitrate resulted as silver nanoparticles were possible through FTIR Analysis. FTIR absorption spectra of bio reduced Ag ions observed by the absorption bands of the region of 1950-600cm$^{-1.}$ These bio reduced nanoparticles are the result of bio reduction of the plant extract and the capping agent which is present in the extract (fig.3).

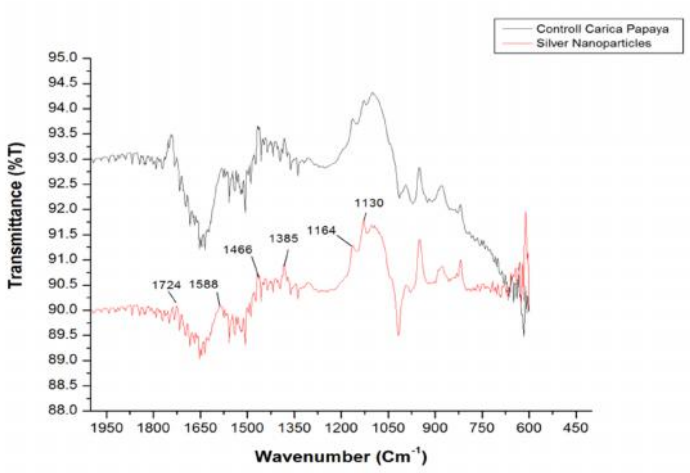

FIGURE 3: FTIR analysis of *Carica papaya* fruit extract

SEM Image showing the size of the silver nanoparticles between the ranges from 0.1µ-10µ under various magnifications. Images at high resolution also showed that silver nanoparticles are well dispersed.

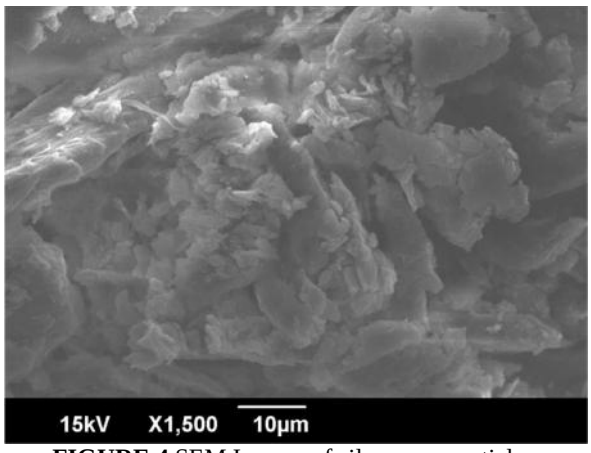

FIGURE 4 SEM Images of silver nanoparticles

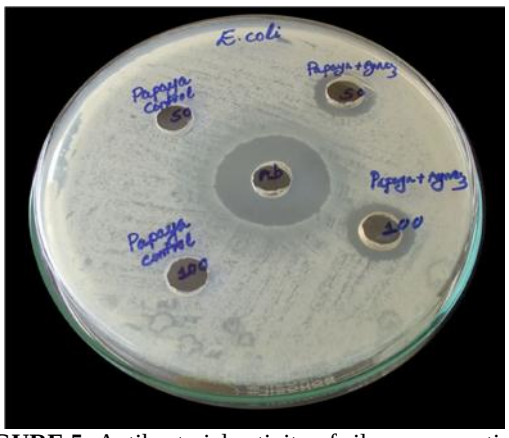

FIGURE 5: Antibacterial activity of silver nanoparticles against *E. coli*

Bactericidal effect on Hospital strain *E. coli* of silver nanoparticles synthesized by raw papaya fruit extract and showed synergetic effect with gentamycin. Synthesized silver nanoparticle showed zone of inhibition against the entire tasked microorganism. Zone of inhibition was found from the range of 1.7mm and 1.9mm with 50µl and 100µl respectively. Synergetic effect of both silver nanoparticles concentration against *E. coli* was found prominent than the effect of antibiotic alone.

TABLE 1: Effect of rang of synthesized silver nanoparticles on the inhibition, growth of *E. coli* in mm

Sample	Volume of sample (µl)	Zone of inhibition (mm)
Gentamycin		2.5
Carica papaya control	50	Nil
Carica papaya control	100	Nil
Carica papaya + Silver nitrate	50	1.7
Carica papaya +Silver nitrate	100	1.9

CONCLUSION

The present study showed a simple rapid and economical rout to synthesize silver nanoparticles. It means for synthesis of silver nanoparticles through Carica papaya fruit extract can be effectively used to follow a greener rout. Control over biological synthesis provides particles with good control over size distribution and shape. Carica papaya fruit extract produced silver nanoparticles have

been used in various applications for human being. Further the above silver nanoparticles revealed to possess an effective antibacterial property against *E. Coli*. Silver nanoparticles synthesized via green rout were highly toxic to pathogenic bacteria, hence has a great potential in biomedical application and a potent antibacterial effect too. This green method resulted many advantages such as ecofriendly, low cost and large scale synthesis of silver nanoparticles.

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