International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 146-152**
http://www.ijcmas.com

Original Research Article

Synthesis of silver nanoparticles from leaf extract of *Psidium guajava* and its antibacterial activity against pathogens

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Keywords

Nanotechnology; Silver Nitrate; *Psidium gujava*; *Staphylococcus aureus*; Reductant.

A B S T R A C T

Recent advances in nanotechnology have enabled us to produce pure silver, as nanoparticles, which are more efficient than silver ions. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. Silver nanoparticles are attractive because they are non-toxic to the human body at low concentrations and have broad spectrum antibacterial actions. New routes to the preparation of these materials extend the choice of properties that can be obtained. In this present study green synthesis silver nanoparticles from aqueous silver nitrate (1mM) through a simple and eco-friendly route using leaf broth of *Psidium guajava* as reductant. The aqueous silver ions when exposed to leaf broth were reduced and resulted in the green synthesis of silver nanoparticle. The bioreduced silver nanoparticle were characterized by UV-Vis spectrophotometer, scanning electron microscope (SEM) and Fourier transform infra-red (FTIR) spectroscopy. The observed peaks in UV a broad spectrum at 460 nm wave length. Size of silver nanoparticles range 0.1µm-0.5µm observed by SEM. The FTIR measurement was carried out to identify the possible biomolecules responsible for efficient stabilization of silver nanoparticles. The synthesized silver nanoparticles were tested against *Staphylococcus aureus* and *E. coli*.

Introduction

Nanotechnology is one of the most active research areas in the modern material science. Based upon their specific characteristics such as size, distribution and morphology nanoparticles have distinct properties compared with the bulk form of the same material. A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm (Packia Lekshmi *et al.,* 2012). Silver nanoparticle can be dissolved in a liquid environment that prevents their agglomeration of entrapped in a matrix that utilizes special drug carrier systems (e.g. the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix). These particles represent an interesting candidate for research as microbicides due to their effectiveness in small doses, minimal toxicity and side effects (Lara *et al.,* 2010). In recent years, resistance to commercially available antimicrobial agents by pathogenic bacteria and fungi has been increasing at an alarming rate and has become a serious problem (Wright, 2005). Microorganisms, such as bacteria, molds, yeasts and viruses, in the living environment are often pathogenic and cause severe infections in human beings. There is a pressing need to search for new antimicrobial agents from natural and inorganic substances (Kim *et al*., 1998; Cho *et al*., 2005). Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. Silver nanoparticles are attractive because they are non-toxic to the human body at low concentrations and have broad spectrum antibacterial actions. The mechanism of action of Silver nanoparticles as an antiviral and antibacterial has been studied against several enveloped viruses. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices. Chemical synthesis of nanoparticles leads to presence of traces of toxic chemical adsorbed on the surface which is undesirable in the medical applications of nanoparticles (Lara *et al.,* 2010). The synthesis of silver nanoparticles from various plants species, the leaf extracts of *Euphorbia hirta* and *Nerium indicum* (Mano Priya *et al*., 2011) papaya fruit extract (Jain *et al*., 2009) *Cinnamomum camphora* (Huang, *et al*., 2007), *Emblica officianalis* (Ankamwar, *et al*., 2005), *Carica papaya* (Devendra Jain, *et al*., 2009), *Parthenium hysterophorus* (Ankamwar, *et al*., 2005), *Diopyros kaki* (Vyom parashar, *et al*., 2009), *Hibiscus rosasinensis* (Mukherjee, *et al*., 2008), *Capsicum annuum* (Harekrishna Bar, *et al*., 2009) and tamarind (Ankamwar, *et al*., 2005) have been reported, the potential plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. The present study was carried out to synthesize and characterize the silver nanoparticles using *Psidium guajava* leaf extract and further synthesized silver nanoparticle were applied to act *S. aureus* and *E. coli* pathogen in laboratory condition.

Materials and Methods

Preparation of leaf extract

The plant material (*Psidium guajava*) was collected fresh from Watrap is a small village located in Virudhunagar District, Tamil Nadu, India. 5 gms of the leaves were surface cleaned under running tap water, followed by distilled water, air dried. The dried and powdered plant materials (100g) were extracted successively with 600ml of ethanol by using soxhlet extraction, for 48hr at a temperature not exceeding at boiling point of the solvent. The extracts were filtered using Whatman No.1 filter paper and then concentrated in vaccum at 40^0C using a Rotary evaporation. The extract was transferred to glass vials at 4^0C before use.

Pathogen and culture condition

Staphylococcus aureus and *E. coli* were taken as target organism. The organism was cultivated in LB broth and grown at 28^0C for 12 hrs at 120 rpm.

Synthesis of silver nanoparticles

0.1M of aqueous solution of Silver nitrate was prepared and used for the synthesis for silver nanoparticles. 10ml of ethanolic leaf extract *Psidium guajava* was added to vigorously stirred 90 ml of aqueous solution of 0.1M silver nitrate and kept at room temperature. Reduction takes place rapidly at 300 k and is completed in 10min as shown by tube light greenish-brown colour of the solution indicating the formation of silver nanoparticle

UV-Visible spectroscopy analysis

The bioreduction of pure Silver nanoparticles are monitored using UV-Vis spectroscopy at regular intervals. During the reduction *Psidium guajava* samples was taken and centrifuged at 12,000 rpm. The supernatant was scanned by UV-300 spectrophotometer.

Agar well diffusion assay

The antagonistic activities of synthesized nanoparticles were tested using Agar well diffusion assay. For evaluation of antibacterial activity Cork barer wells were punctured in LB agar plate sealed with overnight culture of *Staphylococcus aureus* and *E. coli*. About 20 – 100 µl of synthesized silver nanoparticles was administed to well.

Plates were incubated at 28^{0}C for 16 hrs and antibacterial activity was determined by measuring inhibitory zones. To study the synergistic effect of silver nanoparticles and antibiotics, the mixture of 15µl of respective concentrations of silver nanoparticles and 15µl of antibiotics were added into respective wells.

Scanning electron microscope (SEM)

For SEM analysis Silver nanoparticles are completely dried. The dry specimen was mounted on specimen state using anadheome which as epoxy resin or electrically conducture adhesure tape before examination in microscope.

Fourier transform infrared spectroscopy (FTIR)

A known weight of sample (mg) was taken in mortar and pestle and ground with 2.5mg of dry potassium bromide. The powder obtained was filled in 2mm interval diameter micro cup and loaded on FTIR set at $26^{0}C \pm 1^{0}C$. The samples were scanned using infrared in range of 4000-400 cm^{-1} using FTIR. The spectrum obtained was compared with reference chart to identify functional groups present in sample.

Results and Discussion

The green synthesis of Silver nanoparticles through plant extracts were carried out and confirmed by visual observation. The colour was changed greenish–brown colour due to reduction of silver ions. Jain *et al.*, (2009) also observed leaf extracts were mixed with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated that the formation of Ag nanoparticles. It was well known that Silver nanoparticle exhibits greenish–brown colour in aqueous solution due to evitation of plasma on vibrations in silver nanoparticles.

UV-VIS Spectroscopy analysis

The synthesized Silver nanoparticle using *Psidium guajava* plants extracts (Fig. 1) were detected by UV – Vis spectrophotometer. The UV-Vis spectrum

Fig.1 UV – SPEC Absorption Spectrum of Silver Nanoparticles Synthesized by Treating 1mM Aqueous $AgNO_3$ Solution with *Psidium guajava* Extract after 24 hours.

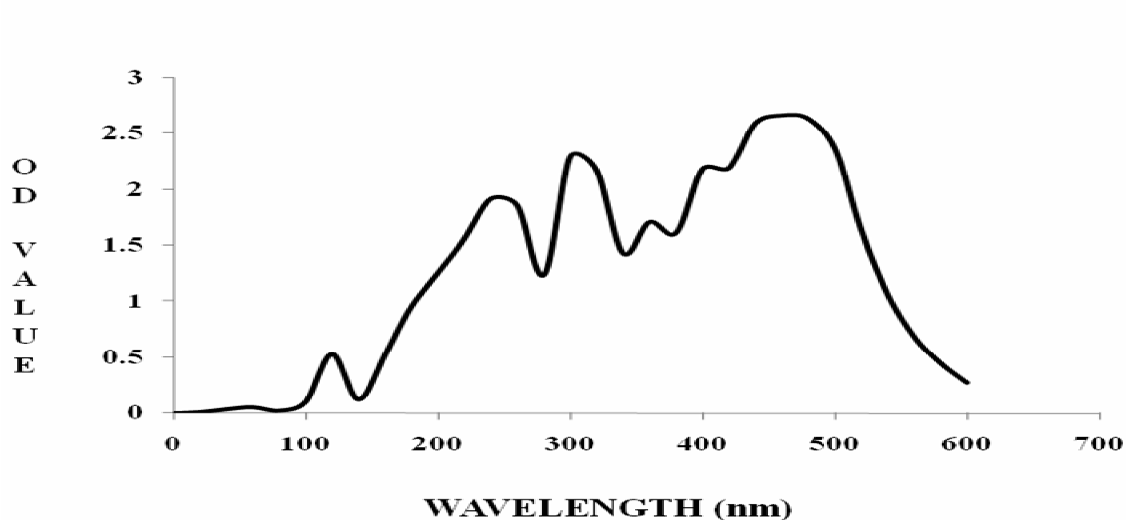


Fig.2 Showing Synthesis of Silver Nanoparticles from *Psidium guajava* plant extract SEM image.

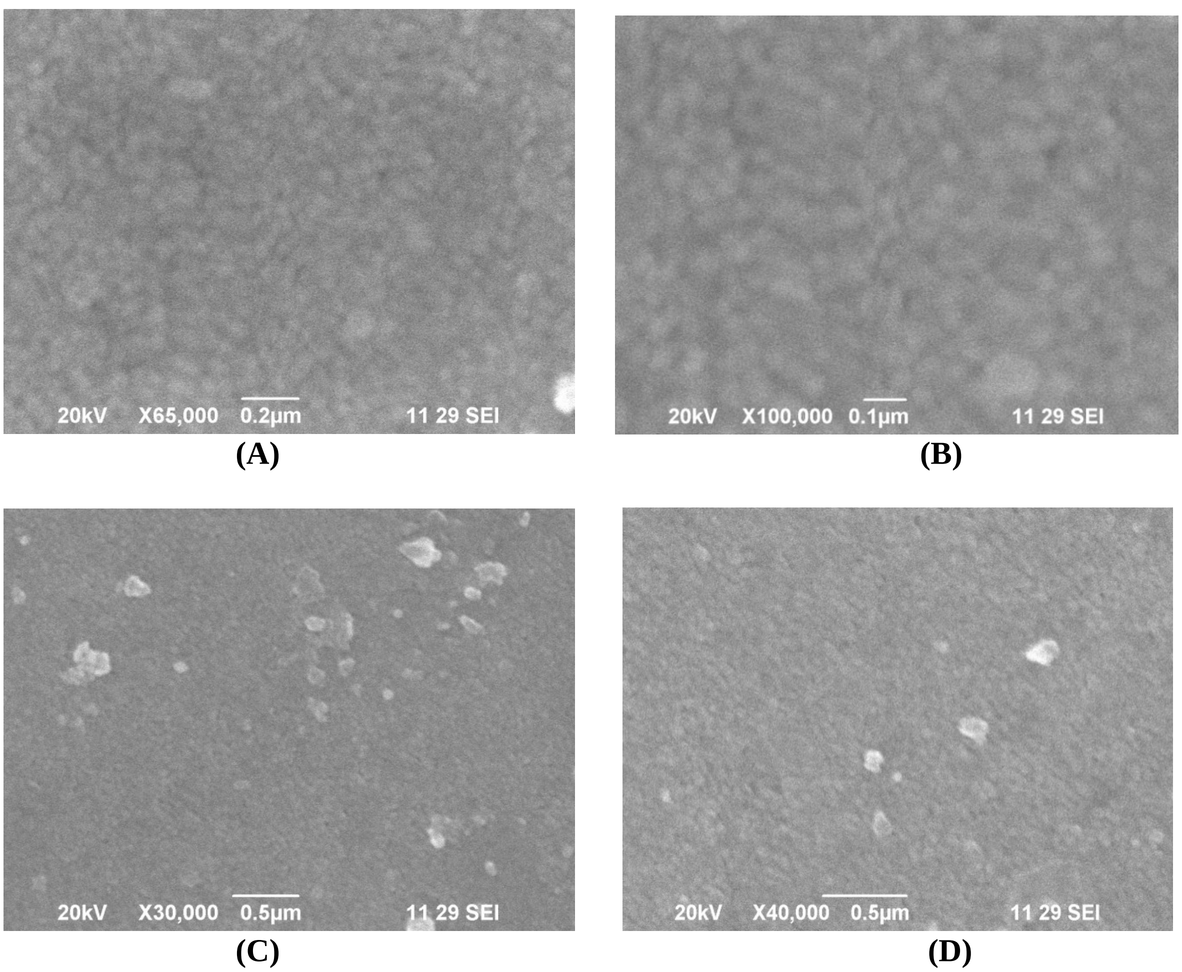
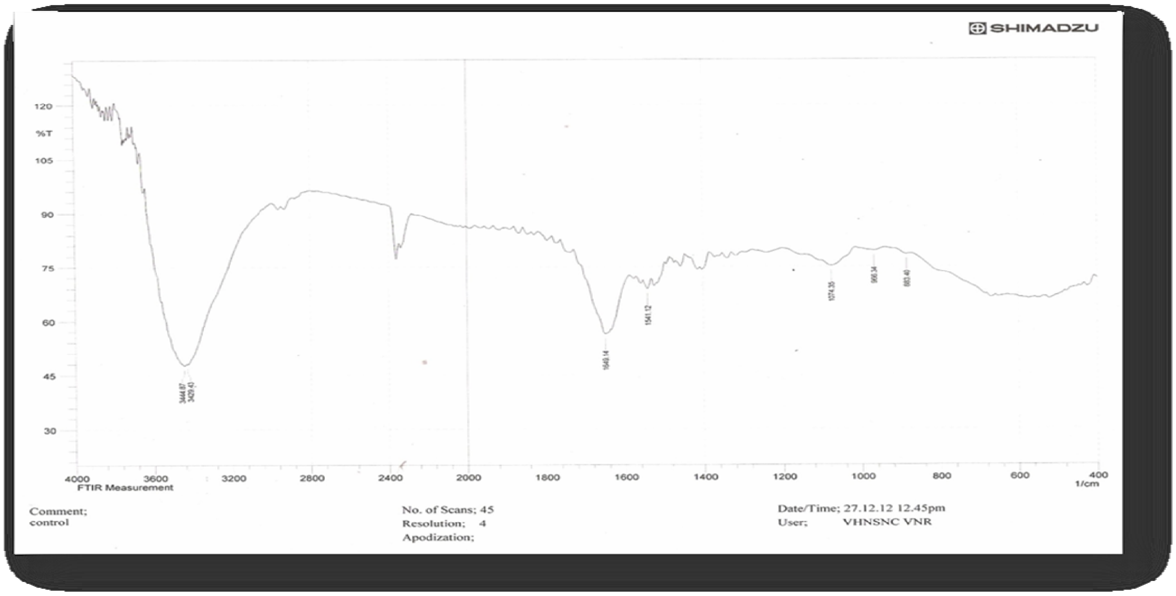


Fig.3 FTIR spectrum of silver nanoparticles synthesized using *Psidium guajava* leaf broth



of colloidal solution of Silver nanoparticles from *Psidium guajava* has maximum absorbance peak at 460 nm, which is proved the synthesis of silver nanoparticles in the colloidal solution. The position and shape of the plasmon absorption depends on the particles size, shape and the dielectric constant of the surrounding medium. The particle showed gradual decrease between 470 - 600nm.

Scanning electron microscope (SEM)

Scanning electron microscope analysis was used to measure the size of silver nanoparticle. In this analysis size of silver nanoparticles was between 0.1µm-0.5µm with different magnifications (Fig. 2).

Identification of functional groups using FTIR

FTIR analysis was used for the characterization of the extract and the resulting nanoparticle (Fig. 3). FTIR absorption spectra of soluble extract reduction of Ag ions. Absorbance bands in the region of 1000-4000 cm^{-1} are 4000, 2000, 1649, 1541, 1074, 966, 883 cm^{-1} . These absorbance bands are known to be associated with the stretching vibrations for O-H(s) stretch, C-H asymmetric stretching, C=C(s) stretch, C=C aromatic ring (s) stretch, C-O(s) stretch, C-O (secondary alcohol), C-H(s) stretch. The total disappearance of this band the bioreduction due to the fact that mainly responsible for the reduction of Ag ions, whereby they themselves to leading a broad peak at 3444 cm^{-1} .

Antibacterial activity of synthesized silver nanoparticle from *Psidium guajava*

The antibacterial activity of the synthesized Silver nanoparticles has been investigated against *Staphylococcus aureus* and *E. coli*. Green synthesis of silver nanoparticles of *Psidium guajava* showed very strong inhibitory actions against *S. aureus* (11mm zone of inhibition) and followed by *E. coli* (15 mm zone of inhibition). Mano priya *et al*., (2011) observed the antimicrobial activity of synthesized Ag nanoparticles against six different bacteria such as *E. coli*, *S. pyrogens, S. auereus, B. Subtilis, S. typhi and Citrobacter* sp. As it showed a clear inhibition zone.

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