GREEN SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES USING PLANT EXTRACTS

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Abstract: *Synthesis of nanosized particles with antibacterial properties is of great interest in the development of new pharmaceutical products. The antimicrobial effects of silver (Ag) ion or salts are well known, but the effects of Ag nanoparticles on microorganisms and antimicrobial mechanism have not been revealed clearly. Stable Ag nanoparticles were prepared and their shape, size and distribution were characterized by UV visible spectroscopy and FT-IR. Bioreduction of silver nitrate was (AgNO3) used for the synthesis of silver nanoparticles with the plant extracts viz. Carica papaya and Leucas aspera. The plant extracts was mixed with AgNO3, incubated for 6 hours and studied the synthesis of nanoparticles by chemical reduction from aqueous solutions of silver nitrate, containing a mixture of hydrazine hydrate and sodium citrate as reductants and sodium dodecyl sulfate as a stabilizer. The formation of plant extract based nanoparticles was confirmed at 271 and 263nm. The antimicrobial activity of Ag nanoparticles was investigated against Shigella flexneri, Vibrio cholerae, Salmonella typhi, Pseudomonas fluorescens and Escherichia coli and antifungal activity against Aspergillus flaves and Candida albicans. The free-radical generation effect of Ag nanoparticles on microbial growth inhibition was investigated by using Bauer method. The results showed reasonable bactericidal activity against Shigella flexneri, Escherichia coli and fungal activity against Aspergillus flaves and suggest that Ag nanoparticles can be used as effective growth inhibitors in microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.*

Key Words: *Silver nanoparticle, Antimicrobial effects, Antifungal effects, Sodium citrate and Bioreduction.*

1. INTRODUCTION:

Silver is a nontoxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms (Jeong *et al*., 2005). Biosynthetic method of nanoparticles has emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nanomaterials. The rate of reduction of metal ions using biological agents is observed to be much quicker with an ambient temperature and pressure conditions (Kaushik *et al*., 2010). The plant materials have been successfully used for silver nanoparticles synthesis, due to their potential medicinal property, huge availability and faster rate of synthesis (Mohanpuria *et al*., 2008). The plants or plants extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes (Valli &Vaseeharan, 2012), because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis (Saxena *et al*., 2012).

Microbial drug resistant is emerged as a major problem in healthcare industry as microbes involve in the change of their metabolism and genetic structure to acquire resistant against the drugs used in the treatment of infectious disease. To overcome microbial drug resistant, scientists are looking forward for the development of alternative and novel drugs. Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials (Jain *et al*., 2008).

Nanotechnology is currently employed as a tool to explorate darkest avenues of medical sciences because silver nanoparticles have been well known for its strong inhibitory and bactericidal effects and can effectively used for the treatment of various infectious diseases (Jeong *et al*., 2005). With high reactivity due to the large surface to volume ratio therefore antimicrobial silver is now used extensively to combat organisms in wounds and burns. The effect of the nanoparticles was found to be significantly more pronounced on MDR strains (Jain *et al*., 2008).

So keeping in view the advantage of silver nanoparticles synthesized from medicinal plants, the present study was based on the synthesis of silver nanoparticles from *Carica papaya* and *Leucas aspera* and checked its antimicrobial activity against *Shigella flexneri, Vibreo cholerae, Salmonella typhi, Pseudomonas florescens, Escherichia coli*, antifungal activity against *Aspergillus flaves* and *Candida albicans.* Characterization of silver nanoparticles was done by UV visible spectrophotometer and FT-IR.

2. MATERIALS AND METHODS:

Synthesis of Silver Nanoparticles from Plants Leaf Extract

Preparation of plants extracts

Fresh leaves of plants were collected from nearby area of Periyakulam, Theni Dist. Leaves were washed thoroughly and allowed for air dry in room temperature. Leaves weighing 15 g were thoroughly washed in distilled water for 5 min, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and were filtered.

Synthesis of silver nanoparticles

15 ml of plant extract was added to the aqueous solution of 1mM Silver Nitrate. Then the sample was incubated in dark for 24 hours. After 24 hours, the sample was measured for its maximum absorbance using UV-Visible spectrophotometry.

Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared (FT-IR) spectra were recorded at room temperature on a Nicolet 6700 FTIR spectrometer. For the FT-IR measurements of capped silver nanoparticles, a small amount of silver nanoparticles (0.01 g) dried at 60 ⁻C for 4 h was mixed with KBr to form a round disk suitable for FT-IR measurements using a Shimadzu DT-50 thermal analyzer.

Antimicrobial Activity

Anti-bacterial activity of aqueous extract was determined by disc diffusion method (Bauer *et al*., 1996) against *S.flexneri, V.cholerae, S.typhi, P.florescens, E.coli*. The culture was inoculated by spread plate method. Amikacin disc was used as standard control and distilled water was used as control for the extract. The plates were then incubated for 24 hours at 37°C. Pathogenic fungal strains were inoculated in potato dextrose agar medium and incubated at 48 hrs. In vitro antifungal activity of plant extracts were determined against *Candida albicans* and *Aspergilus flaves* respectively. Fungal strains were gently swabbed on the surface of the sterile petridishs containing 20 ml Czapex Dox solidified nutrient agar with the help of a sterile cotton swab. Same procedure was followed. Areas of inhibited fungal growth were observed after 48 hrs.

3. RESULTS AND DISCUSSION:

Owing to the applicability of silver nanoparticles in wide sectors, its demand is increasing at an overwhelming rate which has resulted in increased production. Researchers are continuously developing newer methods for synthesis of highly monodispersed silver nanoparticles which are efficient in terms of synthesis rate as well as energy usage. Biological methods have emerged as an alternative to the conventional methods for synthesis of nanoparticles.

Colour Change and UV visible Spectral Study

The silver nanoparticles were synthesized using the *Carica papaya* and *Leucas aspera* extract. The successful synthesis of silver nitrate by *Carica papaya* extract was evident by the formation of dark brown and *Leucas aspera* as dark green colour. However, after addition of silver nitrate and shaking for 25 minutes at room temperature, the emulsion turned to dark green in *L.aspera* (Fig. 1). The colour was changed in the cell free extract when challenged with 1mM $AgNO_3$ from pale yellow to dark brown, attained maximum intensity after 10-12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticles in *Boswellia ovalifoliolata* aqueous extract within 10 min whereas *Shoreatum buggaia* and *Svensonia hyderobadensis* took 15 min to synthesize nanoparticles (Savithramma *et al.,* 2011). In UV visible spectrometer the maximum absorption peak was obtained in *C. papaya* was observed at 271 nm and in *L. aspera* at 263 nm. (Fig. 2).These results agreed with previous work carried out by Mostafa *et al.,* (2014).

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by *C. papaya and L. aspera* were given in (Figure 3). FT-IR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver nanoparticles synthesized from *C. papaya.* The peak IR band observed at 468.7 cm^{-1} is characteristic of the SS stretch aryldisulphides. The strong peak at 623.01 cm^{-1} corresponds to C-H bending. The peak near 1033.85 cm^{-1} is assigned to C-O, alcohol. The observed peak at 1109.07 cm^{-1} denotes C-N stretching vibration. The presence of peak at 1442.75 cm^{-1} could be ascribed to CH_3, bend. The observed band at 1635.64 cm^{-1} is due to aliphatic hydrocarbons, which has been generally assigned to C-9 ketone oxygen coordinated to magnesium; C=O....Mg. The observed band at 3452.58 cm^{-1} is due to symmetric NH_2, N-H and antisymmetric NH_2 stretching vibrations of adenine.

Similar result was reported by Baskaran *et al*.,(2013) in the synthesized AgNPs using *Coleus forskohlii* root extract where the absorption peaks were located at, 500-400 cm^{-1} presence of fatty acids, carbonyl groups, flavanones

amide I band of proteins. The peak near 1033.85 cm^{-1} is assigned to C-O, alcohol. Identical result was reported by Priya, *et al*., (2014) at 1074 cm^{-1} denote C-O, aromatic rings and alkaline bond. Aki, *et al*., (2012) reported that the intense band at 1018 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines.

In *L. aspera* extract synthesized silver nanoparticles exhibited band at 480.28 cm^{-1} corresponds to SS stretch, aryldisulphides. The peak at 630.72 cm^{-1} corresponds to C-H bending. The peak intensities at 1082.07 cm^{-1} corresponds to asymmetrical C-O-C stretching. The presence of bands at 1627.92 cm^{-1} due to fingerprint region of CO, C=O and O-H groups. The absorption peaks at about 2362.8 cm^{-1} could be assigned to OH, carboxylic acid. The intense band at 3450.65 cm^{-1} could be assigned to the OH, alcohol. The presence of bands at 1627.92 cm^{-1} due to fingerprint region of CO, C=O and O-H groups.

The above results corroborate the results by Baskaran *et al*., (2013) in *C. forskohlii* the absorption peak located at 1624 cm^{-1} corresponding to presence of fatty acids, carbonyl groups, flavanones and amide I band of proteins. The intense band at 3450.65 cm^{-1} could be assigned to the OH, alcohol. The same result was reported by Shanmuga prabha *et al*., (2015) in *F.microcarpa* the peak at 3474 cm^{-1} corresponds to O-H stretching, H bonded alcohols and phenols, carbonyl stetching.

Antimicrobial Activity

In the present study, the antibacterial activity of green synthesized silver nanoparticles were tested against *S.flexneri, V.cholerae, S.typhi, P.florescens, E.coli* and the results were shown in (Figure 4&5). The range of inhibition zone of *Carica papaya and Leucas aspera* extract varied from 4mm (*V. cholera*) to 8mm (*S. flexneri*) and 7mm (*P. fluorescens*) to 10mm (*E.coli) (V.cholerae*) respectively. Maximum inhibition zone was exhibited against *E.coli* (8mm) in *C.papaya* and (10mm) in *L.aspera* extract.

This enormous difference may be due to the susceptibility of the organism used in the current study. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. When silver nanoparticles enter the bacterial cell, it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Morones *et al*., (2005); Kvitek *et al*., (2008).

Antifungal activity

In the present study, the antifungal activities of green synthesized silver nanoparticles were tested against *A.flaves* and *C.albicans* and the results were shown in (Figure 6&7).The treated plant *L.aspera* alone inhibited *A.flaves* (8mm). There was no activity observed in *C.papaya* silver nanoparticles.

Figure-1 Adding of AgNO3 and the colour changes of plant extract after 6 hrs of incubation with AgNO3 *Carica papaya* and *Leucas aspera*

Figure-2 The UV spectrum of *C. papaya* and *L. aspera* silver nanoparticles recorded at room temperature

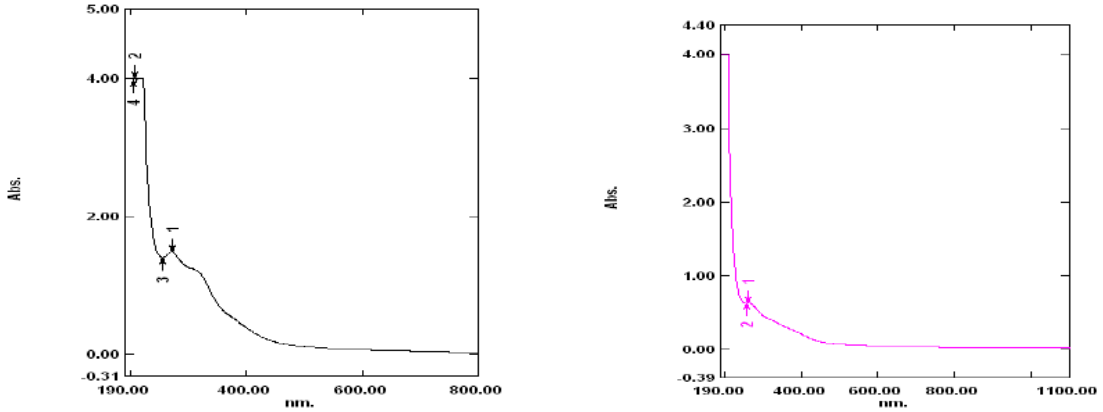


Figure-3 FTIR Spectra of *C. papaya* and *L. aspera*

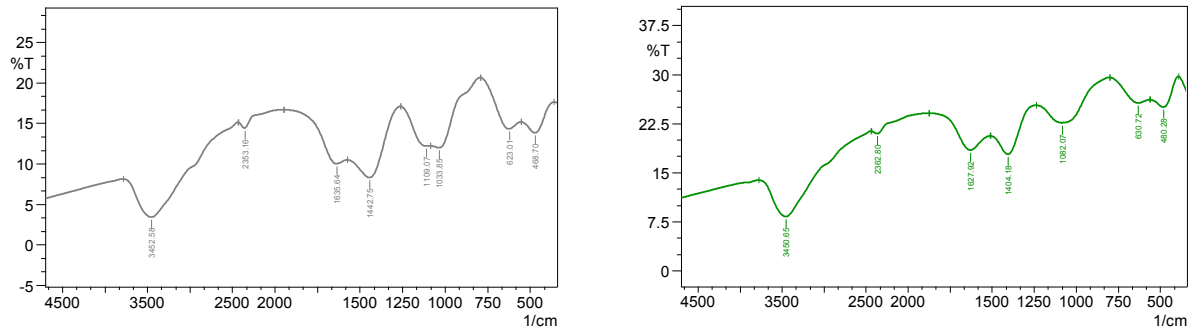


Figure-4 Antibacterial activity of *C.papaya* silver nanoparticles against human pathogens

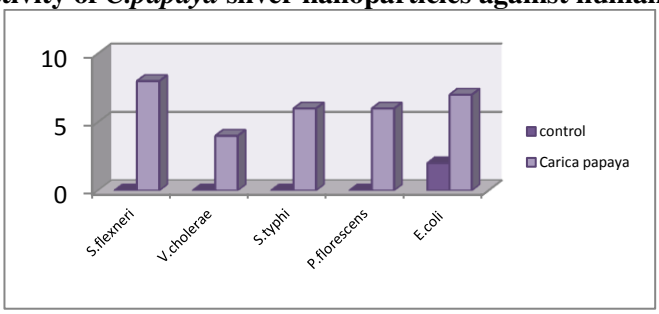


Figure-5 Antibacterial activity of *L.aspera* silver nanoparticles against human pathogens

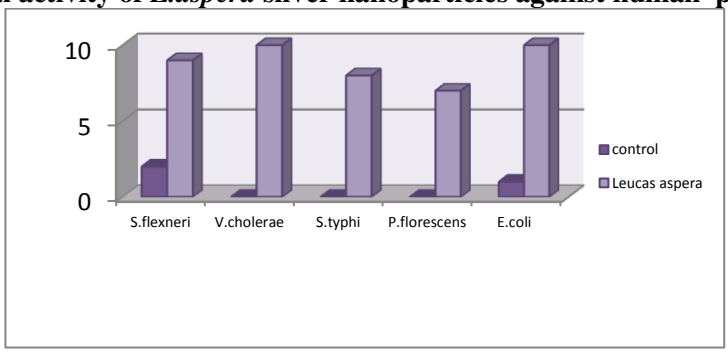


Figure-6 Antifungal activities of *C.papaya* silver nanoparticles against human pathogens

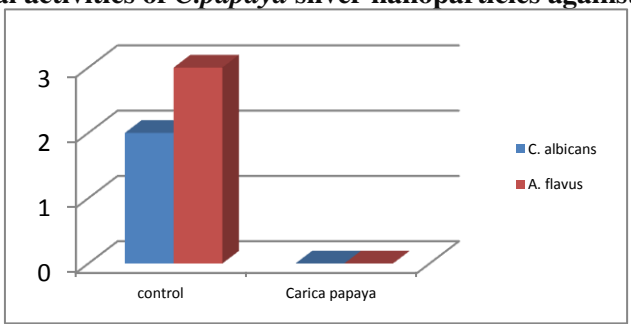
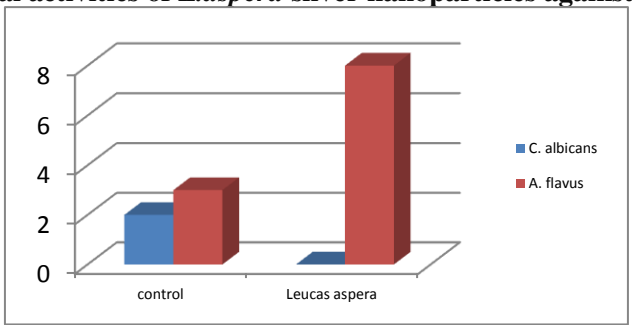


Figure-7 Antifungal activities of *L.aspera* silver nanoparticles against human pathogens



4. CONCLUSION:

It has been concluded that the extract of *Leucas aspera* is capable of producing Ag nanoparticles extracellularly and these nanoparticles are quite stable in solution due to capping likely by the proteins present in the

extract. This is an efficient, eco-friendly and simple process. The AgNPs showed potential antibacterial activity against human pathogens like *S.flexneri* and *E.coli* antifungal activity against *A. flaves.* Therefore, nanoparticles of silver in combination with commercially available antibiotics could be used as an antimicrobial agent after further trials on experimental animals.

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