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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *AZADIRACHTA INDICA* LEAVES EXTRACT AND EVALUATION OF ANTIBACTERIAL ACTIVITIES

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ABSTRACT
Silver nanoparticles were synthesized from reliable and eco-friendly process of nanotechnology using *Azadirachta indica* leaves extract as reducing and capping agent. Popularity behind silver nanoparticles is non-toxic at low concentration and has a nature of antimicrobial actions. In this investigation green synthesis of silver nanoparticles obtained from $AgNo_3$ (1mM) solution through the extract of *Azadirachta indica* leaves. The aqueous silver ions reacted with the broth of leaf extracts were reduced and resulted as silver nanoparticles. The characterization of bioreduced nanoparticles made possible by UV spectrophotometer, FTIR (Fourier transform in-frared spectroscopy) and Scanning electron microscopy (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, *Azadirachta indica* leaves extract, UV-Vis Spectroscopy, FTIR, SEM , Antibacterial activity

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Nanotechnology applications are highly suitable for biological molecules because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which is found to be reliable and ecofriendly. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Silver nanoparticles have ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds. (Ip M *et al.*, 2006) medical devices and implants prepared with silver impregnated polymers. Plants provide a better platform for nano particle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Synthesis of nanoparticles using plant extract is the most adopted method of green eco friendly production of nano particles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites. There have been several experiments performed on the synthesis of silver nano particles using medicinal plants such as *Oryza sativum, Saccharum officinarum, Zea mays, Aloe vera and Azadirachta indica.* Metal nano particles have a high specific surface area and a high fraction of surface atoms. Because of this unique physico chemical characteristics of nano particles including catalytic activity, optical properties, electronic properties, anti bacterial properties and magnetic properties, they are gaining the interest of scientist for their novel methods of synthesis.(Vaidyanathan *et al*. 2009). The silver nanoparticles which has promising antimicrobial properties. Silver nanoparticles are found to be effective as anti-inflammatory, anti-angiogenesis, antiviral, anti-platelet activity and against cancer cells which makes them vital.(Sotiriou *et al*., 2010). It has been also reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentration and without any side effects. Jeong *et al*., 2005. The antimicrobial capacity of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home applications and in medical devices. The experiment carried out for silver Nanoparticles from various plant species, the *Euphorbia hirta* and *Neerium Indicum* (Mano Priya *et al*., 2011) *Emblica officinalis* (Ankamwar *et al*., 2005), *Carica Papaya* fruit extract (Jain *et al*., 2009), *Hibiscus rosa sinensis* (Mukherjee *et al.*, 2008). The aim of the present study is to synthesize silver nanoparticles by green method using aqueous *Azadirachta indica* leaves extract and characterization of these silver nanoparticles and to see its antibacterial activity.

MATERIALS & METHODS

(a)Preparation of plant extract

The *Azadirachta indica* leaves extract was prepared with 10 g of fresh leaves obtained from S.V.R N.S.S college campus, Kottayam, Kerala and which were thoroughly rinsed with deionized water and cut into small pieces. The chopped leaves where boiled in 75ml of deionized water for 3 minutes. The leaf broth was then cooled and filtered yielding 50ml of broth. The colour and translucency of the broth can be observed.

(b)Synthesis of Silver Nanoparticles

Silver nitrate [$AgNO_3$] was obtained from sigma Aldrich and used as received without further purification. 50ml *Azadirachta indica* leaves broth was added to 1mM silver

nitrate and allowed to react at ambient conditions. The observed colour change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles. Further the reduction of the silver ions was monitored over time by UV-visible spectra analysis. The suspension of silver nanoparticles was allowed to settle and the excess liquid was removed.

(c)Characterization of Silver Nanoparticles

(i)UV-vis Analysis

The optical property of silver nanoparticles was determined by UV-vis spectrophotometer (perkin Elmer Lamda 35, Germany). The UV-vis spectra of samples were measured on UV-2600nm.The bio reduction of silver ions in aqueous solution was monitored by UV-vis spectra of the solution between 300-700nm.

(ii)FTIR 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)SEM Analysis

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.

(d) Antibacterial activity

1**. 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 and autoclaved at 15 lbs at 121°C for 15 minutes. Then poured hot agar onto 100mm petriplates.

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

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium in 1000ml distilled water and followed rest of processes as above mentioned.

3.**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. (NCCLS, 1993a). Gentamycin was used as a positive control.

RESULT & DISCUSSION

Visual observation of silver nanoparticles confirmed through the development of greenish aqueous suspension to yellowish brown colour due to the reduction of silver ions (Fig-1). This is the indication of formation of silver nanoparticles.

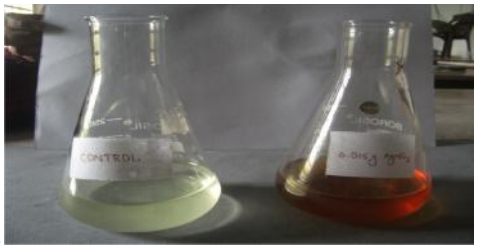

FIGURE 1: Aqueous *Azadirachta indica* leaves extract as control and bio reduction of silver nanoparticle as yellowish brown Color

The synthesized aqueous solution of samples measured through UV-Vis Spectrophotometer. Fig-2(a) showing control and fig-2(b) showing the absorption spectra measured and the absorbance peak observed at 429.50nm and 414.50 respectively, broadening of peak indicated that the particles are polydispersed.

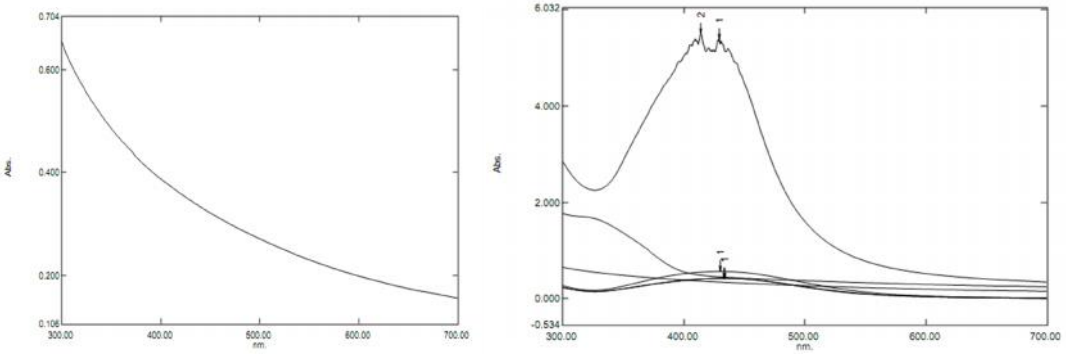

FIGURE 2: UV-vis Analysis (a) control *Azadirachta indica* leaves extract (b) Silver nano particle

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles (fig-3). FTIR absorption spectra of water soluble extracts after reduction of Ag ions are shown in (fig-3).The characterization of bio

reduction of *Azadirachta indica* leaves are possible through FTIR analysis. FTIR absorption spectra of bio reduced Ag ions observed by the absorption bands of the region of 1950-450cm^{-1} are 1730,1452,1374,1098 and 952cm^{-1}. These bio reduced nanoparticles are the result of bio reduction of the plant extracts and the capping agent which is present in the extract (fig-3).

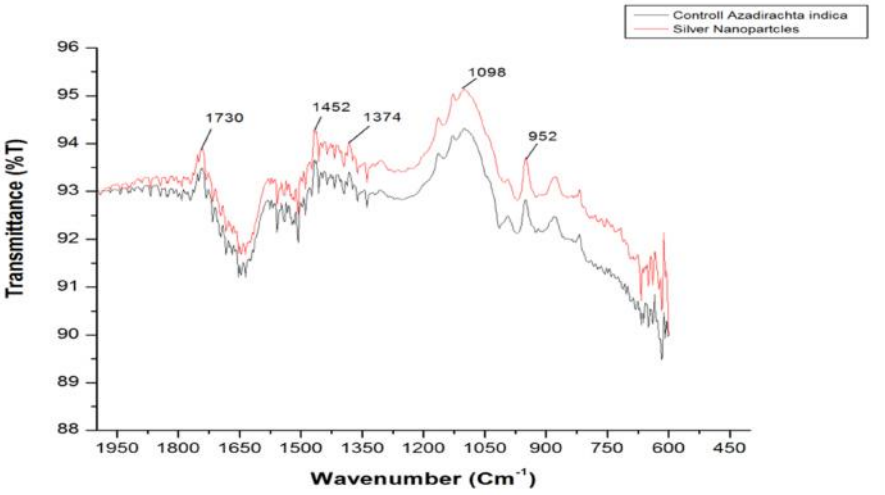

FIGURE 3: FTIR analysis of *Azadirachta indica* leaves extract

SEM Images showing the size of the silver nanoparticles between the ranges from 0.5µ -10µ under various magnifications.

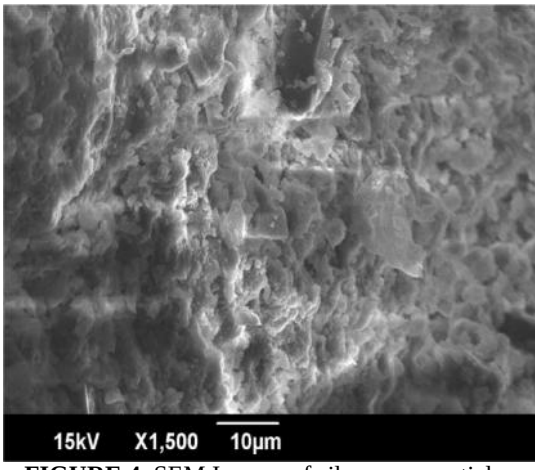

FIGURE 4: SEM Images of silver nanoparticles

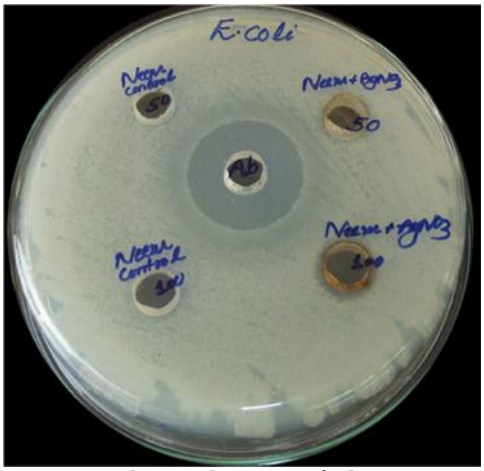

FIGURE 5: Antibacterial activity of silver nanoparticles against *E. coli*

Bactericidal effect on Hospital strain *E. coli* of silver nanoparticles synthesized by *Azadirachta Indica* leaves 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 1.0mm and 1.1mm 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
Azadirachta indica control	50	Nil
Azadirachta indica control	100	Nil
Azadirachta indica + Silvers nitrate	50	1
Azadirachta indica +Silver nitrate	100	1.1

CONCLUSION

The present study showed a simple rapid and economical rout to synthesize silver nanoparticles. For synthesis of silver nanoparticles through *Azadirachta indica* leaves extract can be effectively used to follow a greener rout. Control over biological synthesis provides particles with good control over size distribution and shape. *Azadirachta indica* leaves 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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REFERENCES

National Committee for Clinical Laboratory Standards (1993a) Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA.

Jeong, S.H., Yeo, S.Y. and Yi, S.C. (2005) the effect of filler particles size on the antibacterial properties of compounded polimer or silver fiber 40; 5407-5411.

Ip, M. Lui, S.L., Poon, V.K., Lung, I. and Burd, A. (2006) antimicrobial activities of silver dressing .An in vitro comparison. J medical microbial, 55:59-63 .

Sotiriou, G.A., Pratsinis, S.E. 2010) Antibacterial activity of silver ions and particles. Environ Sci Technol; 44:5649-5654 .

Vaidyanathan, R. Kalishwaralal K, Gopalram S, Gurunathan S (2009) Nanosilver: the burgeoning therapeutic molecule and its green synthesis. Biotechnol Adv; 27:924 – 937

Ankamwar, B., Chaudhary, M., Mural, S (2005) Gold Nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synth.React.Inorg metal Org Nanometal chem.,35,19.*

Jain, D. Kumar Daima, H., Kachhwaha, S., Kothari, S. L. (2009) Synthesis of plant mediated silver nanoparticles using papaya fruit extracts and evaluation of their antimicrobial activities. Digest journal of Nanomaterials and Biostructures, 4(3):557-563.

Mano Priya, M., Karunai selvia, B.A., John Paul, J.A. (2011) Green synthesis of silvernanoparticles from the leaf extracts of Euphorbia hirta and Nerium indicum. Digest journal of Nanomaterials and Biostructures, 6(2): 869-877.

Mukherjee, P., Roy, M., Mandal, B., Dey, G., Mukherjee, P., Ghatak, J. (2008) Green synthesis of highly stabilized Nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus Tasperellum Nanotechnology, 19:75-103.