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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACTS AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES

KEYWORDS: Silver nanoparticles-green synthesis –analysis- antibacterial activity.

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ABSTRACT *The present investigation has been carried out to evaluate the antibacterial activities of Silver nanopartilces synthesized from Azadirachta indica, Tridax procumbens and Aegle marmelos leaves against wound infection causing bacterial pathogens. Antibacterial activity was assessed by well diffusion method. The activities of the AgNps were measured by zone of inhibition and compared with a standard antibiotic Tetracycline. Among the various concentrations (10mg/ml) was found to be very effective. The synthesized AgNps were analysed by colour change, UV Vis spec and SEM. The Green synthesis of silver nanoparticles by the help of plants is a very cost effective, safe, non-toxic, eco-friendly route of synthesis which can be manufactured at a large scale.*

1.Introduction

Wounds can be broadly categorized as acute or chronic. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions. Chronic wounds include leg ulcers, foot ulcers, and pressure sores (Davis M H et al., 1992). Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) has emerged as one of the most reliable tools for fast and easy identification, differentiation and classification of microorganisms (Jana Chalupova et al., 2013).Use of antibiotics is the most simple and common method of treating or preventing wound infection. Resistance to antimicrobial therapies reduces the effectiveness of thesedrug. Increasing incidence of multidrug resistant pathogenic microorganisms has lead to the search for new antimicrobial substances from various sources like the medicinal plants. Antimicrobial activities are due to the secondary metabolites synthesized by the plants such as phenolic compounds. Recently nanotechnology has induced great scientific advancement in the field of medical research and technology. Silver nanoparticles possess an excellent biocompatibility and low toxicity. Silver nanoparticles show potential antimicrobial effects against infectious organisms, including Escherichia coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphillis typhus, and S.aureus.. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. The phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles (Sukumaran Prabhu and Eldho K Poulose., 2012). Biosynthesized silver nanoparticles (AgNPs) are usually characterized by X-ray diffraction (XRD), atomic absorption spectroscopy (AAS) and Fourier transform infrared spectroscopy (FT-IR). UV-vis spectroscopy showed that the surface plasmon resonance (SPR) at 425 nm (Akl M. Awwad et al., January 2013). UV-Vis spectra recorded during synthesis of nanoparticles shows an absorption maximum at 440 nm, which is typically attributed to plasmon resonance of silver nanoparticles. Analysis of Ag-Nps using SEM image showed a clear image of highly dense Ag nanoparticles, which are almost spherical in size(Behera S.S et al., 2013). Eco friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. This study revealed that the antimicrobial activity of silver nanoparticles synthesized from plant extracts of Azadirachta indica ,Tridax procumbens and Aegle marmelos against wound pathogens

2. Materials and methods:

a. Isolation and Identification of pathogens from wound infections:

Wound swabs were collected and inoculated into Blood agar and Mac Conkey agar plates and incubated at 37 °C for overnight (18- 24 hours). The isolates were send for Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry MALDI TOF analysis for the species level identification. The analysis was done by Bruker Daltonik MALDI Biotyper.

b.Bioynthesis of silver nanoparticles by plant extracts:

Aqueous plant extract of Azadirachta indica ,Tridax procumbens and Aegle marmelos was added to 1mM silver nitrate solution (1:5). The flasks were covered with aluminum foil and incubated in the shaker at 150 rpm at 37 °C for 38 hours (Rajasekar et al., 2013).

c.Characterization of synthesized nanoparticles:

Silver nanoparticle synthesized was confirmed and characterized by following methods; colour change, UV-visible spectroscopy and SEM analysis (Rajasekar et al., 2013).

d.Antibacterial assay of silver nanoparticles:

Antibacterial assay of silver nanoparticles was done by well diffusion method. Nutrient agar plates were swabbed with test organisms. About 30µl of different concentrations of silver nanoparticles (1, 5, 10, 15 and 20 mg/ml) was added to wells and the plates were incubated at 37^{0}C for 24hrs. After incubation, the zone of inhibition was observed and measured in mm (Rajasekar et al., 2013).

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3.Results:

a.Isolation and Identification of pathogens from wound infections :

Following incubation of blood agar and Mac Conkey agar plates colonies were observed the isolates were sent MALDI TOF analysis for the species level identification. The isolates identified are Staphylococcus aureus(C1), Providencia rettgeri(C2), E. coli (C3), Klebsiella variicola(C4) and Pseudomonas aeruginos(C5). In recent years (MALDI-TOF-MS) has proved a rapid and reliable method for the identification of bacteria and yeasts (C5) that have already been isolated(Sanchez et al..2014).

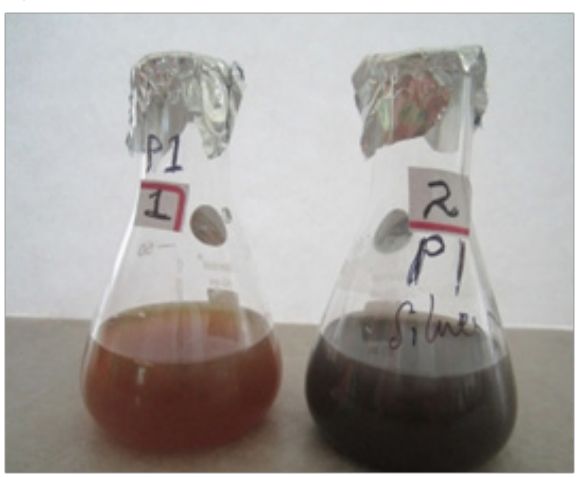
b.Bioynthesis of silver nanoparticles by plant extracts:

Bioynthesis of silver nanoparticles by plant extracts was confirmed as follows:

1.Colour change

Silver nanoparticles (AgNPs) appear reddish brown in colour in aqueous medium as a result of surface plasmon vibrations. As the different leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from light yellowish to reddish brown and finally to colloidal brown indicating AgNP formation(Figure:1).

Figure 1: Silver nanoparticle production



1: Before addition of silver nitrate 2: After addition

2. UV-Visible spectroscopic analysis

UV–vis spectroscopy is an indirect method to examine the bioreduction of Ag nanoparticles from aqueous AgNO3 solution. The Ag nanoparticles exhibited an absorbance peak around 446 nm characteristic of Ag nanoparticle, its surface Plasmon absorbance and due to different shapes of Ag nanoparticles

3. SEM analysis

The micrographs of nanoparticles obtained in the filtrate showed that silver nanoparticles are spherical shaped well distributed without aggregation in solution with an average size of about 35-65 nm. Azadirachta indica , Tridax procumbens and Aegle marmelos extracts formed approximately spherical, triangular and cuboidal AgNPs, respectively. This may be due to availability of different quantity and nature of capping agents present in the different leaf extracts(Figure:2)

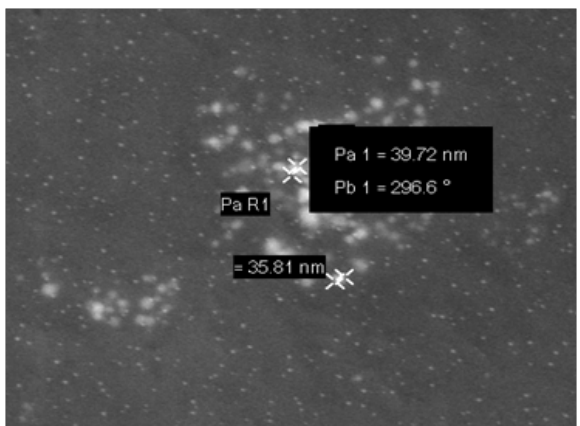


Figure 2: SEM image of silver nanoparticle of Tridax procumbens

4.Antibacterial activity of silver nanoparticles

Antimicrobial activity of silver nanoparticles synthesized by plant powders was investigated against various pathogenic organisms using the well diffusion method. The diameter of inhibition zones (mm) around each well with silver nanoparticle solutions is represented in Table:1 The highest antimicrobial activity of silver nanoparticles synthesized by Tridax procumbens against Pseudomonas aeruginosa .Simlarly Azadirachta indica silver nanoparticle did not show any significant sensitiveness to any pathogens.

Table: 1 Antibacterial activity of silver nanoparticles

Isolates	SNP P1 10 mg/ml	SNP P2 10 mg/ml	SNP P3 10 mg/ml	1 mM Silver nitrate	Tetracycline 30 µg/ml
C1	R	R	R	12 mm	32 mm
C2	R	R	14mm	13 mm	R
C3	R	14 mm	R	15 mm	12 mm
C4	R	R	16mm	9 mm	12 mm
C5	R	18 mm	R	17 mm	19 mm

R: resistance

5. Conclusion:

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of silver nanoparticles obtained from bio reduction of silver nitrate solutions using Azadirachta indica, Tridax procumbens and Aegle marmelos leaf extracts. AgNPs obtained from them also varied in antibacterial activity against pathogens due to different concentration of Ag in the nanoparticles. AgNPs have been appropriately characterized using UV-vis spectroscopy and SEM. Biological synthesized silver nanoparticles could be of immense use in the medical field for their efficient antimicrobial function.

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