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GREEN SYNTHESIS OF MULTIFUNCTIONAL SILVER NANOPARTICLES

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ABSTRACT

In the present investigation, we report the green synthesis of multifunctional silver nanoparticles (SNPs) using agro-waste material (paddy straw), a good alternative to the electrochemical methods. The synthesised SNPs were characterized by UV-visible spectrophotometry, Scanning electron microscopy (SEM) and Fourier transform - infrared spectroscopy (FTIR). UV-visible absorption scan of SNP revealed a broad peak at 420nm indicative of the surface plasmon resonance using 10 mM silver nitrate with the reaction time of 24 h. The synthesized silver nanoparticles were of size ranges from 50-70 nm with 67%. The silver nanoparticles were investigated to evaluate the anti-microbial activity against clinical pathogens, efficacy in sewage water treatment and in biofilm degradation. The nanoparticles showed promising compatibility with these diverse functionalities which would transform it to a multifunctional nanoparticles biomedical agent potential for future clinical use.

KEYWORDS: anti-microbial; biofilm degradation; biomedical agent; green synthesis; nano-particle.

INTRODUCTION

Nanotechnology is a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. The nanoparticles can play a crucial role in the field of nanomedicines such as health care and medicine diagnostic and screening purposes and drug delivery systems. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, they are gaining the interest of scientist for their novel methods of synthesis. [1]

Recently, biosynthetic methods employing either microorganisms or plants extract have emerged as an alternative to more complex chemical synthetic procedures to obtain nanomaterials. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, it requires capping agents for size stabilization of the nanoparticles that are toxic and lead to non-ecofriendly by products. The need for environmental non-toxic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals. Thus, there is an increasing need for “Green Nanotechnology”.[2]

The major advantage of using plant extracts for 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. [1]. With the huge plant diversity much more plant species are in the way to be exploited and reported in future era toward rapid and single step protocol with green principle. The plant phytochemicals like terpenoids, flavonoids, alkaloids present in the aqueous leaf extract with antioxidant property were accountable for the preparation of metal nanoparticles.[2]

However, limited literature is available on silver nanoparticles (SNP) synthesis by agrowastes with diverse applications. Hence in the present investigation, we report a simple economic synthesis of silver nanoparticles by an environmental friendly procedure involving the in situ reduction of Ag by agro waste (paddy straw) extract and evaluation of the multi-applications of the synthesised silver nanoparticles.

MATERIALS AND METHODS

Synthesis of silver nanoparticles (SNP)

Approximately 5 gm of thoroughly washed paddy straw (collected from the local market) was boiled in 100 ml of distilled water for 10 min. The suspension was cooled and filtered. Silver nanoparticle synthesis was carried out by mixing 90 ml of the filtrate with 10 ml of aqueous solution of 10 mM $AgNO_3$. The mixture was incubated till a colour change was obtained and centrifuged at 10000 rpm for 20 min. The pellet obtained was washed repeatedly with sterile distilled water, dried and finely powdered for characterization.[2] The possibility of controlling the reaction rate was further investigated by using various concentrations of substrate $AgNO_3$ (0.5 – 25 mM).

Characterization of SNP

The synthesised SNPs were characterized by UV-visible spectrophotometry, Scanning electron microscopy (SEM) and FTIR spectroscopy. UV-vis spectral analysis was done by using Spectrophotometer (Chemito UV2300) by measuring the absorbance from 300 - 600 nm. Scanning Electron Microscope (SEM) analysis was carried out to characterize particle shape, distribution and approximate size. Dry powdered sample was coated with 80% gold and 20% palladium with quorum SC7620 sputter coater to make them conductive, and analysed with Zeiss Evo 18, Scanning Electron Microscope. FTIR (Fourier Transform-Infrared spectroscopy) analysis of the dried SNP was done using Shimadzu Fourier transform infrared spectrometer and spectrum was recorded in the range of 500-4000cm-1 at a resolution of 4 cm^{-1}.

Applications of SNP

Evaluation of antimicrobial activity

The antibacterial activity of silver nanoparticles was carried out using the well diffusion assay against test pathogens.[3] The antifungal activity of silver nanoparticles was carried out using *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., and *Aspergillus niger* as test cultures. Wells were punched into the PDA plates inoculated with the respective fungal cultures and SNP solutions (10µg mL^{-1}) were added in the respective wells. The plates were incubated and inhibition diameters were measured. Well without SNP solutions served as control.[4]

Determination of Minimum Inhibitory Concentration (MIC) of test pathogens

The Minimum inhibitory concentration (MIC) of SNP against the test pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* was calculated by micro broth dilution method in 96 well micro-titre plates using resazurin indicator solution.[5] The lowest concentration at which no colour change occurred was recorded as the MIC against the respective clinical pathogens.

Biofilm inhibition

Biofilm inhibition assay was carried out using *Bacillus subtilis.* [6] The percentage inhibition of biofilm activity was calculated using the following equation.

$$\text{Percentage inhibition} = \frac{\text{(O.D of sample – O.D of control)}}{\text{O.D of control}} \times 100$$

Waste water treatment using SNP coated stones

The colloidal solutions of SNP were coated onto stones to check their efficacy in water treatment. Sterile stones coated with SNP were dipped in 100mL domestic sewage water sample with CFU more than 10^{12}, incubated overnight, spread plated on nutrient agar plates and observed for the reduction in bacterial growth.[7]

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles (SNP)

It was observed that the colour of the solution turned from bright orange to dark brown after 24 h, which indicated the formation of silver nanoparticles (Fig 1). Currently, the mechanism of biological nanoparticle synthesis is not fully understood. Recent results with *Capsicum annuum L*. extract indicate that the proteins which have amine groups play a reducing and controlling role in the formation of SNPs in the solution. Therefore, more elaborate studies are required to elucidate the mechanism of biological nanoparticle synthesis.[8] The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra showed maximum absorbance at 420 nm, which varied with different concentrations of silver nitrate (Fig. 2). The optimum concentration of $AgNO_3$for the synthesis was found to be 10mM. Though further increase in the concentration of $AgNO_3$ reduced the time required for nanoparticle production by the extract, higher concentration higher pH leads to aggregation of nanoparticles which causes them to precipitate out of the solution.

The UV–vis spectra showed maximum absorbance at 420 nm is a characteristic of this nobel metal particle which is due to the Surface Plasmon Resonance of silver nanoparticles (Fig 2).[9] SEM analysis shows the presence of the synthesized silver nanoparticles of size ranges from 50-70 nm (Fig 3). FTIR measurements were carried out to identify the biomolecules responsible for capping and efficient stabilization of the synthesised NPs. The FTIR spectra of SNP (Fig 4) showed bands between 3400–3250 cm^{-1} which corresponds to N–H stretch 1˚, 2˚ amines and amides. Band at 1650 cm^{-1} corresponds to N–H bend, 1˚ amines. Bands between 1335–1000 cm^{-1} correspond to C–N stretch aromatic amines, C–O stretch alcohols, carboxylic acids, esters, ethers.

Fig. 1: Colour change during biosynthesise of SNP.

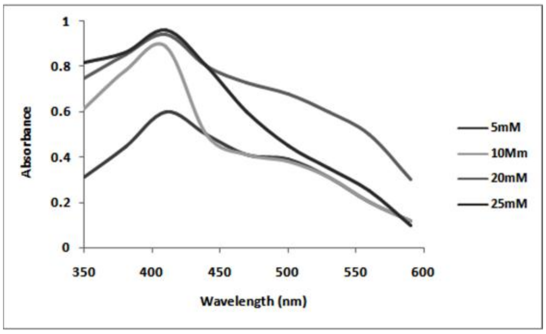

Fig. 2: UV-Vis spectrum of SNP.

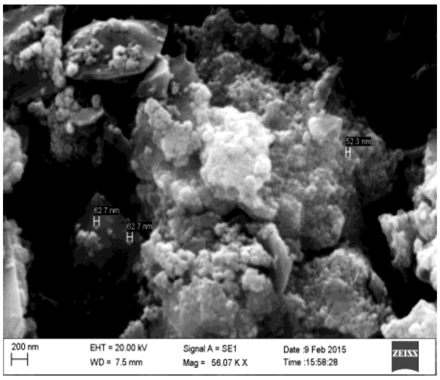

Fig. 3: SEM analysis.

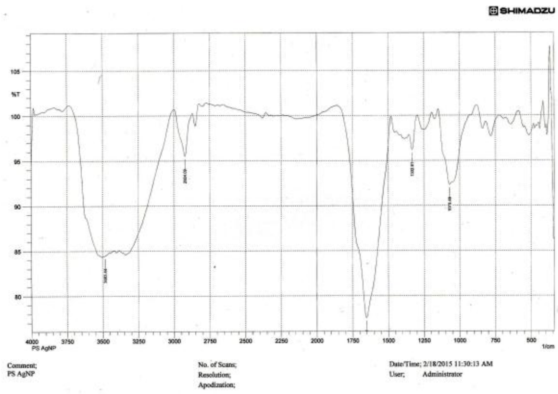

Fig. 4: FTIR spectrum.

Applications of SNP

Antimicrobial activity

The antimicrobial activity of silver nanoparticles synthesized by green route was investigated against various pathogenic organisms and the result is shown in Table. Antimicrobial activity depends on the size and shape of the silver nanoparticles. Small size nanoparticles have large surface area ensuring the inhibition of microbial growth.[10] Due to the abundance of sulphur containing proteins on the bacterial cell membrane, nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It has been hypothesized that nanoparticles primarily affect the functions of membrane bound enzymes resulting in the loss of cellular integrity and osmotic culminating in acute toxicity to fungal cells.[11]

Table 1: Antimicrobial activity of SNP.

Test cultures	Zone of inhibition (mm)
Antibacterial activity	
S. aureus	18.3 ± 1.50
Bacillius subtilis	20.0 ± 2.0
Proteus vulgaris	18.0 ± 1.0
E.coli	18.6 ± 0.55
Paddy straw extract (control)	0
$AgNO_3$ (control)	9.2 ± 1.0
Antifungal activity	
***Rhizopus* sp.**	13.3 ± 0.57
***Fusarium* sp**	12.6 ± 0.57
Aspergilus niger	13.0 ± 0.0
***Penicillium* sp.**	13.3 ±0.57
Paddy straw extract (control)	0
$AgNO_3$ (control)	6.4 ± 1.0

*Well diffusion method on LB agar /PDA plate for antibacterial /antifungal activity respectively and the zone of clearance was measured after the incubation period. Values are presented as mean ± SD of the three triplicates of the experiments.

Minimum Inhibitory Concentration (MIC) of test pathogens

The results MIC (Fig 5) suggest that SNPs exhibited excellent bactericidal effects against test cultures. Though many reports showed the antibacterial effect of SNP synthesized from microbes, the present study emphasises the elimination of the complicated process of maintaining the microbial culture for nanoparticles synthesis thus reducing the cost of nanoparticle synthesis.

Biofilm Inhibition

Biofilm infections are extremely challenging to treat because antimicrobials are less effective. The presence of biofilms causes numerous problems in the field of medicine as well as marine bioufouling, it interferes with the clinical therapy of chronic and wound-related infections as well as persistent infections of various medical devices. Although numerous strategies have been established and are currently in use to control biofilms, the search for novel, natural and effective antibiofilm agents still continues. In the present study, the use of nanoparticles as alternatives to control biofilms has been explored. Compared to the control, preformed *Bacillus subtilis* biofilms treated with SNP (12 µg mL^{-1}) for 24h showed inhibition of 78.92%. Silver nanoparticles have been shown to modify the surface properties of bacterial cells and reduce their adhesive properties.[6]

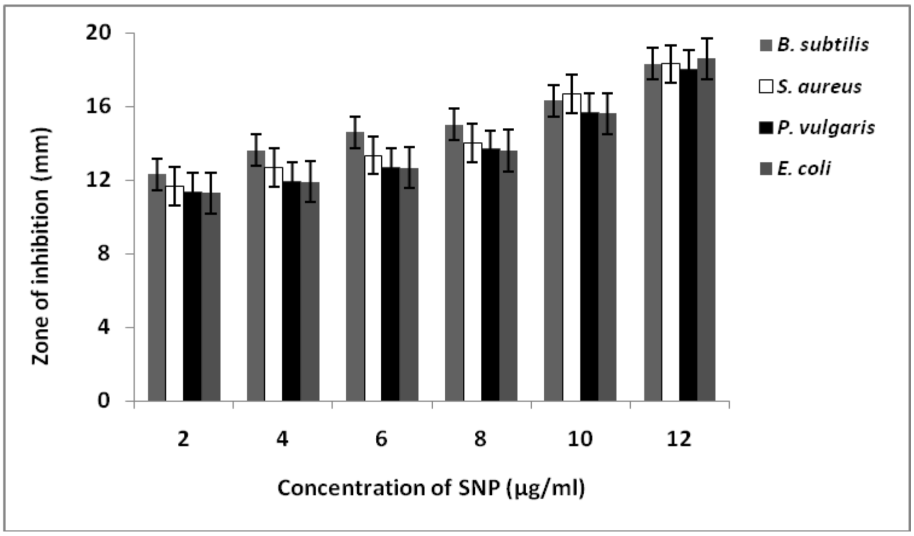

Fig. 5: Dose-dependent antimicrobial activity of SNP synthesised from paddy straw extract.Error bars are standard error of the mean (n=3)

The result of the present study reveals that biologically synthesized silver nanoparticles not only effectively inhibited the growth of the bacteria, but also wiped out the biofilm formed by it which may due to be the presence of water channels though out the biofilm. Since in all biofilms water channels (pores) are present for nutrient transportation, SNPs may directly diffuse through the exopolysaccharide layer through the pores and may impart anti-microbial function. This study thus demonstrates the futuristic application of silver nanoparticles as an potential anti-biofilm agent. Similar report by Kalishwaralal *et al*,[12] showed that SNPs eliminated the biofilm formed previously besides inhibiting the formation of biofilm in existing bacteria.

Waste water treatment using SNP coated stones

The application of silver nanoparticles as an effective agent for sewage water treatment clearly shows no bacterial growth on the plate spread plated with water sample treated with the stones coated with SNP as compared to the control plate (Fig 6). Thus agro-waste generated silver nanoparticles can be effectively used in sewage water treatment, as well where stones can be used in the form of a filter, through which water when passed, can be purified.

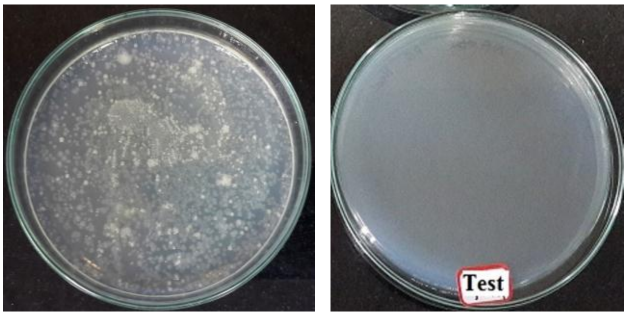

Fig. 6: Sewage water treatment using SNP.

CONCLUSION

In conclusion, it has been demonstrated that the extract of paddy straw is capable of producing silver nanoparticles. The biosynthesized silver nanoparticles showed excellent antimicrobial activity, in water treatment and biofilm inhibition property. Thus the biologically synthesized silver nanoparticles could be of immense use in medical field. The present study paves the way for future therapeutic applications of nanoparticles in biology and medicine.

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