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SYNTHESIS OF SILVER NANOPARTICLES USING PLUMERIA RUBRA EXTRACTS AND TO STUDY ITS ANTI-BACTERIAL PROPERTIES

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ABSTRACT

Silver nanoparticles have found applications in catalysis, optics, electronics and other areas due to their unique size-dependent optical, electrical and magnetic properties. Currently most of the applications of silver nanoparticles are in antibacterial/antifungal agents in biotechnology and bioengineering, textile engineering, water treatment and silver-based consumer products. In this study, silver nanoparticles were successfully prepared from $AgNO_3$ using *Plumeria rubra* extract. The products were characterized by FTIR (Fourier-transform IR) and SEM (Scanning electron microscopy) analysis. The size of the spherical silver nanoparticles was found to be in the range of 70.2 to 127.2 nm. The results revealed that AgNP is an effective broad-spectrum antimicrobial agent, which exhibited an effective antibacterial property.

1. INTRODUCTION

The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Nanomaterials such as Ag, Au, Pt, ZnO and Pd have been synthesized by different methods, including hard template [1], using bacteria [2], fungi [3], and plants [4,5],. Among these, silver nanoparticles play a significant role in the field of biology and medicine due to its attractive physiochemical properties. The highly reactive metal oxide nanoparticles exhibit excellent bactericidal action against Gram-positive and Gram-negative bacteria [6]. The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material. Sondi and Sondi [2004] studied the antimicrobial activity of silver nanoparticles against *Escherichia coli* as a model of Gram-negative bacteria.

Although most of the studies are focused on nanoparticle applications, studies describing the impact of nanoparticles on human health and the possible application of nanoparticles as anticancerous drug are limited (Hsin *et al* 2008 and Sondi *et al* 2004). Whereas, the present study was aimed in the synthesis of silver nanoparticles using the shade dried *Plumeria rubra* plant extract and evaluates its antimicrobial activity.

2. MATERIAL AND METHOD

2.1 Collection of Plant Material And Preparation of The Extract

Freshly collected *Plumeria rubra* leaves were shade dried and powdered. 5 g of the leaf powder was boiled for 10 min. in 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters, stored in refrigerated condition and used for the further study.

2.2 Synthesis of Silver Nanoparticles

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Plumeria rubra* extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag^+ ions and incubated overnight at room temperature in dark.

2.3 UV-Vis Spectra Analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction medium after overnight incubation, after diluting a small aliquot of the sample into distilled water. Silver nanoparticles (AgNPs) are soluble in distilled water and the colour changes were observed visually. A yellowish brown colouration was noticed at the synthesis phase. The concentration of AgNP produced was measured using a Systronics UV double-beam spectrophotometer (model 2201), at a resolution of 1 nm, between 200 and 600 nm using 10-mm-optical-path-length quartz cuvettes.

2.4 FTIR (Fourier-TRANSFORM Ir)

Studies on the samples were carried out using Nicolet Impact 400 FTIR spectroscopy to ensure the formation of silver nanoparticles.

2.5 SEM Analysis of Silver Nanoparticles

A scanning electron microscope (JEOL 6380A; Tokyo, Japan) was used to record the micrograph images of synthesized AgNPs.

2.5 Antimicrobial Assay

The study was performed by using the agar-disc assay method. The solidified LB agar plates were swabbed with 100 μl of *Escherichia coli* and solidified NB agar plates were swabbed with 100 μl of *Staphylococcus aureus*, *Pseudomonas aureginosa* and *Micrococcus luteus* [10^8 cfu (colony-forming units)]. For this assay paper discs were prepared, paper discs were soaked in 20µl of 1mM $AgNO_3$, plant extract, silver nanoparticle and tetracycline respectively. The above treated paper discs were dried in the hot air oven and placed on the bacterial sample inoculated plates. An untreated paper disc was placed at the centre as a positive control. The above steps were repeated for agar plates swabbed with all the four different bacterial samples. The plates were then incubated overnight at 37 ◦C and the antimicrobial effect was determined by measuring the diameter of the zone of inhibition.

3. RESULT AND DISCUSSION

The chemical reduction of aqueous solution of silver nitrate is one of the most widely used methods for the synthesis of silver colloids. In the present study, the formation of silver nanoparticles by *Plumeria rubra* extract was investigated. The appearance of a yellowish brown colour in the reaction vessels suggested the formation of silver nanoparticles (Ahmad et al, 2003). The plant extract after addition of aqueous 1 mm silver nitrate was subjected to optical measurements by UV-vis spectrophotometer. This analysis showed an absorbance peak at 420 nm, which was specific for the Ag nanoparticles.

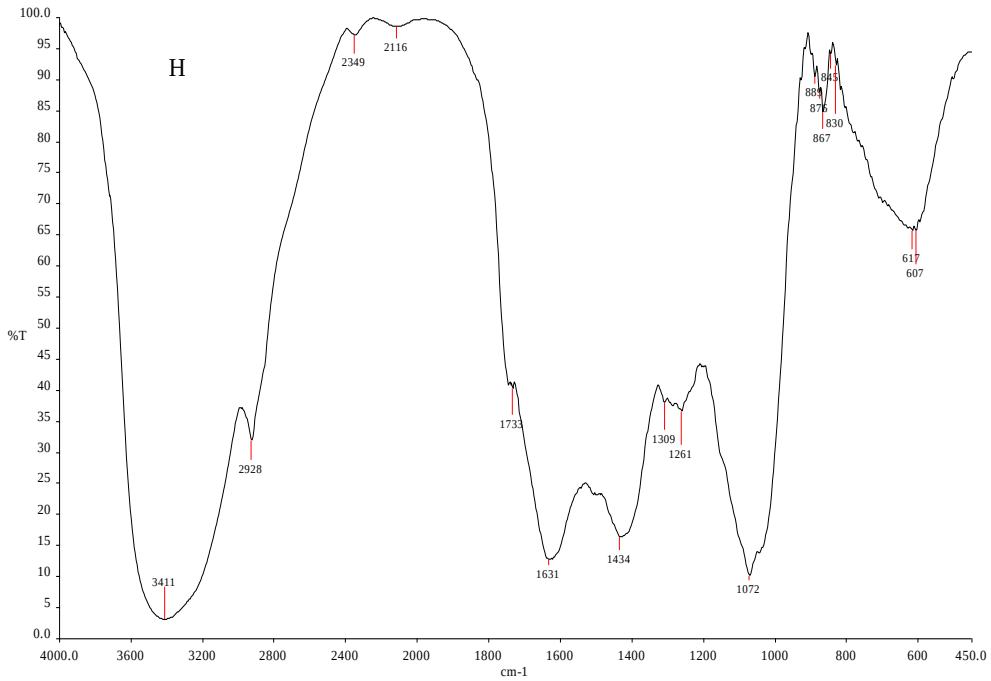

Fig 1. FTIR Spectra of Silver Nanoparticles By Plumeria Rubra Extract.

The FTIR spectrum (Fig.1) of Ag nanoparticles showed distinct peaks 3411 cm^{-1}, which assigned for OH streaching vibration of alcohol and phenolic compounds, the peak at 2928 cm^{-1} represent C-H stretching mode in alkanes. The distinct peaks 1631 cm^{-1}, which is attributed to N-H bending vibrations of amides, 1434 cm^{-1} represents C=C stretching mode in aromatic compounds. Peaks between 1261-1309 cm^{-1} represent the involvement of C-O in plane vibrations of carboxylic acids, the above peaks indicating the involvement of aromatic compounds present in this plant for the reduction of silver ions into nanoparticles.

The sizes of the spherical Ag nanoparticles were found to be in the range of 70.2 to 127.2 nm (Fig.2).

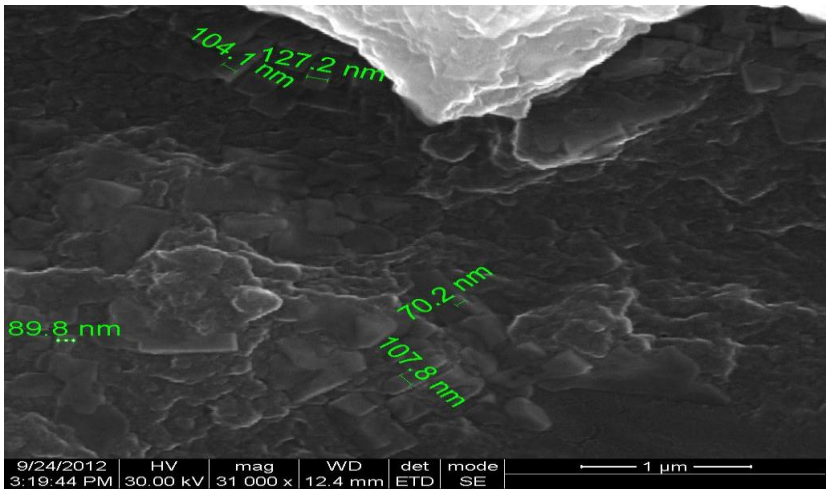

Fig.2 SEM Image of Silver Nanoparticles By Plumeria Rubra

Thus, the zone of inhibition was observed in the next day, by observing the zone of inhibition formed around the respective paper disc it was clearly shown that the prepared nanoparticle have antibacterial properties. It shows a better effect and activity than silver nitrate, which poses a risk. The zone of inhibition of the nanoparticle for the gram negative bacteria like *P.aeruginosa* is greater than that of the other bacteria used for the present study(Fig.3). That implies that it shows near antibiotic effect against these microorganisms. Appreciable antimicrobial activity equal to the antibiotic was also observed in the nanoparticles against *P.aeruginosa* only.

Fig.3 Antimicrobial Effect of Silver.

Table 1: Antimicrobial Activity of AgNP (Average of Three Replicates)

Organism	$AgNO_3$	Plant Extract (mm)	Nanoparticle (mm)	Antibiotic (mm)
Staphylococcus aureus	9mm	-	14mm	20mm
Micrococcus luteus	7mm	-	10mm	17mm
Eschericia coli	-	-	7mm	15mm
Pseudomonas aeruginosa	10mm	-	12mm	12mm

CONCLUSION

The present study demonstrated the green synthesis of silver nanoparticles using the extract of *Plumeria rubra* in room temperature without using any templates, additives or accelerants.

Nanoparticles

In the present study silver nanoparticles have exhibited a significant antimicrobial effect against *P.aeruginosa.* Hence, it is suggested that the silver nanoparticles prepared using the

extract of *Plumeria rubra* can be used as an efficient antimicrobial agent against various other sensitive group of bacteria. It can also be screened against various types of clinically challenging MDR strains.

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