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Green Synthesis of Silver Nanoparticles Using *Sphaeranthus Indicus* Leaf Extract and Their Antibacterial Activity

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Abstract
The present work deals with Sphaeranthus indicus leaf extract used as a capping and reducing agent for silver nanoparticles (Ag NPs) synthesis. Synthesized nanoparticles were characterized by UV-Vis, FT-IR, XRD, SEM with EDX and TEM analysis. The UV–Visible spectra of Ag NPs exhibited a surface plasmon resonance peak at 424 nm. FT-IR analysis determined the vibration functional group to responsible for formation of Ag NPs. XRD patterns revealed that face-center cubic phase structure. SEM images clearly showed spherical nature of the Ag NPs. The EDX analysis showed the complete elemental composition of the synthesized Ag NPs. TEM images of Ag NPs revealed the spherical shapes with an average particle size of 20 nm. Synthesized Ag NPs was investigated against Gram positive and negative bacterial strains were tested with different concentrations of 10, 30, 50, 70 and 100 μL. The zone of inhibition varied significantly in a dose-dependent manner. In future, it can be used for against human pathogenic bacterial strains as well as to photo-catalytic, textile fabric and co-formulate detergents.

Keywords: Sphaeranthus indicus, XRD, TEM, Antibacterial activity, Zone of inhibition

1. INTRODUCTION

Past two decades, modern world consume the downstream process of nanoscience and technology for counterpart in day to day lifestyle. In this point of nanotechnology, 'high surface to volume ratio' plays a significant role of nanomaterials when compare to bulk materials. Because, it's contains different physicochemical properties at nano scale level (1-100 nm) [1]. Recently, researchers focus on synthesis of silver nanoparticles understanding the knowledge about it is used for versatile applications such as food, cosmetics, biomedical and pharmaceutical [2].

Sphaeranthus indicus L. (Asteraceae) is 20–50 cm in height and grows widely in the paddy fields. It is distributed in tropical Asia, Africa and Australia. It has been used in Indian traditional system of Ayurvedic medicine in the treatment of skin infections, glandular, swellings, bronchitis, jaundice and nervous depression. Phytochemical analysis of the plant contains sphaeranaine alkaloid, sesquiterpene glycosidesphaeranthanolide, and eudesmanoidesesquiterpene possessing

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immunostimulating activity. The plant leaves contain the glycoside isoflavone. Extracts of *S. indicus* also have been reported for anti- inflammatory, wound healing and antioxidant activities. Recently, its leaf extract used for gold nanoparticles synthesis [3].

Intent of our research, synthesis of Ag NPs was using *S. indicus* leaf extract. Synthesized Ag NPs were characterized by structural and optical properties. In addition to that, Ag NPs were investigated against gram positive and negative bacterial strains.

2. MATERIAL AND METHODS

2.1. Sample collection and identification:

Healthy plants of *Sphaeranthus indicus* were collected from the Alagappa University, Botanical Garden, Karaikudi. Tamil Nadu.

2.2. Synthesis of Ag NPs

10 g of *S. indicus* fresh leaves were transferred into100 mL of boiling double distilled water and kept for 10 min. After that, the hot leaf extract was filtered through Whatman filter paper no. 1., and was then used to further process. The reaction was carried out in 250 mL conical flask containing 100 mL of 1 mM $AgNO_3$ solution plus 10 mL of *S. indicus* leaf extract and stirred well for 30 min. After 30 min, the colourless solution changed to brown color, indicating the synthesis of Ag NPs (pH 5.2) .

2.3. Characterization of Ag NPs

UV-visible spectrum of the synthesized silver nanoparticles was recorded by Shimadzu spectrophotometer (Model UV-1800 Japan). Fourier transform infrared spectroscopy (FTIR-Thermo Nicolet 380, USA) analysis was carried out in the range of 400–4000 cm^{-1}. XRD pattern was recorded using Cu Kα radiation (λ=1.54060 Å) with nickel monochromator in the 2θ range from 10° to 80° using X'pert PRO PAN analytical diffractometer. Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis by (Instrument model: FEI Quanta 250, Czech Republic) operated at an accelerating voltage of 10 kV. HR-TEM images of the Ag NPs were recorded using a JEOL JEM 3010 instrument with a UHR pole piece electron microscope operating at 200 kV.

2.4. Antibacterial activity

The antibacterial activity of the synthesized Ag NPs was examined against three Gram-positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* MTCC 96, *Streptococcus pneumoniae* MTCC 1936) and two Gram-negative bacteria of (*Escherichia coli* MTCC 40 and *Klebsiella pneumoniae* (MTCC 432) by disc diffusion method. The bacterial strains were grown in nutrient broth at 37°C until the bacterial suspension has reached 1.5×10^8 CFU/mL. Approximately, 20 mL of molten nutrient agar was poured into the Petri dishes. Cotton swab sticks were immersed in bacterial suspension and spread out evenly on nutrient agar plates. The discs loaded with 10, 30, 50, 70 and 100 μL of Ag NPs were placed over the medium using sterile forceps. Plant leaf extract used as negative control and Gentamicin 30 mcg/disc used as positive control. Plates were incubated for 24 h at 37°C. The inhibition zone of each disc was measured [4,5].

3. RESULTS AND DISCUSSION

3.1. UV-Visible, FT-IR and XRD analysis of Ag NPs

The recorded UV-Visible spectrum showed an absorption peak at 424 nm, which is correspond to the wavelength of the surface plasmon resonance of Ag NPs (Fig.1) [5,6]. FTIR spectroscopy revealed the functional groups from the *S. indicus* extract, which can be responsible for Ag NPs reduction and stabilization. FT-IR spectrum of *S. indicus* extract and Ag NPs showed bands at 3422, 2919, 1621, 1385 and 1087 cm^{-1} indicating OH stretching, aliphatic C-H stretching, –C=O stretching, NO_2 stretching, and–C-O-C stretching (Fig. 2) [3]. Hence, the main component of isoflavone glycosides present in the leaf extract of *S. indicus* are prime responsible for the observed reduction and capping during the synthesis of Ag NPs. The XRD patterns showed angles (2θ) of 38.09, 44.59, 64.67 and 77.54 corresponding to (111), (200) (220) and (311) planes of the Ag NPs (Fig. 3) [2]. All reflections can be indexed to Face-Centered-Cubic (FCC) nature of synthesized Ag NPs, in good agreement with standard data (JCPDS Card No: 03-0921). From the XRD data, the calculated the average crystallite size was estimated 18.00 nm.

3.2. SEM with EDX and TEM analysis of Ag NPs

The SEM image (Fig. 4) further ascertained that the Ag NPs are predominantly spherical in morphology with the sizes ranging from 20 to 40 nm and has an average size of about 30 nm. Energy dispersive X-ray spectroscopy (EDX) (Fig. 5) illustrated the chemical nature of synthesized Ag NPs using *S. indicus* leaf extract. The peak obtained at the energy of 3.0 keV for Ag and also some weak peaks for C and Al have also been found. We observed a significant peak corresponding to Al in the sample, which is attributed to the aluminum foil substrate. The emission energy at 3.0 keV indicates the reduction of Ag ions to element of silver [5]. HR-TEM images further ascertain that the Ag NPs are predominantly spherical morphology with their sizes ranging from 20 to 40 nm and calculated the mean values of particles size at 20 nm (Data not shown).

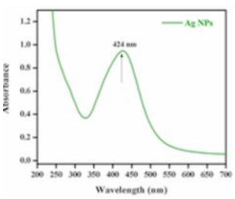

Fig.1

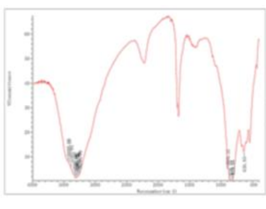
Fig.2

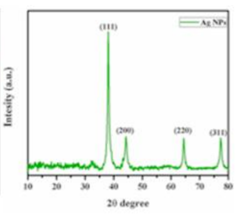

Fig.3

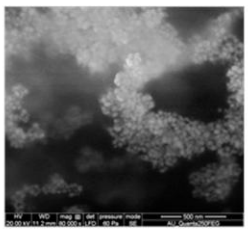
Fig.4

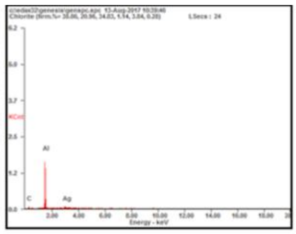

Fig.5

Fig.1. UV-Visible spectrum synthesized Ag NPs, **Fig.2** FT-IR spectrum of Ag NPs using *S. indicus* leaf extract, **Fig.3** XRD analysis of Ag NPs, **Fig. 4.** SEM analysis of Ag NPs, **Fig. 5** EDX analysis of Ag NPs

3.3. Antibacterial activity of Ag NPs

In order to evaluate the antibacterial activity of synthesized Ag NPs were treated at different concentration. Obtained results clearly showed that significantly reduced the bacterial growth at 100 µL of Ag NPs (Data not shown) when compare to all other treatments of bacterial strains. Hitherto, actual mechanisms of silver nanoparticles were against antibacterial activity are not well understood and still persists. Generally, Ag NPs are positively charged and easy to bond with negative charged bacterial cell wall of peptidoglycans by electrostatic interaction [5,6,7].

4. CONCLUSION

In our findings concluded that, synthesis of green-synthesized Ag NPs using *S. indicus* leaf extract. The SEM images substantiated that the particles are spherical shaped with the range from 20 to 40 and average size of 30 nm. The antibacterial activity showed higher concentration at 100 µL of Ag NPs to produce the most significant effects against the gram positive and negative bacteria. Furthermore, the bactericidal efficacy of Ag NPs can be useful to develop new eco-friendly antibacterial products to be used for photocatalytic, textile fabric applications, as well as to co-formulate detergents.

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