

NOVEL SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUTION OF SILVER NANOPARTICLES

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ABSTRACT

The present investigation evaluates green synthesis & characterization of silver Nanoparticles & its antimicrobial activity by using *Sonneratia alba* leaf extracts. The deionized water was used as solvent to prepare crude leaf extract of plant leaves such as *Sonneratia alba*. Synthesized silver Nanoparticles were investigated using UV-visible spectrophotometry, Fourier transform infrared (FTIR) Spectroscopy, Field emission scanning electron microscopy (FESEM), X-Ray Diffraction (XRD), Energy dispersive Spectroscopy (EDS). Absorption Spectrum at 445 nm range confirmed the synthesis of AgNPs, FESEM images revealed that the synthesized AgNPs had spherical shape & all are aggregated. XRD pattern indicated the crystalline nature of synthesized AgNPs. EDS result confirmed the presence of 100% silver element in the produced NPs from *Sonneratia alba* leaf extract. The FTIR analysis indicates the presence of phytochemical group in synthesized nanoparticle of different plant extract. Green biosynthesis of AgNPs by sonochemical method appears to be rapid, reliable, non-toxic, and ecofriendly. Silver nanoparticle assessed enhance inhibitory activity against *E. coli* (gram negative), *Pseudomonas aeruginosa* (gram negative) and *Bacillus spp.* (gram positive) bacteria. The MIC value of silver nanoparticles was found to be between 200 to 400 µg/ml.

Keywords: Silver nanoparticles (Ag NPs), Sonochemical method, Energy dispersive method, x-ray diffraction, minimum inhibitory concentration.

INTRODUCTION

Nanotechnology is one of the most active areas of research for developing material with high

efficiency and cost effectively. [1-3] Nanomaterial is the material which exist in nanometer scale that is 1-100nm with at least one

dimension. Nanomaterials are of interest because at this scale unique optical, magnetic, electrical and other properties emerge. These emergent properties have potential for great impact in electronics, medicines & other fields. [4-5]

For synthesis of nanomaterial, basically two main approaches can be used i.e. Top-Down (physical) & Bottom –up (chemical) approach. There are several methods for synthesis of nanomaterial such as sol-Gel, Hydrothermal coprecipitation, Microwave & sonochemical methods.[9] Among these, we have to select sonochemical method for synthesis of silver Nanoparticles because this method is very easy, required less equipment, energy & time. In this method we do not require stirring and heating for long time. Sonochemical is understanding the effect of ultrasound in forming acoustic cavitation in liquid resulting in the initiation or enhancement of a chemical activity in the solution. [10] In this technique the reactivity of the precursors is enhanced by taking the advantages that large amount of energy can be released when bubbles burst in ultrasonic waves in a frequency range of nearly 20 kHz – 2 MHz

Nanotechnology is being Developed for research activities and various applications in humans, generally the nanoparticle synthesized from chemical method, but these approaches of synthesis are medically non-application due to contamination from precursor chemical. Today, various types of nanoparticles can be synthesized by green approach.[1]

Green synthesis of nanoparticles is obtained a lot of attention due to cost-efficient and green approach. Among the different sources available plants are viewed because their potential for biological reduction and stabilization. Plant are serves as source of many biochemical compounds. [8,11]

The silver nanoparticle getting extra and more attention due to its huge application. The silver nanoparticles are universal antimicrobial substance in province in biology and medicine because their strong antimicrobial activity

against microbial species. The silver nanoparticles are numerous applications, in medicine as a antibacterial, antifungal, antioxidant, antiviral, and anti-inflammatory due to their dressing, ointment, implant coating, solar technology, engine bearing, water purification [17-21], in electronic, catalysis, food container, part in clothing. [12-13]

For this synthesis we used **SONOCHEMICAL** method, due to nanostructure prepared are uniformly sized nanoparticle in short time and utilizing less energy. High reaction rate achieved by using these sonochemical method.[10]

In present work, we synthesizing silver nanoparticle by the green chemical method. In this synthesis we use the plant leaf extract of *Sonneratia Alba* as reducing and capping agent. Metal and metal oxide NPs synthesised by green approached have been long been used due to its antibacterial properties. [5,14-16] Synthesized silver nanoparticle was screened for their antimicrobial activity against three bacteria. properties. recently improvement of silver nanoparticle Ag NPs obtained by reduction of silver nitrate solution with an electron beam are more effective at killing all kinds of bacteria, including gram negative type, that are not harmed by conventional antibacterial agents. Ag NPs sprays can be used to kill bacteria on shoes, socks, phone and even computer keyboard, keep the smelling sweet and prevent the spread of infection. Ag NPs can also useful against infected skin wounds by reducing antibacterial activities. These results confirmed that Ag NPs could provide a safer alternative to conventional antibacterial agents.[5]

MATERIAL AND METHODS

Silver salt (Silver Nitrate- AgNO_3) of analytical grade were purchased from Sigma-Aldrich. with the highest existing purity were used without further purification.

SAMPLE COLLECTION AND PREPARATION

Sonneratia alba leaf were collected from mangroves habitat in Revdanda near Alibag, Raigad District. The leaf samples were transported in polythene bags. The leaves were cleaned by using distilled water three to four times to remove dirt. Samples were dried in shed. The dried leaves grinded and used for further lab

analysis.

PREPARATION OF PLANT EXTRACT

Fresh leaves of Sonneratia Alba were collected. The Leaves were washed with distilled water, shaded dried and powdered to use. The powdered leaves of about 3 gm are boiled for 20 min into 70 ml deionized water. Filter with ordinary and then with Whatman filter paper. The resulting plant extract was used for further experiments.

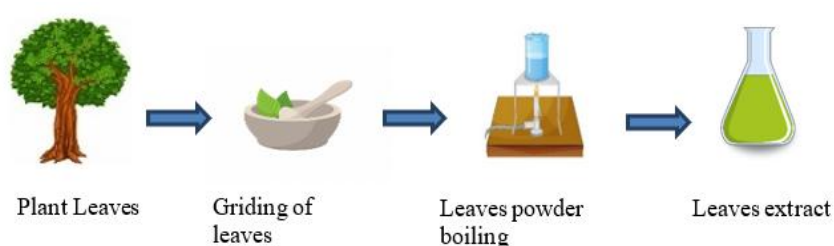
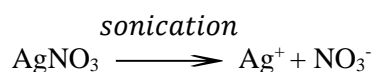


Figure. 1 Flow chart of leaves extract

SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT

For synthesis of silver nanoparticles AgNO_3 of 0.01 M precursor solution sonicate for about 20 min. After sonication of metal salt solution add 2 to 3 ml plant extract in a beaker at room Reactions:

temperature, brown color precipitate was observed in solution, then kept solution as it is for incubation (2 hour). After that precipitate was centrifuge for 20 min. Remove supernant water by using dropper. Then dry precipitate in oven.



RESULTS AND DISCUSSION

CHARACTERIZATION OF SILVER NANOPARTICLES

The biosynthesized silver nanoparticles were characterized by optical technique.

UV-VISIBLE SPECTROSCOPY

Principle of UV spectrophotometer is based on lambert beer's law. Which states that absorbance is directly proportional to the concentration of

sample solution to be analyzed.

Instrumental and analytical condition:

The primary characterization of synthesized silver nanoparticle was carried out using double beam UV spectrophotometer (Shimadzu-1800) with 1 cm quartz cell. The wavelength 200nm to 800nm selected for the analysis of the silver nanoparticle prepared from plant extract of Sonneratia We have to observed absorption spectrum at **445 nm**. It is confirmed that silver

nanoparticles were synthesized by using *Sonneratia alba* leaf extract.

The Colour change observed due to reduction Ag^+ was monitored by using UV-visible

spectrometry. The standard silver nanoparticle wavelength range are 400nm-450nm. The silver nanoparticle peak observed at **445 nm** as shown in fig. 2.

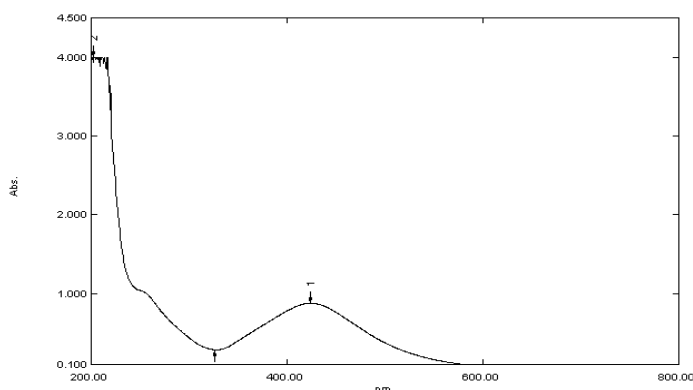


Figure: 2 - visible spectra of silver nanoparticles

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is most powerful tool for identifying the types of chemical bonds (Functional groups) present in the compound. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared adsorption spectrum, the chemical bonds in a molecule can be determined.

The silver nanoparticle prepared from plant extract were used for FTIR analysis. The product of the sample was loaded in FTIR spectroscopy

(Shimadzu, IR Affinity 1, Japan), with scan range from 400 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1} .

The FTIR of silver nanoparticle synthesizes using plant extract of *Sonneratia Alba* are showed different functional groups. The FTIR spectra had major vibration modes at 410.84 (Ag metal stretching), 1365.50 (C-O stretching), 2981.95 (C-H stretching), 3705.26 (N-H stretching) as shown in fig. 3 All these spectra represent different functional groups. FTIR Studies proposed that the presence of phytochemical group in prepared silver nanoparticles.

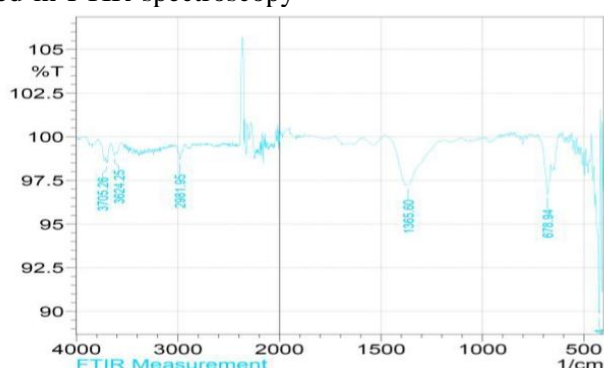


Figure: 3 FTIR of biosynthesised silver Nanoparticle using aqueous leaf extract of *Sonneratia alba* plant.

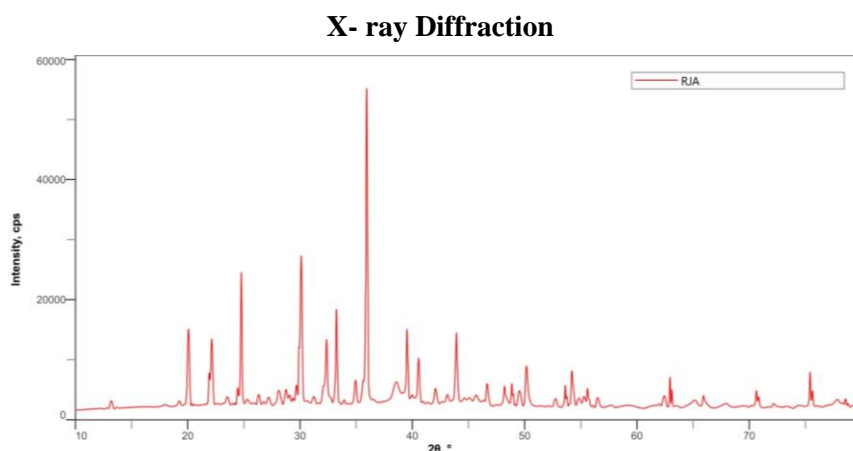


Figure: 4 XRD pattern of biosynthesized silver Nanoparticle using aqueous leaf extract of *Sonneratia alba* plant.

Structure analysis and crystalline size of synthesized silver nanoparticles were carried out by XRD. The XRD analysis of synthesized silver Nanoparticles showed diffraction peak at $2\theta = 20.22^\circ, 24.98^\circ, 32.36^\circ, 35.93^\circ, 43.93^\circ, 50.16^\circ, 54.81^\circ$ respectively as shown in fig. 4. When compared with the standard, the obtained XRD spectrum confirmed that the synthesized silver Nanoparticles were in nanocrystal form and crystalline in nature. The peak can be assigned to the planes (200), (210), (211), (220), (221), (221), (222) of silver crystal respectively. The result indicated that the silver Nanoparticles are simple Cubic and crystalline in nature.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

FESEM microscope work with electrons instead of light. These electrons are produced by field emission source. The object scanned by electrons according to zig-zag pattern. A FESEM is visualize very small topographic details on the surface. The FESEM offer highest magnification as possible. The FESEM which produces, less electrostatically distorted image with spatial resolution to 1.5nm to that is 3 to 6 times better than conventional SEM

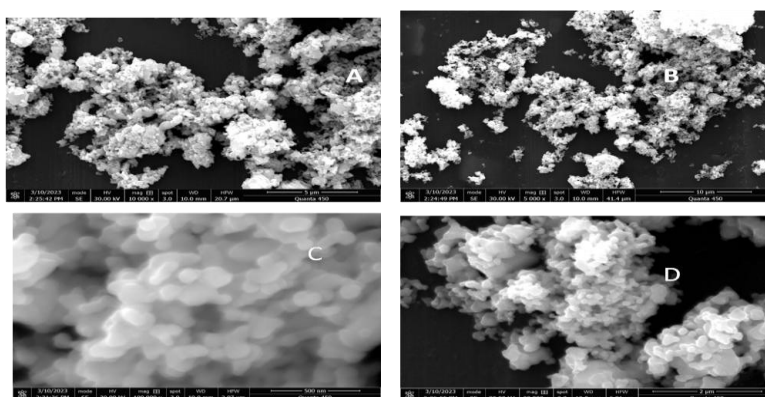


Figure: 5 – FESEM Images

FESEM is an advanced technology used to capture the microstructure image of nanomaterials. FESEM clearly shows the presence of synthesized nanoparticles. The

nanoparticles were spherical in shape. Most of the nanoparticle were aggregated as shown in fig. 5.

EDX ANALYSIS

EDX is microanalysis technique of elemental analysis. The basic principle of Energy Dispersive spectroscopy (EDX) a generation of X-ray from a specimen through the electron beam. The X-ray are generated according to the characteristic and nature of elements present in

the sample.

The EDX was used to carry out the elemental analysis of silver nanoparticle. The EDX analyses the composition and quantity of heavy metals ions in nanoparticles present on the sample surface shown in figure.6. The EDX spectrum states that presence of spherical shaped silver nanoparticle prepared by the green synthesis.

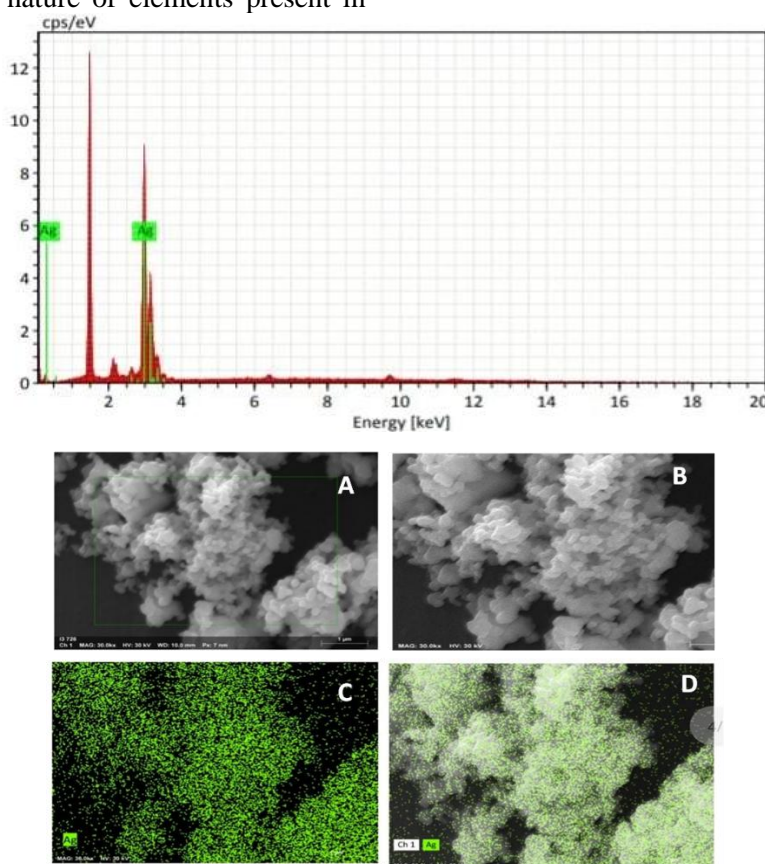


Figure: 6 Energy Dispersive Spectroscopy (EDX) Analysis of Silver Nanoparticle

ANTIMICROBIAL ACTIVITY

The antimicrobial activity testing was carried out by the Disc diffusion Assay (Growth media: Sterile nutrient Agar at pH-6.7). The antimicrobial activity was measured by analyzing the zone of inhibition formed around the test sample. All the samples were tested in three

times. Green synthesized silver nanoparticle assessed enhance inhibitory activity against *E. coli* (gram negative), *Pseudomonas aeruginosa* (gram negative) and *Bacillus spp.* (gram positive) bacteria as shown in table:1.

Sample Id	Inhibition zone diameter(mm) against pathogen		
	E. coli	Pseudomonas aeruginosa	Bacillus spp.
Plant extract	00	00	00
NPs after addition of plant extract	19	15	19
Silver Nanoparticle	24	22	16.5

Table : 1 Enhance inhibitory activity

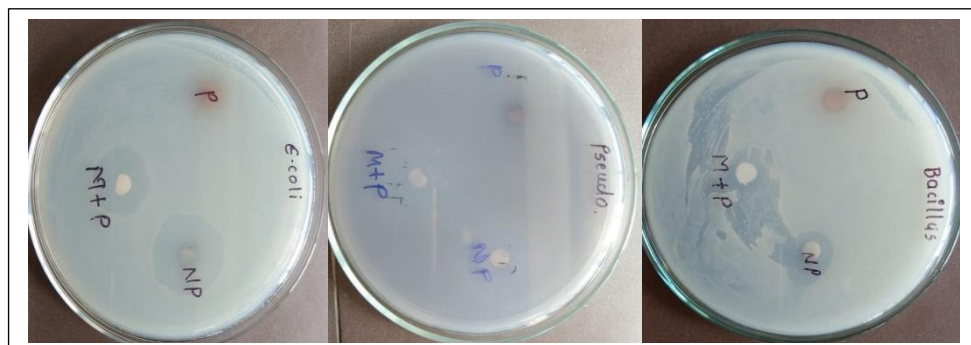


Figure: 7- Antimicrobial activity

The plant extract of Marine *Sonneratia Alba* has no antimicrobial activity, liquid nanoparticle in solution and dried nanoparticle has antimicrobial activity present. The liquid solution of nanoparticle showed the zone of inhibition for *E. coli* 19 mm, *Pseudomonas aeruginosa* 15 mm and *Bacillus spp.* 19 mm. The Nanoparticle (dried) show zone of inhibition for *E. coli* 24 mm, *Pseudomonas aeruginosa* 22 mm, *Bacillus spp.* 16.5 mm as shown in fig. 7.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of silver nanoparticle was determined

using a broth microdilution method. Bacterial suspension 1 ml was poured into each well plates, then different concentrations (0.2-0.8mg/ml) of silver nanoparticle was used to MIC experiment. The microtiter plate was incubated at 37⁰ C for 24 hr. The result showed by the MIC values of silver nanoparticle against *E. coli* and *Pseudomonas aeruginosa* bacteria varied, and the MIC value depended upon the bacterial strains. The MIC reveal that synthesized silver nanoparticles exhibited antimicrobial properties. The MIC value of silver nanoparticles was found to be between 200 to 400 µg/ml as shown in table No:2

Concentration of compound (µg/mL)	Minimum inhibitory concentration (µg/mL) against Bacteria Growth		
		<i>Pseudomonas a. (A₆₀₀)</i>	<i>E. coli (A₆₀₀)</i>
200	+	0.47	0.64
400	-	0.00	0.00
600	-	0.00	0.00
800	-	0.00	0.00
1000	-	0.00	0.00

Table :2 Minimum inhibitory concentration (µg/mL) against Bacteria Growth

CONCLUSION

The silver nanoparticle where successfully synthesized by mixing the AgNO₃ and *Sonneratia*

alba plant extracts separately, gentle stirring and placing room temperature at dark conditions. The synthesized silver nanoparticles showed a change in color from light yellow to dark brown. The

UV-visible spectrum observed at 445 nm.

The FTIR analysis indicates the presence of phytochemical group in synthesized nanoparticle of different plant extract. Whereas the silver nanoparticle of plant extract of Marine *Sonneratia Alba* showed maximum transmittance at 3699.47 (N-H stretching), 2989.66 (C-H stretching), 1379.1 (C-O stretching), 410.84 (Ag metal).

The FESEM analysis revealed that the synthesized silver nanoparticle exhibits spherical shape and particle agglomeration. The EDX spectrum states that presence of spherical shaped silver nanoparticle prepared by the green synthesis.

The synthesized silver nanoparticle its own antimicrobial property against pathogen such as *E. coli*, *Pseudomonas aeruginosa*, *Bacillus* spp. The plant extract of Marine *Sonneratia Alba* has no antimicrobial activity, liquid nanoparticle solution and dried nanoparticle has antimicrobial activity is present. The liquid solution of nanoparticle the zone of inhibition for *E. coli* 19 mm, for *Pseudomonas aeruginosa* 15 mm and for *Bacillus* spp. 19 mm. The Nanoparticle (dried) show zone of inhibition for *E. coli* 24 mm, *Pseudomonas aeruginosa* 22 mm, *Bacillus* spp. 16.5 mm. The MIC reveal that synthesized silver nanoparticles exhibited antimicrobial properties. The MIC value of silver nanoparticles was found to be between 200 to 400 µg/ml.

support.

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The silver nanoparticle successfully synthesized by using *Sonneratia alba* leaf extracts following green approach that is without involving harmful chemicals. The nanoparticle synthesized is eco-friendly, quick, cost-efficient, simple protocol, energy efficient. The synthesized silver nanoparticle also showed efficient antimicrobial activity against human lungs infections and urinary tract infections

The silver nanoparticles are numerous applications, in medicine as antibacterial, antifungal, antioxidant, antiviral, anti-septic and anti-inflammatory due to their properties. recently improvement of silver nanoparticle expending, they are used as wound dressing, ointment, implant coating, solar technology, engine bearing, water purification, in electronics, catalysis, food container, fabric-coating, dye reduction etc.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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