Investigation of Antibacterial Efficacy of Phyto-Engineered Hybrid Nanoparticles

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Abstract **- An eco-friendly green strategy is presented for the synthesis of Ag-Ni hybrid nanoparticles taking a medicinal plant, Aerva lanata for phytoreduction of the precursor salt solutions. The synthesized nanoparticles are characterized by instrumental techniques such as UV-Visible, FTIR, EDX, FESEM, XRD and HRTEM. These particles are found to possess significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria on investigation by Agar Well Diffusion method.**

Index Terms **- Hybrid nanoparticles (HNPs), Aerva lanata (AL), Gram-positive bacteria, Gram-negative bacteria, minimum inhibitory concentration (MIC).**

1.INTRODUCTION

Nanotechnology involves the manipulation of materials having one of the dimensions in the range of 1 nm-100 nm [1]. It has its applicability in different fields like biology, chemistry, physics, engineering and medicine [2].There are two types of methods generally adopted for fabricating nanomaterials: top-down and bottom-up methods. In top down method we transform material progressively from bulk substrate until the desired nanomaterial is obtained. Bottom-up strategies are employed starting from the atomic or molecular precursors and by gradually assembling it until the preferred structure is formed [3].

Remarkable expansion of nanotechnology has spread out its application in biomedical sciences, nutrition, energy sciences, nanobiotechnology, cosmetics, mechanics, optics, chemical industries, drug-gene delivery [4]. Alloying of two different metals in nanosize may enhance the characteristics of their corresponding monometallic nanoparticles. These hybrid nanoparticles show greater stability, catalytic activity than monometallic nanoparticles [5]. Generally nanometals are synthesized by physical or chemical reduction methods but they are hazardous and expensive. In contrast, plant mediated green methods are eco-friendly, cheaper and benign for the synthesis of nanometals. Secondary metabolites present in plant extract will act as bio-reducing and capping agents for the synthesized nanoparticles [6].

Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Organic compounds used for disinfection have notable disadvantages including toxicity to the human body and therefore the interest in inorganic nanoparticles has increased as they are benign to greater extent [7]. Nanoparticles are increasingly used to target bacteria as an alternative to antibiotics [8].Traditional methods like herbal extracts used to the synthesize nanometal particles have shown extensive consent in medicine. These synthesized nanometal particles have great bactericidal activity than bulk metals because of its adsorption at bacterial surface [9].Metals like silver, copper, gold, nickel etc., in the nanodimensions are hypothesized to be able to participate in sub-cellular reactions as their size is comparable to biological molecules [10]. Bimetallic nanoparticles composed of two different metals have drawn a greater interest than the monometallic nanopaticles due to the properties differ from pure elemental particles include unique size dependent, optical, electronic, thermal, catalytic and biological effects [11]. Plant mediated green synthesized HNPs have increased attention towards their antimicrobial properties as they contain bioactive phytochemicals as stabilizing and capping agents.

In this present study, an effortless and robust green method is reported for the synthesis of Ag-Ni hybrid nanoparticles (HNPs) by using leaf extract of *Aerva lanata* as a reducing and capping agent. The synthesized HNPs are studied for their antibacterial

activity against Gram-positive, Gram- negative bacteria.

2. EXPERIMENTAL

2.1. Materials: Chemical reagents used (silver nitrate and nickel nitrate) in this study are of analytical grade. Deionized water is used to clean glassware, to prepare chemical solutions and for experimental procedure. Fresh leaves of *Aerva lanata* are collected from agricultural lands in Nellimarla village, Vizianagaram district, Andhra Pradesh state of India.

2.2. Preparation of *Aerva lanata* leaf extract: 100 g of fresh leaves are weighed and thoroughly washed with running tap water to remove detritus on surface of leaves followed by deionized water to get rid of other contaminants from leaves and dried up under shade for ten days. These leaves are cut into tiny pieces and made homogenized powder by using home blender. The procured powder is placed in refrigerator at 4 ºC which is kept in an air tight container. Now 200 mL deionized water is taken in 500 mL beaker to this 10 g stored powder is weighed and added. The contents in the beaker are heated for 20 minutes at 60 ºC with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled concoction is filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4 ºC. This is taken as leaf extract throughout the experiment.

2.3. Synthesis of Ag-Ni bimetallic nanoparticles:
Equimolar (25 mM) concentrations of silver nitrate and nickel nitrate aqueous solutions were prepared separately in 100 ml volumetric flasks by dissolving 0.4246 g, 0.7267 g weight of $AgNO_3$ and $Ni(NO_3)_2$ in deionized water respectively. Synthesis of Ag-Ni HNPs was done by taking 100mL of $AgNO_3$ solution in a 500 mL beaker, to this 90ml of leaf extract, 100mL of $Ni(NO_3)_2$ solution were added by drop wise in simultaneous addition process. After this addition the beaker was placed on a magnetic stirrer for continuous agitation. This mixture was stirred at 70ºC for 70 minutes at pH 8 on magnetic stirrer. These synthesized HNPs were separated out by doing centrifugation at 5000 rpm for 40 minutes. The obtained HNPs were washed with deionized water 2 times to remove unwanted constituents and dried in oven at 80 ºC for two hours. The resultant HNPs particles were collected (Figure: (1)) and used for characterization.

Figure (1): Synthesis of Ag-Ni HNPs from precursor solutions

2.4. Characterization
The synthesized HNPs are characterized by various instrumental techniques. UV-Visible analysis indicates the formation of HNPs by SPR band at band at around 438 nm. The FTIR spectrum of Ag-Ni HNPs exhibits major peak positions at 3212 cm^{-1}, 3416 cm^{-1} and 3382 cm^{-1}which indicate the N-H stretching vibrations of amines and O-H stretching of hydroxyl groups of alcohols and phenols. Intense peak at 1640 cm^{-1} is due to C=O stretching of amide group. Very small peak at 602 cm^{-1} indicates the presence of C-Cl group

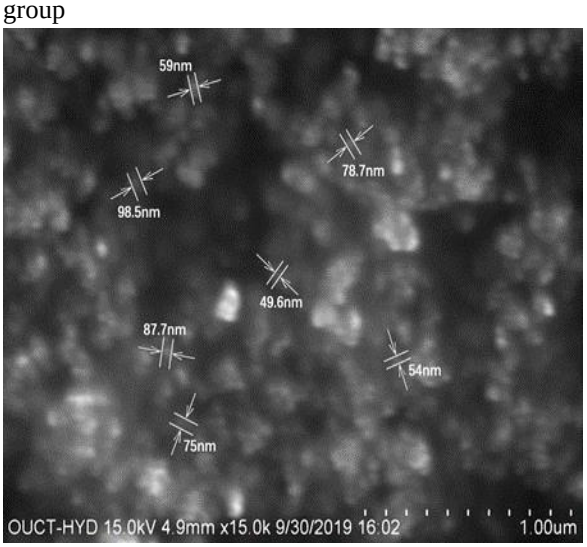

Figure (2): FESEM Image of HNPs

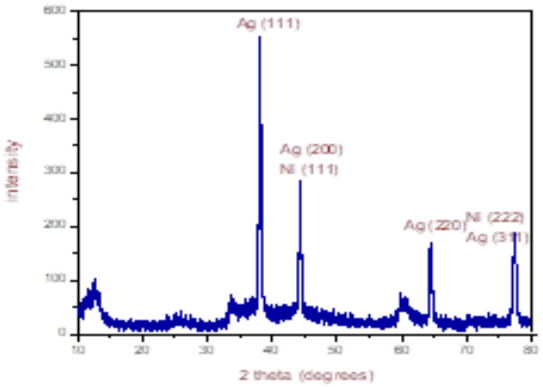

Figure (3): XRD Image of HNPs

3. INVESTIGATION OF ANTIBACTERIAL ACTIVITY

3.1 Reagents and Materials

Microorganisms

(Source of strains- IMTECH, Chandigarh, India)

1) *Bacillus subtilis* MTCC211
2) *Escherichia coli* MTCC443
3) *Staphylococcus aureus* MTCC6908
4) *Pseudomonas aeruginosa* MTCC2581

3.2 Antimicrobial activity

Antimicrobial activities of the compounds investigated are evaluated by agar-well diffusion method. The standardized cultures of test bacteria are first evenly spread onto the surface of Mueller Hinton Agar plates using sterile cotton swabs. Five wells (6 mm diameter) are made in each plate with sterile cork borer. Twenty microlitres of the nanocompound and positive control are added in wells. Streptomycin (10 µg) is used as reference antibiotic. Diffusion of compounds, antibiotic and DMSO were allowed at room temperature for 1 h. All of the plates are then covered with lids and incubated at 37 ºC for 24 h. After incubation, plates are observed for zone of bacterial growth inhibition. The size of inhibition zones is measured and antimicrobial activity of the compounds is expressed in terms of the average diameter of inhibition zone in millimeters. Those compounds that are unable to exhibit inhibition zone (inhibition zone diameter less than 6 mm) are considered non-active. The compound is tested in triplicate with two independent experiments and the mean values of inhibition zone diameters are taken.

4. RESULTS AND DISCUSSIONS

Ag-Ni HNPs are studied for their antimicrobial activity against two-gram positive bacteria and two gram negative bacteria. In case of gram-positive bacteria, the test organisms are *Staphylococcus aureus* and *Bacillus subtilis.* The nanocompound shows significant antibacterial activity against these bacteria in all the four concentrations under study (Figure (4), Table. 1). Ag-Ni HNPs demonstrate significant activity against the two selected gram-positive bacteria, 15 mm against *S. aureus* and 16 mm against *B. subtilis* at 1 mg concentration. In case of gram-negative bacteria, the test organisms were *Pseudomonas aerugisona* and *Escherichia coli.* The compound shows antibacterial activity against these two bacteria in all the four concentrations studied (Figure (5), Table. 2). Ag-Ni nanocompound demonstrates remarkable activity against the two selected gram-positive bacteria, 13 mm against *P. aeruginosa* and 14 mm against *E. coli* at 1mg concentration.

5.CONCLUSIONS

These findings demonstrate that green synthesized Ag-Ni hybrid nanoparticles are found to possess moderate anti-bacterial activity against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) respectively. This antimicrobial activity of nanoparticles is attributed to the capped plant secondary metabolites that are present on their surface. With the future expansion of this present work in mind, considering the evolution of new drug resistant strains, the synthesized HNPs may be administered against those strains and they may be also examined and reported in the future. The future research may be directed for the genetic manipulation of plants to increase the metal tolerance and metal accumulation capacities.

Table 1: Antibacterial activities of nanocompound against gram positive bacteria

S. No	Compound Name	Zone of inhibition (mm)							
		Gram positive *(Staphylococcus aureus)*				Gram positive *(Bacillus subtilis)*			
		1mg	0.75mg	0.5mg	0.25mg	1mg	0.75mg	0.5mg	0.25mg

1	Ag Ni	15	12	11	9	16	14	12	10
2	Streptomycin	29				31			

Table 2: Antibacterial activities of nanocompound against gram negative bacteria

S. No	Compound Name	Zone of inhibition (mm)							
		Gram negative *(Pseudomonas aerugisona)*				Gram negative *(Escherichia coli)*			
		1mg	0.75mg	0.5mg	0.25mg	1mg	0.75mg	0.5mg	0.25mg
1	Ag Ni	13	10	9	8	14	13	11	10
2	Streptomycin	26				27			

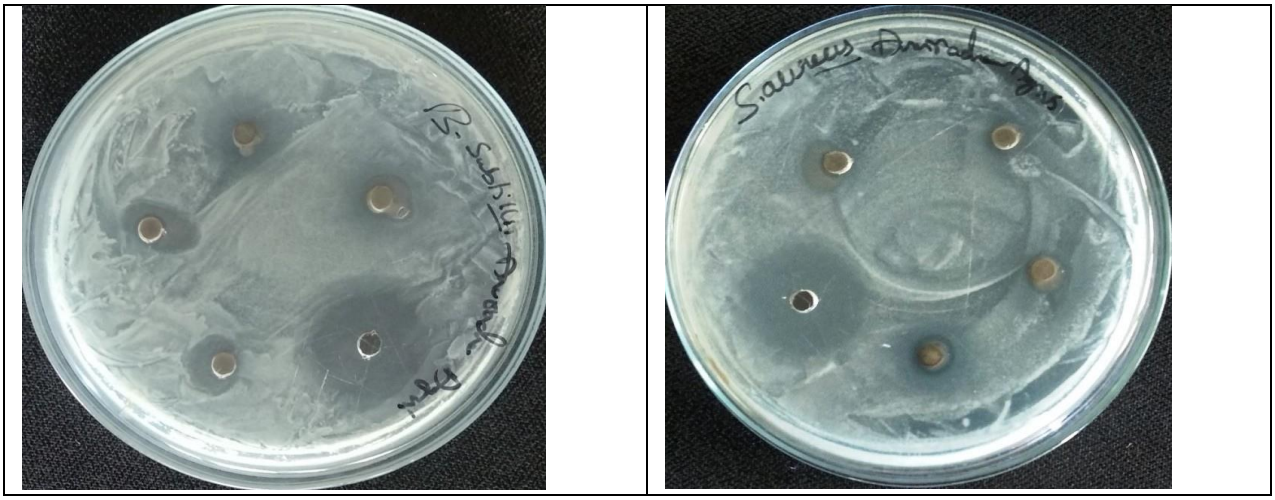

Figure (4): Antibacterial activities of HNPs against gram positive bacteria

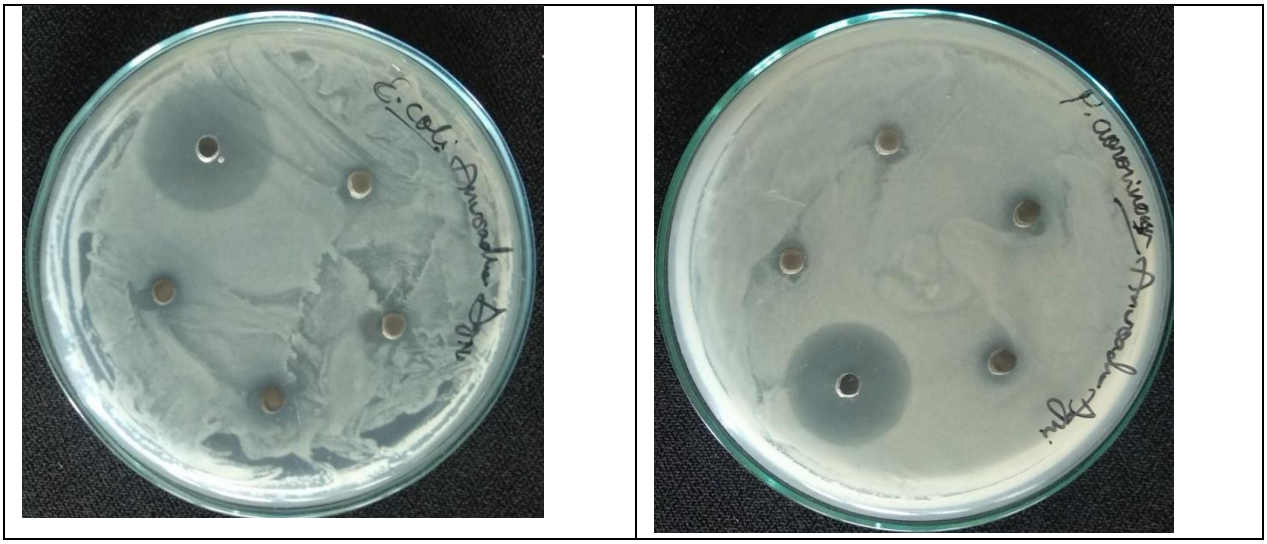

Figure (5): Antibacterial activities of HNPs against gram negative bacteria

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