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IN VITRO ANTIBACTERIAL STUDIES OF PHYTOFABRICATED Ag-Co HYBRID NANOPARTICLES

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ABSTRACT

An ecologically benign green strategy is presented for the synthesis of Ag-Co hybrid nanoparticles taking a medicinal plant, *Aerva lanata* for the phyto reduction of the precursor salt solutions. The formed nanoparticles are characterized by instrumental techniques such as UV-Visible, FTIR, EDX, FESEM and HRTEM. These particles are found to possess significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria, which is investigated by Well Diffusion method.

KEY WORDS

Hybrid nanoparticles (HNPs), *Aerva lanata (*AL), antibacterial, Gram-positive, Gram-negative, zone of inhibition.

INTRODUCTION

Nanotechnology involves the manipulation of materials having one of the dimensions in the range of 0.1-100 nm [1]. It has its applicability in different fields like biology, chemistry, physics, engineering and medicine [2]. There are two types of strategies generally adopted for fabricating nanomaterials, namely top-down methods 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 (**Figure. 1(a), 1(b)**) [3].

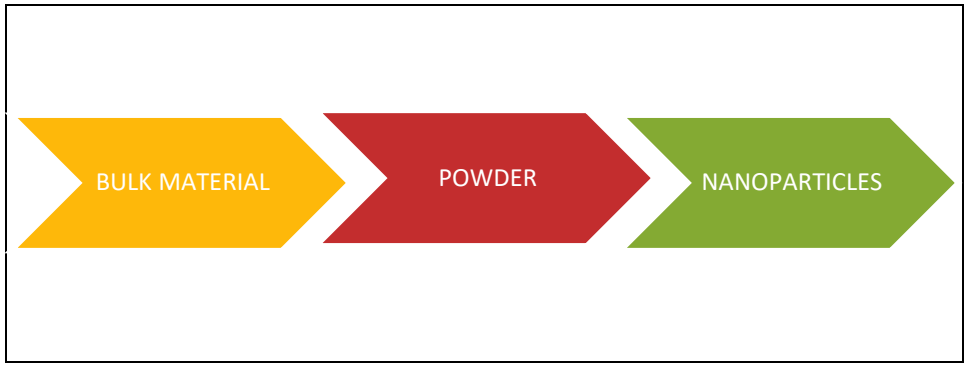

Figure: 1(a). Top-Down approach

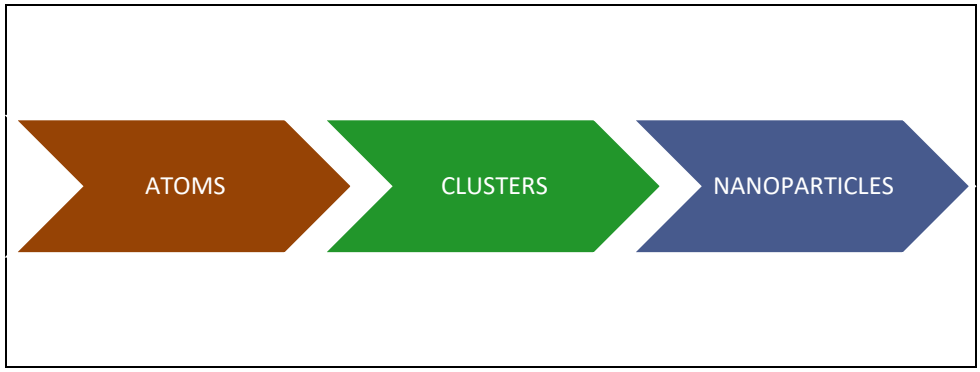

Figure 1(b): Bottom-Up approach

Remarkable expansion of nanotechnology has spread out its application in multiple fields such as biomedical sciences, nutrition, energy sciences, nano-biotechnology, cosmetics, mechanics, optics, chemical industries, drug-gene delivery [4]. Alloying of two different metals in nanosize may enhance their characteristics than that of their corresponding monometallic nanoparticles. These hybrid nanoparticles show greater stability, mechanical strength and catalytic activity than monometallic nanoparticles [5]. Generally nanometals are synthesized by physical or chemical reduction methods which 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 [6].

Antibacterial agents are very important in the textile industry, water disinfection, medicine, and food packaging. Organic compounds used for disinfection have some disadvantages including toxicity to the human body. Therefore the interest in inorganic nanoparticles has been increased as they are benign to a 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 their ease of adsorption at bacterial surface [9]. Nanometals like silver, copper, gold, etc., are assumed 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 greater interest than the monometallic nanopaticles as the properties differ from pure elemental particles include unique size dependent, optical, electronics, thermal, catalytic and biological effects. Hence they are being used as antimicrobial agents. Plant mediated green synthesized hybrid nanoparicles 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-Co hybrid nanoparticles (HNPs) by using leaf extract of *Areva 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:

Chemicals and apparatus required

- Silver nitrate and cobalt nitrate of analytical grade.
- Digital weighing balance
- Magnetic stirrer
- Centrifuge machine
- Hot air oven
- pH papers
- Whatmann-1 filter papers
- Leaves of *Aerva lanata*
- 0.1 N HCl
- 0.1 N NaOH

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 of S.Kota, Vizianagaram district, Andhra Pradesh state, 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 10 days. These leaves are cut into tiny pieces and made homogeniozed 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 deionised 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 two times with Whatman No.1 filter paper and reserved in refrigerator at 4 ºC. This is taken as leaf extract throughout the experiment (**Figure: 2(a), 2(b)**).

Figure 2(a): *Aerva lanata* plant

Figure 2(b): leaf extract

2.3. Synthesis of Ag-Co bimetallic nanoparticles:

Equimolar (20 mM) concentrations of silver nitrate and cobalt nitrate aqueous solutions are prepared in deionized water respectively. Synthesis of Ag-Co BMNPs is done by taking 100 mL of $AgNO_3$ solution in a 500 mL beaker, to this 100 mL of leaf extract, 100 mL of $Co(NO_3)_2$ solution are added by drop wise in simultaneous addition process. After this addition the beaker is placed on a magnetic stirrer for continuous agitation. This mixture is stirred at 75 ºC for one hour at pH 8 on magnetic stirrer. These synthesized HNPs are separated out by centrifugation at 5000 rpm for 30 minutes. The obtained HNPs are washed with deionizer water twice to remove unwanted constituents and dried in oven at 80 ºC for two hours. The resultant HNPs particles are collected and used for characterization.

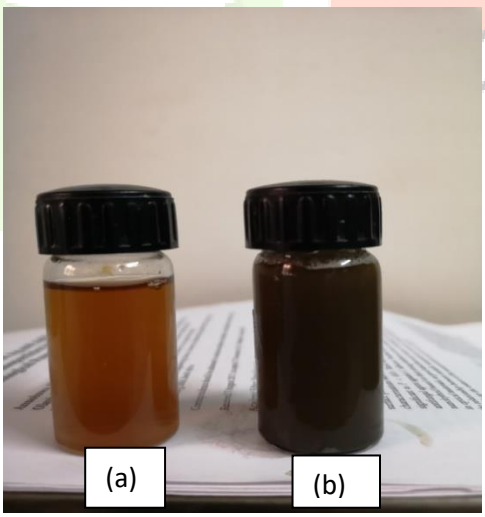

Figure 3: Precursor solutions (a) before (b) after the bioreduction

2.4 Characterization

The synthesized HNPs are characterized by various instrumental techniques. UV-Visible analysis (Figure 4) indicates the formation of HNPs by SPR band at band at around 436 nm [11]. The FTIR spectrum of Ag-Co 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.

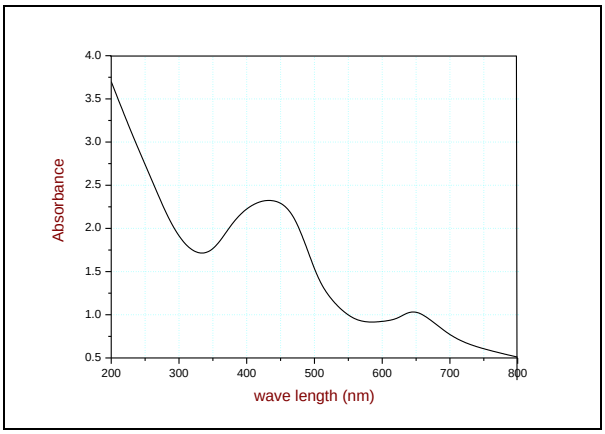

Figure 4: UV-Visible spectrum of Ag-Co HNPs

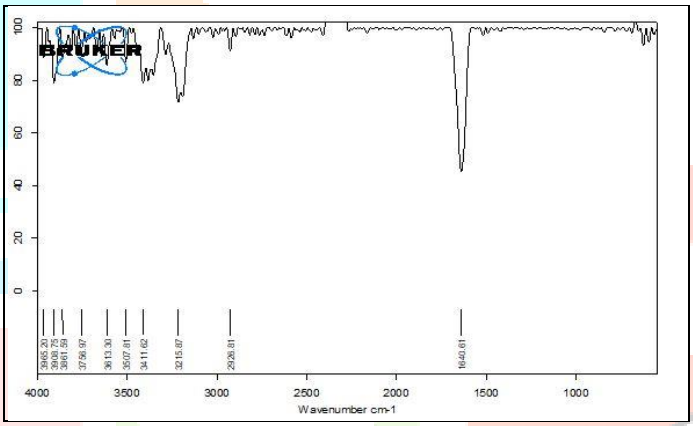

Figure 5: FTIR spectrum of Ag-Co HNPs

From energy dispersive X-ray analysis (EDX), we can analyze all the elements present in the HNPs which indicate the existence of Ag and Co which confirms the formation of Ag-Co hybrid nanoparticles. This is also supported by the EDX study which gives quantitative data of silver and cobalt compositions in HNPs**.** By Field Emission Scanning electron microscopic (FESEM) images of Ag-Co HNPs, it can be clearly noted that the prepared Ag-Co hybrid nanoparticles are in the size range between 50 and 100 nm in diameter.

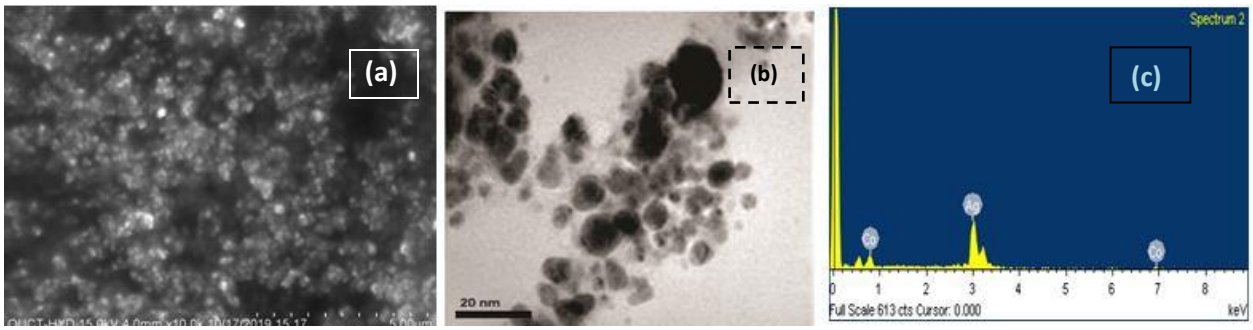

Figure 6 : (a) FESEM image (b) HRTEM image (c) EDX spectrum

HRTEM shows that Ag-Co HNPs are figured with spherical morphology and crystalline structure below 100 nm in size. More specifically, the two metal nanospheres are shown and spoted adjacent to each other. It is also in strong agreement with the micrographs from FESEM analysis. Powder XRD analysis (Figure 7) confirms that HNPs have FCC crystal structure with average particle diameter of 24.4 nm.

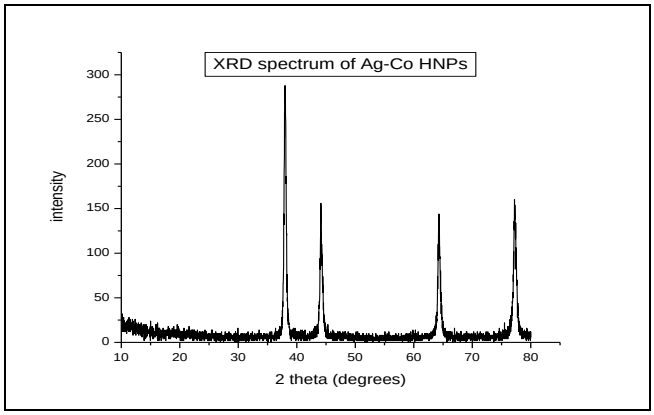

Figure 7: Powder XRD spectrum

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Reagents and Materials

Microorganisms used are obtained from IMTECH, Chandigarh, India

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

Experimental Determination

Antibacterial activity of the HNPs is evaluated by agar-well diffusion method [12]. 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 each of the compound and positive control is added in wells. Streptomycin (10 µg) is used as reference antibiotic. Diffusion of compounds, antibiotic and DMSO are allowed at room temperature for 1 hour. All of the plates are then covered with lids and incubated at 37 °C for 24 hours. 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 zone of inhibition 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 average values of diameters of inhibition zones are considered.

RESULTS AND DISCUSSIONS

Ag-Co HNPs are studied for their antimicrobial activity against two gram positive bacteria, two gram negative bacteria. In case of gram positive bacteria the test organisms were *Staphylococcus aureus* and *Bacillus subtilis.* The nano compound shows significant antibacterial activity against these two bacteria in all the four concentrations studied. HNPs demonstrate high activity against the two selected gram positive bacteria, 22 mm against *S. aureus* and 28 mm against *B. subtilis.*

In case of gram negative bacteria the test organisms were *Pseudomonas aerugisona* and *Escherichia coli.* The HNPs show substantial antibacterial activity against these two bacteria in all the four concentrations studied and demonstrate high activity against the two selected gram positive bacteria, 22 mm against *P. aeruginosa* and 27 mm against *E. coli* at 1mg concentration.

Table 1: Antibacterial activity 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-Co HNPs**	22	21	18	14	28	26	22	19
2	**Streptomycin**	31				32			

Table 2: Antibacterial activity 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-Co HNPs**	22	19	13	12	27	25	22	13
2	**Streptomycin**	28				30			

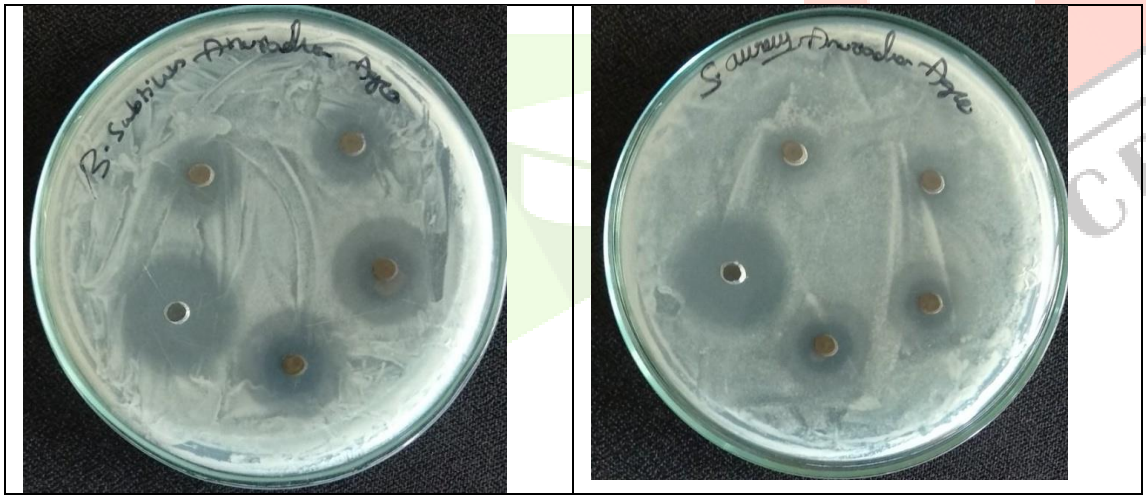

Figure 8: Antibacterial activities of Ag-Co HNPs against Gram-positive bacteria

Figure 9: Antibacterial activities of Ag-Co HNPs against Gram-negative bacteria

CONCLUSIONS

An ecologically innocuous method is projected to synthesize Ag-Co bimetallic nanoparticles from *Areva lanata* leaf extract. From UV-Visible analysis it is proved that the particles are in nanoscale as per the positions of the Surface Plasmon Resonance (SPR) bands. FTIR data confirms the presence of secondary metabolites of phyto molecules as the bio reducing and capping agents of the formed nanoparticles. XRD, SEM and HRTEM results evince that Ag-Co HNPs are in spherical morphology and cubic crystalline structure with size between 30-100 nm. The findings of the well diffusion method demonstrate that *Aerva lanata* leaf mediated green synthesized Ag-Co hybrid nanoparticles are found to possess significant antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) respectively. This antimicrobial activity is attributed to the capped plant secondary metabolites that are present on the surface of the hybrid nanoparticles.

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