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Syngonium podophyllum-based ZnO/Ag Nanocomposites: Biogenic Synthesis and Antimicrobial activity Against Bacterial Isolates and *Saccharomyces cerevisiae*

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ABSTRACT

There is a growing need for engineers to develop effective, powerful, and economical nanocomposites with effective antimicrobial effects. The co-precipitation method was used to fabricate Zinc oxide/Silver nanocomposites (ZnO/Ag NCs). FTIR analysis confirms the presence of a functional group responsible for the stabilization and reduction of fabricated NCs. TEM and SEM analysis showed the internal morphology of the ZnO/Ag NCs as nearly spherical and the average crystallite size of NCs 16.64nm. The NCs are polycrystalline, as demonstrated by the selected area electron diffraction (SAED) pattern. The NCs show good antimicrobial action against Prokaryotic and Eukaryotic organisms. The Minimum inhibitory concentration (MIC) of the NCs are 12.50 and 50 µg/mL for *E. coli* and *S. aureus* respectively. The finding reveals, ZnO/Ag is found to be an efficient combat agent against prokaryotic and eukaryotic organisms and may be employed as an antimicrobial in the future.

Keywords: Nanocomposites, ZnO/Ag, Antibacterial activity, Minimum Inhibitory concentration.

INTRODUCTION

NCs that comprise two or more various components have been fabricated and have become increasingly popular in recent decades. Due to the fact that their special multifunctional nano-assembled system concurrently displays fresh and improved features, nanoscale materials have gained tremendous popularity as advanced nanomaterials for various applications, including biomedicine, catalysis, electronics, and photonics.[1] There has been a long history of using metals as antimicrobial agents in ancient times. To treat bacterial diseases, a vast range of medications have been developed, including silver, copper, zinc, and arsenic. Oxidative stress kills pathogens in eukaryotic cells generated by zinc, silver, and copper, which are associated with the process.[2] There has been a recent increase in microbial resistance to conventional anti-bacterial drugs due to their widespread use. Globally, the majority of population deaths are going to be primary cause by

this situation and it is approaching a perilous level. besides, designing and creating new antibiotics have been slowed by financial constraints, existing regulatory obstacles and scientific impediments.[3] Under its stability and antibacterial activity, nanotechnology-based antibacterial approaches have gained much attention as novel strategies for eradicating bacteria. They supply antibacterial advantages through the unique shape, size, and structure of their material. Some nanomaterials are inherently incapable of killing germs. Although, Due to their form, size, and surface charge, silver nanoparticles are able to affect the integrity of bacterial cell membranes, respiration, and ATP production.[4] A nanoscale particle size comparable to that of the microorganisms that live inside the body and environment has rendered nano-ZnO an extraordinary material with marvelous antibacterial activity. It can therefore enter the cell wall of bacteria and damage multiple parts of their structure, including their DNA and proteins. In addition, Larger surface area, surface charge density, shape, crystal flaws, and optical characteristics are also thought to contribute to ZnO antibacterial properties.[5]

The antibacterial properties of both Ag and ZnO particles appear satisfactory, but they agglomerate when used individually, reducing their effectiveness. By using noble metals like silver, ZnO chemical stability and antibacterial capabilities can be improved (Ag). Various nano ZnO/Ag preparation techniques have been reported in recent years, including photocatalysis[6], photothermal/ photodynamic therapy[4], and antimicrobial activity.[7-6]

This study presents a method for preparing zinc oxide/silver nanocomposites (ZnO/ Ag NCs) by co-precipitation. Nanocomposites were characterized by understanding their morphology and structure. The investigation was conducted using a well diffusion approach for antimicrobial properties of the synthesized samples. Antibacterial activity of ZnO is improved by surface modification with Ag nanoparticles.

EXPERIMENTAL

Reagents

Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) (CAS7447-20-0) were brought from Merck chemicals. silver nitrate ($AgNO_3$) (CAS 7761-88-8) was used as a silver source, sodium hydroxide (NaOH) (CAS 1310-73-2) was used to keep the basic pH, all chemicals used were high purity grade and bought from Sigma Aldrich. Muller Hinton Agar, Yeast extract peptone dextrose, and Luria broth media were obtained from Hi Media. Double-deionized water was used in all experiments, along with autoclaved borosilicate glassware. Whatman paper grade 1 from GE Healthcare Wipro Pvt. Ltd. filtered the sample.

Method of synthesis

Preparation of *Syngonium podophyllum* aqueous extract

Using distilled water and tap water, *Syngonium podophyllum* leaves were rinsed four times for complete removal of dirt or dust particles and semi-air dried to remove the external moisture of the leaves before chopping the leaves into small pieces. The leaves weighing 25 g were heated on a Soxhlet apparatus at 50°C for 30 min after being suspended in 250 mL of double-distilled water in a flask. Mixture was set aside to cool down at room temperature before filtration by Whatman filter paper no.1 to eliminate the solid residues of the extract. After that, the mixture was used, and the remaining was stayed at 4°C for later work.

Formulation of ZnO NPs and other nanocomposites

For nanoparticle synthesis: 100 mL of double-distilled water were used to prepare 1mM of $ZnSO_4.7H_2O$ and $AgNO_3$. The prepared sample was reduced with arrowhead leaf extract to form Zinc oxide and silver nanoparticles in the ratio of 1:2 respectively.

Fabrication of ZnO/Ag binary nanocomposite

These reduced Ag metal nanoparticles were further poured into the matrix of synthesized ZnO while stirring at 10000rpm at 80°C for 1 hours. After that, the pH was raised to 8 by adding dropwise, 5 M NaOH solution. Room temperature was used to rest the solutions for 18 hours. To confiscate impurities, ethanol followed by distilled water was added to the solution and was centrifugated at 7000rpm for 8 min, After every wash, the supernatant was removed until pH comes to neutral. The pellet obtained was dried at 50°C. The ultimate greyish-black powder was mashed in mortar and pestle and subjected to characterization for confirmation of the formed sample.

Characterization of nanocomposites

The morphologies and the crystal

composition of the nanocomposites were characterized with a Transmission Electron Microscope (TEM) Model No. JEM 2100 Make JEOL, Japan to obtain the internal morphology size SEM analysis was carried out by model number JSM 6510LV, made by JEOL in Japan. A Rigaku X-ray diffractometer determine the X-ray diffraction patterns (XRD) (Japan).[8] Organic compounds were classified using FTIR (Bruker Tensor37).[9]

The Antimicrobial potential of ZnO/Ag nanocomposites

Antibacterial properties of the synthesized ZnO/Ag nanocomposites were studied method by Romana *et.al.,* with few modifications.[10] A standard well diffusion process was used to assess the antimicrobic sensitivity of the *Escherichia coli* and *Staphylococcus aureus* isolates including *Saccharomyces cerevisiae* yeast. All of the materials and apparatus used in these studies were autoclave and sterilized for 20 min at 121°C. In 180mm diameter petri plates with a diameter of 4mm, sterilized Muller Hinton agar (Hi-Media, Mumbai) and yeast extract peptone dextrose for yeast were added, and the plates were left to set. The isolates of *S. aureus* and *E. coli* were grown in 20 mL of Luria Broth and kept at 37°C overnight prior to use. After pouring 2 µL of bacteria on Mueller Hinton Agar (MHA) plates and yeast suspension on YEPD petri plates respectively. A sterile glass rod spreader was rotated several times to ensure the consistent spreading of the inoculant on top of the side of the petri plate. A 6 mm-diameter well was created on each plate using a cork borer. The different concentrations of 20, 40, and 60 µg/mL ZnO/Ag nanocomposites were placed in each well and incubation for 18 h for bacterial culture at 37°C and 48 h for yeast culture.[11] For the bacterial culture, the antibiotic ampicillin served as a positive control.[12] Scales were used to measure the clear zone of inhibition surrounding the wells to determine the sensitivity. The studies were repeated twice to calculate the zone of inhibition and drop handling errors.

Minimum inhibitory concentration (MIC)

MIC of fabricated nanocomposites were evaluated by the broth dilution technique in 96 well plates. In a nutshell, Luria broth (LB) was made with consecutive two-fold concentrations of the manufactured ZnO/Ag nanocomposites ranging from 200.0 to.75 µg/mL. A suspension of young colonies of the tested bacteria was used to create the inoculum, and the turbidness was altered to meet the 0.5 McFarland standard, resulting in a bacterial concentration of 1.5×108 CFU/mL. Each tube had 1 mL of LB supplemented with varying quantities of nanocomposites, and 100 mL of the diluted solution was added to each tube. This suspension was diluted to 106 CFU/mL in LB medium. In a shaker incubator, at 37°C, each test tube was incubated overnight. The term "MIC" refers to the lowest amount at which no bacterial growth was seen.[13]

RESULTS AND DISCUSSION

An in-depth study of fabrication of ZnO/Ag nanocomposites through Co-precipitation technique using *Syngonium podophyllum* as reductant and stabilizing agent.

Structural analyses (XRD)

On the synthesized nanocomposites, an XRD examination was done to determine their phase characteristics. The ZnO/Ag NCs XRD spectrum is shown in Fig.1(b) 31.76°, 34.44°, 36.24°, 47.56°, 56.66°, 62.90°, 66.42°, 68.04°, 72.64°, and 76.98° equivalent to the crystal lattice of (100), (002), (101), (102), (110), (103), (200), (112) has been listed to the structure of wurtzite ZnO(*) (JCPDS No. 89-1397).[14] Besides, doping with Ag salt results in four additional peaks in the ZnO/Ag nanocomposite, characteristics peaks at 38°, 44.3°, 64.3° and 77.74° Ag (#) were listed to cubic form in (JCPDS No. 89-3722)[15] for ZnO/Ag NCs relating to the crystal planes of (111), (200), (220) and (311) respectively as shown in Fig. 1(b). With no peaks connected to other phases or contaminants, the XRD patterns show the samples excellent purity. ZnO/Ag NCs were found to consist solely of ZnO and Ag phases. These pure peaks prove the synthesis of ZnO decorated Ag nanocomposites. The average size of ZnO/Ag NCs was determined by applying equation Debye–Scherrer[16]

$$n = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

n denotes to Average Crystallite size
K = with a range of 0.9 to 1(Scherrer Constant),
λ is denoted as 1.5418 Å X-ray wavelength
β represents Full width half maxima
θ = Bragg angle

It is estimated that ZnO/Ag composites have an average size of 24.39nm using the Scherrer equation.

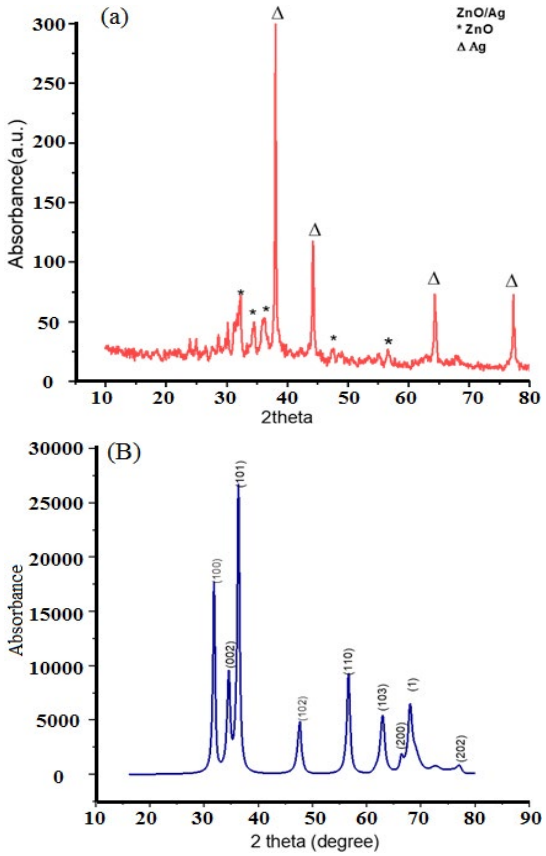

Fig. 1. XRD analysis of fabricated (a) ZnO/Ag Nanocomposites; (b) XRD pattern of synthesized ZnONPs

Vibrational analysis (FTIR)

From Fig. 2 it was clear that FTIR vibrational analysis was used to examine the organic composition of the fabricated ZnO/Ag nanocomposites by identifying functional groups. A C=O appeared at peak 1392 cm^{-1},[9] and C=O stretch vibrations of carboxylic acids contributed to a peak at 1739 cm^{-1}.[17] The strong peak at 2112 cm^{-1} can be ascribed as C≡O.[18] The vibration of the NED functional groups was associated with a band that was identified at 2384 cm^{-1}.[19] A band peak at 2656 cm^{-1} is associated to carboxylic acid stretch in the O-H direction.[20] whereas, peak around 1000 cm^{-1} is accredited to the presence of metal oxide.[21] According to the ZnO/Ag FT-IR spectra the peaks between 3200 and 3600 cm^{-1}, corresponds to the stretching vibration of the inter-molecular bond (O-H) that exists in between zinc oxide oxygen and the adsorbed water molecule.[22] The fabrication of ZnO results in a prominent band at 650 cm^{-1}, It is strongly consistent with the available research.

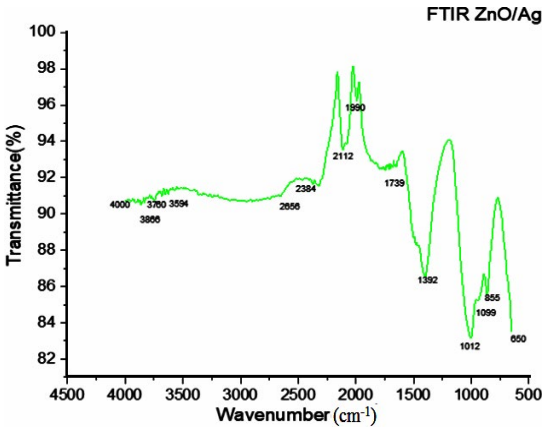

Fig. 2. FTIR analysis of fabricated ZnO/Ag

Analysis of morphology, size, and pattern (SEM, TEM, SAED pattern)

SEM morphology of fabricated AgNPs, ZnO and ZnO/Ag NCs in Fig. (3a,b,c) respectively, showed that majority of nanoparticles were spherical shape. Whereas fabricated ZnONPs are pure sphere-shaped. Another interesting part of the fabrication is knowing how the size, shape, surface, and aggregation state of the Ag and ZnO nanoparticles change after blending. According to the SEM analysis, the AgNPs are spherical, but ZnO shows the structure is purely spherical. The ZnO/Ag nanocomposite shows a transitional sphere-like structure. The synthesized ZnONPs in Fig. 3(b) were completely spherical, whereas the synthesized AgNPs in Fig. 3(a) were almost spherical and diameter of AgNPs ranges from 4-58nm.

To define possible role of supernatant biomolecules in the fabrication, reducing, and stabilizing AgNPs, ZnO, and ZnO/Ag TEM analysis was performed. The TEM analysis in Fig. 4(a) revealed that fabricated ZnO/Ag nanocomposites are transitional sphere shaped with an average size of the fabricated nanocomposite were 16.64nm depicted in Fig. 4(c). As ZnO-NPs and Ag NPS both are synthesized in an aqueous medium, they have a high surface energy. It may be possible because of combining nanoparticles as composites which densified the fine gaps between them.[23]

The SAED pattern in Fig. 4(b) shows that the particles have a substantially crystalline appearance and also display a configuration of rings including spots, which suggests that nanocomposites have a higher grain dimension, regular morphology, and polycrystalline in structure.[23]

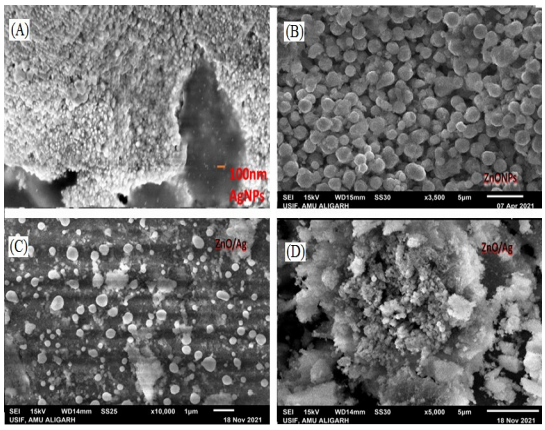

Fig. 3. SEM analysis (a) AgNPs; (b) ZnO; (c) and (d) ZnO/Ag synthesized from Syngonium podophyllum

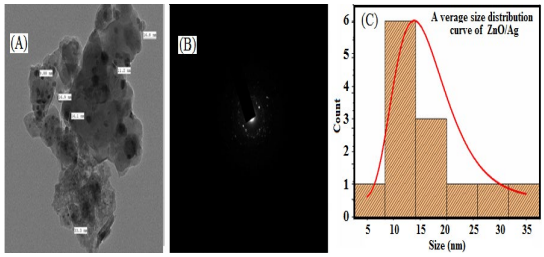

Fig. 4(a). TEM analysis of ZnO/Ag; (b) SAED pattern of ZnO/Ag; (c) Average size distribution curve

Applications

Antibacterial Analysis

Antimicrobial potential was evaluated by using the well diffusion technique for *E. coli,* and *S. aureus* onto MHA plates, as well as YEPD media for saccharomyces cerevisiae. Each inhibition zone for nanocomposites and commercially available antibiotics has been visualized in terms of its antimicrobial activity. Ampicillin was used as a positive control against bacterial species because of its high antibacterial performance. According to this study, the quantity of ZnO/Ag NCs affects the antimicrobial potential of *E. coli*, *S. aureus*, and *S. cerevisiae*. The antibacterial action that results from the ZnO/Ag NCs distribution on the agar surface limits microbial growth in the space that is already occupied by them.[24] The MIC of the biosynthesized ZnO/Ag NCs against *Staphylococcus aureus* and *Escherichia coli* was assessed at various concentrations. The MIC study was done by broth dilution technique.[13] The lowest inhibitory concentrations of nanocomposites, which ranged from 12.50 to 50 µg/mL and was liable for preventing the growth of isolated strains, was determined (MIC). After 5 h of treatment, fabricated ZnO/Ag nanocomposites exhibited approximately 75% toxicity against tested isolates. As a result of this antibacterial study, fabricated ZnO/Ag nanocomposites showed potential effectiveness against isolates as an antimicrobial. Silver and ZnO nanoparticles were already widely recognized to have antibacterial properties.

Anti-yeast analysis

The Zone of Inhibition proved *S. cerevisiae* sensitivity to ZnO/Ag NCs (Table 1). Thus, the antimicrobic potential of ZnO/Ag changes with the concentration of NCs. As the concentration of NCs increases the zone around the well subsequently increases. The result revealed that the anti-yeast activity is dose-dependent (NCs).

Table 1: Antimicrobial potentials of ZnO/Ag NCs

Micro organism	various concentration of ZnO/Ag NCs for ZOI mm			
	+ve control/Antibiotic	20µgm/L	40µgm/L	60µgm/L
E. coli	23	10±0.3	12±0.6	13±0.2
S. aureus	0	8±0.8	10±0.5	11±0.5
S. cerevisiae	-	12±0.1	12±0.5	13±0.7

CONCLUSION

The current study focused on the fabrication of ZnO/Ag nanocomposites by co-precipitation with Syngonium podophyllum aqueous leaves extract, and their antimicrobic sensitivity against *E. coli, S. aureus*, and yeast *S. cerevisiae*. ZnO/Ag NCs synthesized showed smaller sizes based on XRD, SEM, and TEM analyses. The SAED pattern showed the polycrystallinity of the nanocomposites. FTIR analysis suggested that the functional group, for example flavonoids, terpenoids, etc. exhibit in leaf extract are liable for the reducing and stabilization of the nanocomposites.A possible antiseptic resultant can be obtained from the green fabricated ZnO/Ag nanocomposites due to their promising characteristics and their favorable antimicrobial efficiency against tested pathogens.

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