Synthesis and Characterization of Ag_2O and MnO doped Ag_2O nanocomposites for Antibacterial activity against E.Coli.

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Abstract: Synthesizing the doped material for industrial or clinical surfaces to avoid microbes, particularly antibiotic-resistant strains, which has emerged in growing the interest in development of effective antibacterial agents. In this work, it is elucidated the antibacterial activity of MnO-doped Ag_2O nanoparticles. Synthesis was followed by the coprecipitation method is adopted to synthesize the nanoparticle and the characterization is done to analyse the crystallinity, size, morphology and elemental composition by different methods. The solution is made for Ag2O and MnO-doped Ag_2O nanoparticles of two different concentration-to-volume ratios of 5% and 10%, and the antimicrobial activities is studied for *E. Coli* bacteria. MnO doped Ag_2O nanoparticles exhibits a better antibacterial activity.

1. Introduction:

A class of materials known as nanoparticles (NPs), has diameters ranging from 1 to 100nm. Since it has high surface-to-volume ratios, nanoparticles are used in catalysts in many industries [1]. Nanoparticles exhibit distinctive and intriguing physicochemical and biological properties [2]. Nanoparticles draw more attention due to their small size, mass-to-charge ratio, volume-to-surface ratio, optical absorption, and electrical conductivity that changes the chemical, physical, and biological properties of the material derived from the bulk materials[3]. Nanoparticles can be synthesized in various ways, and the specific synthesis methods by varying concentrations can lead to different types of structures and morphologies for the required research of interest[4]. Extensive research has been done to investigate the potential uses of nanoparticles in electronic devices, cancer therapies, biosensors, and antimicrobial applications [5], [6]. Apart from medical applications, Metallic nanoparticles have increased the market value for its other applications like cloths, photography, mirror, optics, electronics, food preservatives, and packing[7], [8].

Nanoparticles play an essential role in the field of antimicrobial applications due to their unique properties, which include high surface area, tuneable size and shape, which has the potential to interact with microorganisms at the nanoscale [9]. In some cases, nanoparticles are coated on to the surfaces of medical equipment, which releases antimicrobial ions or exhibits direct contact-killing properties [10]. In some nanoparticles, certain metals and metal oxide nanoparticles are incorporated into polymers and other materials to create antimicrobial nanocomposites [11]. These materials are used in medical devices, wound dressings, and food packing to prevent bacterial contamination [12]. The metal-nanoparticles, which include gold, silver, and metal oxides like titanium dioxides, iron oxide, organic nanoparticles like dendrimers, micelles, liposomes, and polymer nanoparticles, are generally focused on targeted drug delivery systems, etc., [13]–[15]

Manganese is the most plentiful element on earth as the twelfth most frequent element on the globe and the third most common transition element after iron and titanium [16]. For fish and other terrestrial animals to reproduce, stay healthy and thrive, manganese is an essential micronutrient[17]. Also, it is also used in water treatment and purification due it's chemical and physical properties[18], [19]. Due to their extensive structural and compositional diversity, Mn-oxides have attracted particular attention among the various 3d transition metal-oxides such as MnO, MnO_2, Mn_2O_3 , and Mn_3O_4 and these Mn-oxide NPs give sustainable nanotechnology in all fields. Mn-oxides can be used in drug deliver, optical electronics, magnetic materials, optical electronics, ion-sieves, and Batteries[20], [21]. Since, Toxicity of Mn-oxide is much lesser when compared to other metals, Mn-Oxides are widely used in medical field and food industry[22].

Silver nanoparticles are generally excellent at fighting a variety of microbes, including bacteria, fungus, and viruses[23]. Additionally, metallic silver is a powerful antibacterial agent with a high potential for antimicrobial activity that has recently been examined[24], [25]. But as antibiotics became more widely used, use of silver in medicine steadily declined[26]. The effectiveness of silver antibacterial properties has increased further with creation of nanomaterials with sizes in the nano range. High bactericidal action was demonstrated by silver nanoparticles against both gram-positive and gram-negative bacteria[27]. Silver nanoparticles are powerful against the antimicrobial agents against MDR bacteria like *methicillin resistant Streptococcus Pyogens*, *Ampicillin resistant Escherichia coli*, *Vancomycin resistant Staphylococcus aureus*.[28] Nanoparticles have capable of penetrating bacteria cells and acts as a catalyst, to inactive enzymes that microorganisms need for their metabolism by interacting with thiol groups[25]

2. Synthesis of $MnSO_4$ doped Ag

10ml of 0.01M $MnSO_4$ and was 5ml of 0.01M $MnSO_4$ mixed with 45mL of 0.01M $AgNO_3$ and sonicated separately for 30mins. Further, to the reaction mixture, 0.1 M NH_4OH was added dropwise with continuous stirring until the pH of the solution reached 8, The mixture was allowed to still overnight at 1200 rpm. The resultant precipitate was centrifuged and washed with water. The obtained precipitate was dried in an oven at 180 ^oC.

Antibacterial activity

The synthesized Ag_2O and MnO doped Ag2O nanoparticles were evaluated for antibacterial activity by the disk diffusion method. Antibacterial activity performed by nutrient agar medium, the antimicrobial activity assay was evaluated against gram-negative bacteria *E. coli*. Microbial cultures were subcultured on nutrient agar and uniformly swabbed on individual plates. Fresh bacterial culture at concentration 1.5 × 108 CFU/mL was gently spread on the agar Petri dishes evenly using a sterile swab. After that, the wells of 6 mm diameter were punctured., Fifty microliters of synthesised nanoparticles were added into wells and the plates were incubated at 37 °C for 24 h. After 24 h of incubation, the zone of inhibition was recorded by measuring in millimeters (mm). Gentamicin 0.1 ug/mL was used as the standard antibiotic. The antibacterial activity was studied using the diameter of the zone of inhibition with deionized water as the negative control, gentamicin as the positive control for *E.coli.*

Characterisation of Nanoparticles:

Powder X-ray diffraction (XRD) on a Bruker X-ray diffractometer was used to analyse the synthesized nanoparticles' structural analysis and phase purity. Analysis at room temperature with a 0.02° step size and two values ranging from 10° to 80° using CuK radiation with a wavelength of 1.541874. The surface charge, polydispersity index (PDI), and hydrodynamic diameter of the produced nanoparticles were determined using the dynamic light scattering (DLS) approach. The morphology and distribution of the synthesised materials were investigated using scanning electron microscopy (SEM: EVO LS 15 model, Carl Zeiss, Germany) and energy dispersive X-ray (EDX analyser from HITACHI Noran system 7, USA).

A. X-ray diffraction (XRD).

Figure 1informates on the XRD peaks and d_{hkl} values plotted with origin pro using the data received. XRD characterized the crystal structure of the MnO-doped Ag_2ONP. The XRD peak of the synthesized MnO-doped Ag_2ONp was compared with the Ag_2O JCPDS file No. 04-0783. Other peaks at 21.5508^o, 29.5394^o and 32.6610^o were found other than Ag_2O peaks. The same, when compared with the JCPDS 04-0836 pertaining to MnO np's showed similar peaks thereby explaining the presence of Manganese in silver oxide lattice. The size of the synthesized nanoparticles was evaluated using Debye–Scherrer equation, and the average crystallite size was 37.38nm. The data about average crystallite size, d_{hkl}, and respective hkl values are tabulated in Table 1.

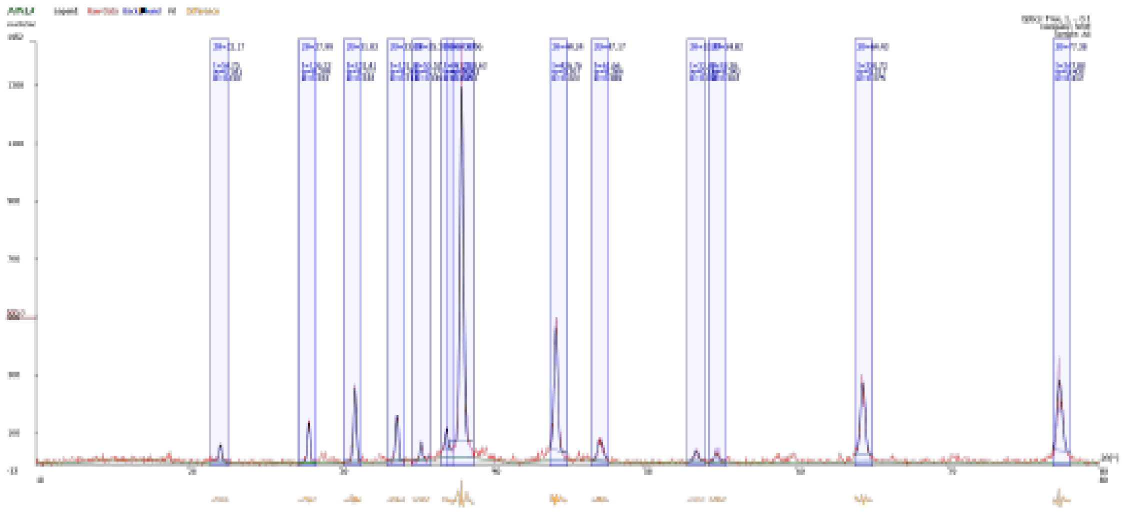


Fig 1: XRD spectra of synthesized Ag_2O Nanoparticles.

***T*able 1:** XRD details of synthesized Ag2O/ MnO Nanoparticles.

2θ (°)	θ (°)	FWHM	D (nm)	h,k,l
21.5508	10.78	0.2701	31.27	1,0,0
29.5394	14.77	0.2188	39.22	1,1,0
32.661	16.33	0.1691	51.13	0,0,2
38.0353	19.02	0.235	37.35	1,0,1
44.2215	22.11	0.2651	33.78	2,0,0
64.4004	32.20	0.311	31.53	2,2,0
77.3745	38.69	0.3936	27.01	3,1,1
Average Crystallite size =			**37.38 nm**	

B. Morphological analysis

The surface morphology of the produced samples was examined using SEM. SEM has shown that Ag_2O (Fig 2a) grain size changes with MnO -doped Ag_2O (Fig 2b). Additionally, differences in grain size, shape, and orientation with cross-network of nanorods can be seen in SEM micrographs of MnO -Ag_2O NPs samples.

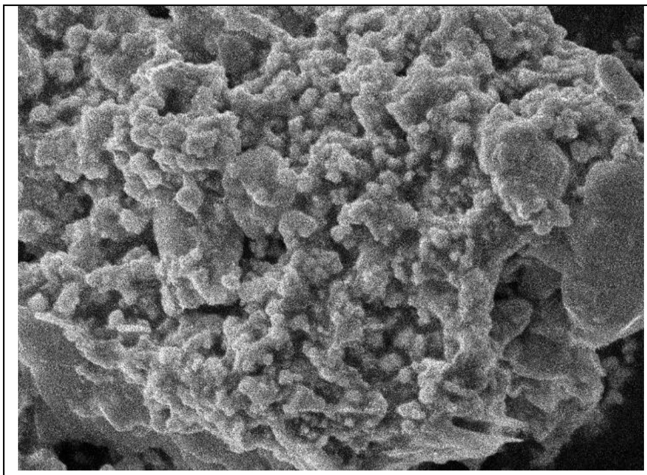
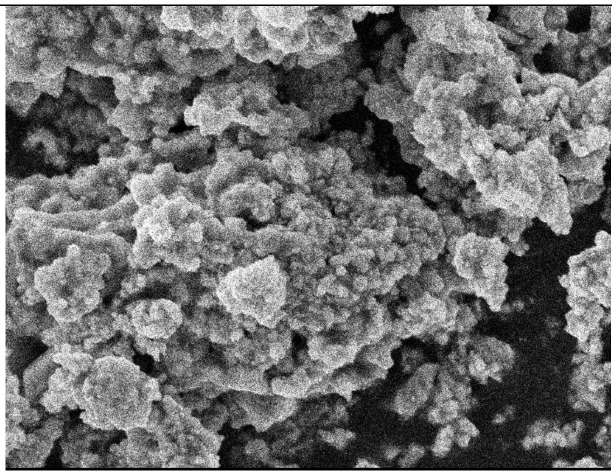
	

Fig 2a. SEM of Ag_2O nanoparticles.	**Fig 2b.** SEM of MnO-doped Ag_2O nanoparticles.

C. Morphological analysis

Energy-dispersive X-ray spectroscopy (EDS) images of the synthesized samples are given in Figure 3. The result exhibits the formation of Ag_2O nanoparticles, a strong peak near 3.0 eV, which is doped with MnO, a weak peak near 1.0 eV. The inset of Figure 3 shows the presence of % weight of Ag, Mn, and O.

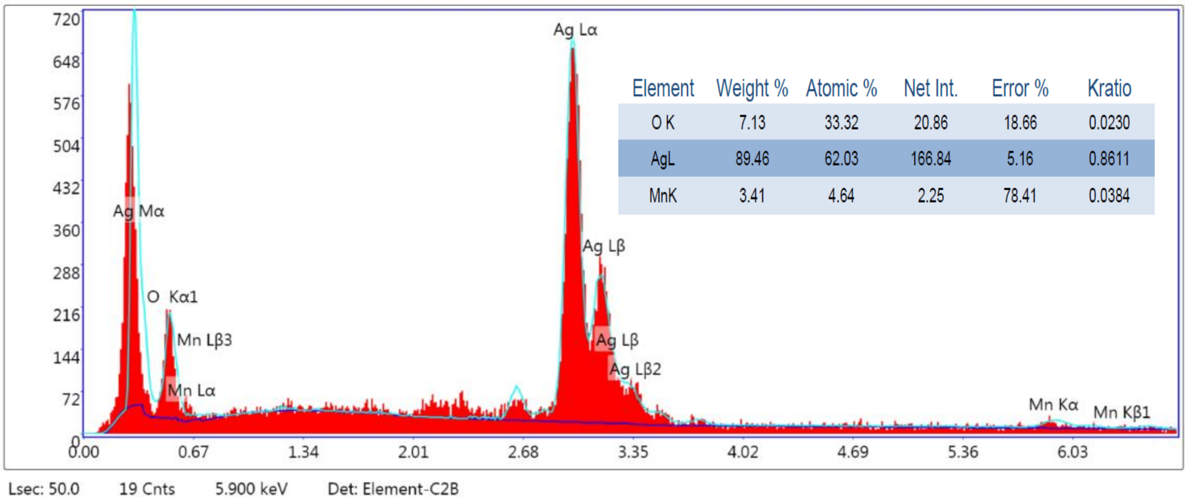


Fig 3: Energy-dispersive X-ray spectroscopy (EDS) of MnO doped Ag_2O nanoparticles.

Antibacterial activity

The synthesized Ag_2O and MnO-doped Ag_2O nanoparticles were evaluated for inhibition of antibacterial activity by the disk diffusion method. Antibacterial activity was performed by nutrient agar medium, the antimicrobial activity assay was evaluated against gram-negative bacteria *E. fecalis* and E. Coli. Microbial cultures were subcultured on nutrient agar and uniformly swabbed on individual plates. Nanoparticles of were impregnated on 6mm filter paper discs, dried and placed on the culture plate and incubated at 37°C for 24h. The antibacterial activity was studied using the diameter of the zone of inhibition with deionized water as the negative control and gentamicin as the positive control for E.Coli

Fig 4: Antibacterial activity against *E. Coli* bacterial strain

The effect of antibacterial activity by Ag_2O NPs and MnO- Ag_2O NPs against E. fecalis and E.Coli bacterial strain is shown in Figure 4, and the ZOI is tabulated in Table 2. For both bacterial strains, the MnO-Ag_2O np's have provided better resistance when compared to Ag_2O np's. The activity was compared with 50 µl of 0.1mg/ml of standard and nanocomposite solution with 100 µl and 50 µl of 0.1mg/ml nanoparticles.

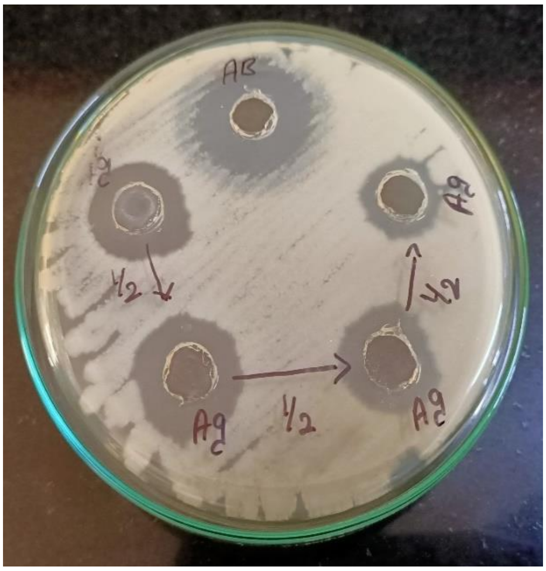
Table 2: Effect of Ag2O NPs and Ag2O/MnO nanocomposites on antibacterial activity**.**

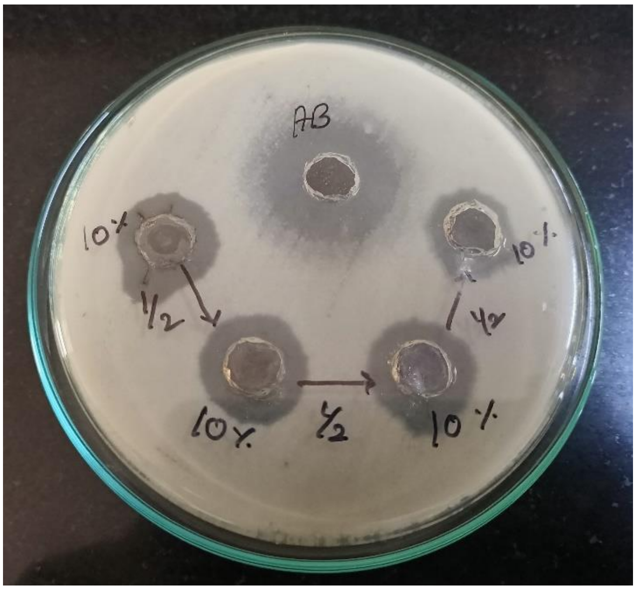
Compund (0.1ug/ml)	***E. Coli***
Gentamycin	26 ± 0.5
Ag	18 ± 0.5
AgO 5% MnO	20 ± 0.5
AgO 10% MnO	18 ± 0.5

Conc in micromolar	1 uM	0.5 UM	0.25uM	0.125 uM
Ag	24 ± 0.5	22 ± 0.5	16 ± 0.5	12 ± 0.5
AgO 5% MnO	26 ± 0.5	24 ± 0.5	16 ± 0.5	12 ± 0.5
AgO 10% MnO	26 ± 0.5	24 ± 0.5	20 ± 0.5	14 ± 0.5
MIC in mm				

3. Conclusion:

In this present work, the co-precipitation method is adopted for the synthesis of Mn-doped Ag nanoparticles of two different concentration-to-volume ratios of 5% and 10%, and the antimicrobial activities is studied for *E. Coli* bacteria. And it is found that the antibacterial activity of the synthesized nanoparticles has proved the inhibition activity against the potent bacteria *E. Coli.* Thus, the present research gave a positive output to serve this in various applications in the field of medicinal and biological applications.





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