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Antimicrobial activity of the novel metal oxide nanoparticles against selected human pathogenic bacteria

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Abstract : Antibacterial activities of the selected metal oxide nanoparticles viz., lead oxide (PbO), silver oxide (Ag₂O), tin oxide (SnO₂), alpha manganese sesquioxide (α -Mn₂O₃), cerium oxide (CeO₂), silver doped tin oxide (Ag-SnO₂), silver doped alpha manganese sesquioxide (Ag- α -Mn₂O₃), silver doped lead oxide (Ag-PbO) and silver doped cerium oxide (Ag-CeO₂) have been successfully investigated against human pathogens, viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The antibacterial studies of the selected nanoparticles are primarily investigated by the well diffusion process. Different concentrations of the nanoparticles are analyzed by the minimal inhibitory concentration (MIC) technique. Among the metal oxide nanoparticles, the Ag-doped CeO₂ nanoparticles showed maximum inhibition against *E.coli*, followed by against *P. aeruginosa*, *P. mirabilis* and *K. pneumoniae*. Further, the MIC studies showed that, the Ag-CeO₂ nanoparticles exhibited maximum inhibition against human pathogenic bacteria.

Keywords: Metal oxide; Nanoparticles; Agar well diffusion method; MIC technique

1. Introduction

In general run of things, human beings and animals are consistently infected by microorganisms available in their living environments. As a result of the existence and developed in the various number of antibiotic-resistant microorganisms, many research groups have been investigated the different process to create the powerful antimicrobial factors to get control of the resistances of these microorganisms [1]. In particular, the metal oxide nanoparticles have attracted a lot of interest in pharmaceutical fields [2]. Nowadays, nanocrystalline metal based particles have existed as new antimicrobial factors with special biological, chemical and physical characteristics [3]. Importances of the nanocrystalline metal oxide particles are low toxic, able to resist and remain unaffected by heat and suitability for pharmaceutical applications [4-5]. In the earlier studies showed that the nanocrystalline silver and gold particles have different antibacterial properties [6]. Moreover, the titanium oxide, cadmium oxide, iron oxide and zinc oxide nanomaterials have successfully investigated for the antimicrobial activity [7-9]. For the most part, the gram negative *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* are largely utilized in bacterial studies, due to these pathogens reside on the body surface of mammals and also create infection to them. Thus, the gram negative strains are selected for this antibacterial studies.

In this current work, metal oxide nanoparticles such as PbO, Ag₂O, SnO₂, α -Mn₂O₃, CeO₂, Ag-SnO₂, Ag- α -Mn₂O₃, Ag-PbO and Ag-CeO₂ are studied for their antibacterial activities against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*.



2. Materials and Methods

2.1 Agar Well Diffusion Method

The antibacterial activity of the selected metal oxide nanomaterials was carried out by the well diffusion process. The nanocrystalline metal oxide samples stock solution (1mg/ml), and dilutions of the stock solution containing 0.125, 0.250, 0.5 and 1.0 mg/ml were prepared in dimethyl sulfoxide (DMSO). The prepared inoculums were adjusted to the McFarland standard 0.5 Scale and the Lawn culture was made on the Mueller Hinton Agar (MHA) plates. Triplicates plate was swabbed with the overnight culture of pathogenic bacteria viz., *E. coli*, *K. pneumoniae*; *P. aeruginosa* and *P. mirabilis*. The prepared extracts were added in the respective wells on the swabbed plates. Chloramphenicol and Gentamycin were used as the standard reference drugs (30 µg). The plates were incubated at 37°C for 24 hours. After the incubation, the zone of inhibition was measured in millimeters and compared with the standard antibiotic discs.

2.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was measured by the micro-dilution process using the Mueller Hinton (MH) broth. The nanoparticles stock solution (1mg/mL) was dissolved in DMSO, and the dilutions of the stock solution containing 800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml were prepared in the MH broth. 100µl of each dilution was added in the corresponding wells and 2µl of broth culture was added in each well. The microtiter plates were incubated at 37°C for 24 hrs. The properties of selected nine metal oxide nanoparticles are listed in Table 1.

Table 1 Properties of metal oxide nanoparticles

Metal oxide nanomaterials	Colour of the samples	Form	Particle size (nm) (calculated from TEM images)
PbO	Orange	Powder	52.4
Ag ₂ O	Ash	Powder	32.5
SnO ₂	Pale yellow	Powder	11.6
α-Mn ₂ O ₃	Black	Powder	30.2
CeO ₂	Yellow	Powder	12.4
Ag-SnO ₂	Yellowish black	Powder	10.1
Ag-α-Mn ₂ O ₃	Brownish black	Powder	28.5
Ag-PbO	Brownish orange	Powder	50.7
Ag-CeO ₂	Yellowish grey	Powder	10.9

3. Results and Discussion

Of the selected nanocrystalline metal oxide particles, the Ag-doped CeO₂ nanoparticles exhibited high sensitivity at a concentration of 1mg/ml against *E.coli* (16mm), *K. pneumoniae* (13mm), *P. aeruginosa* (14mm) and *P. mirabilis* (14mm), respectively as shown in Fig. 1(a-d). This is due to the particle size, morphology and crystalline structure of the synthesized Ag-doped CeO₂ samples. The expected formation mechanism for the cell lyses is that, the nanocrystalline Ag-doped CeO₂ release ions which react with the thiol groups of the protein available in the cell wall inactivate the protein and reduce the cell permeability, which extends to cellular death of the pathogens [10].

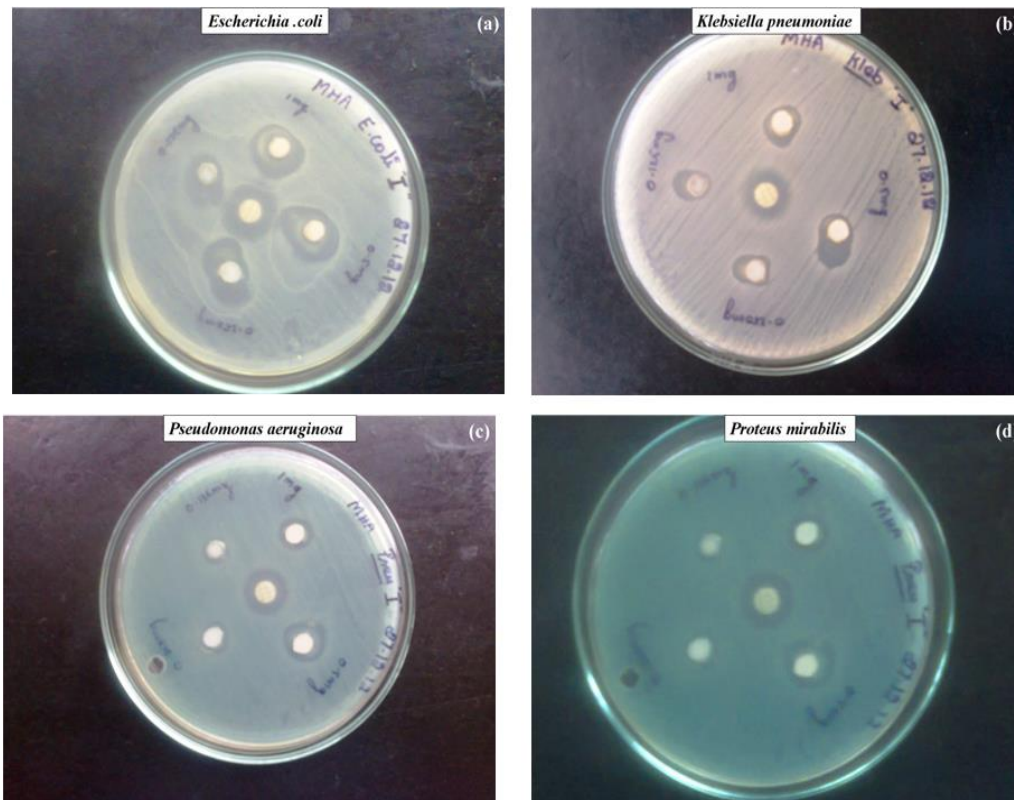


Figure 1 Zone of inhibition of Ag-doped CeO₂ nanoparticles against (a) *E. coli*, (b) *K. pneumoniae*, (c) *P. aeruginosa* and (d) *P. mirabilis* by Agar well diffusion method

Table 2 Zone of inhibition of Ag-doped CeO₂ and Ag₂O nanoparticles against human pathogenic bacteria

Organisms	Concentrations of Ag–CeO ₂ nanoparticles(mg)				Concentrations of Ag ₂ O nanoparticles (mg)			
	0.125	0.25	0.5	1	0.125	0.25	0.5	1
<i>E.coli</i>	10mm	13mm	15mm	16mm	–	–	–	–
<i>K. pneumoniae</i>	9mm	10mm	12mm	13mm	–	–	–	–
<i>P. aeruginosa</i>	8mm	10mm	13mm	14mm	9mm	11mm	12mm	15mm
<i>P. mirabilis</i>	9mm	10mm	12mm	14mm	–	–	–	–

Table 3 Zone of inhibition of Ag-doped SnO₂ and Ag-doped α-Mn₂O₃ nanoparticles against human pathogenic bacteria

Organisms	Concentrations of Ag–SnO ₂ nanoparticles (mg)				Concentrations of Ag–α-Mn ₂ O ₃ nanoparticles (mg)			
	0.125	0.25	0.5	1	0.125	0.25	0.5	1
<i>E.coli</i>	–	–	–	–	–	–	–	11mm
<i>K. pneumoniae</i>	–	–	–	–	–	–	–	–
<i>P. aeruginosa</i>	–	–	–	10mm	–	–	–	–
<i>P. mirabilis</i>	–	–	–	–	–	–	–	–

The zone of inhibition of the chosen nanomaterials against pathogens is displayed in Tables (2–3). Further, it reveals that the Ag₂O (15mm) and Ag-doped SnO₂ (10mm) demonstrated high sensitivity for *Pseudomonas aeruginosa*, whereas the Ag-doped α-Mn₂O₃ nanoparticles exhibited high sensitivity (11mm) for *E-coli*.

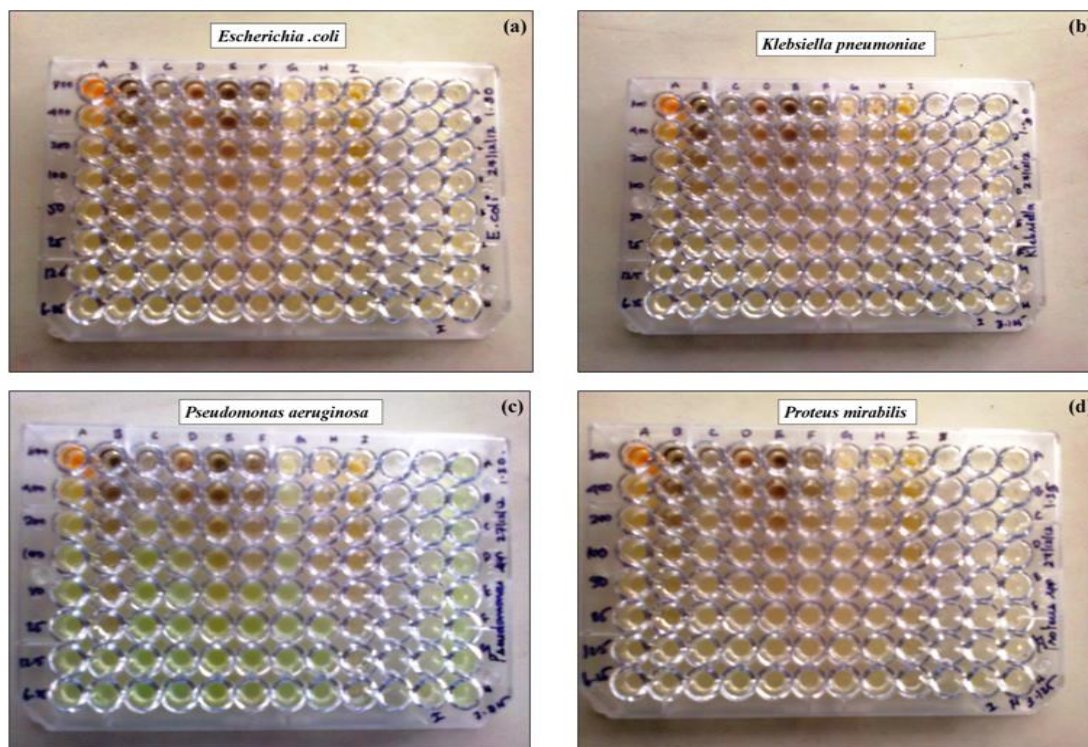


Figure 2 (a-d) MIC results of (A) PbO, (B) Ag₂O (C) SnO₂ (D) α-Mn₂O₃ (E) CeO₂ (F) Ag-SnO₂ (G) Ag-α-Mn₂O₃ (H) Ag-PbO and (I) Ag-CeO₂ nanoparticles

Fig. 2(a-d) shows the MIC results of the selected metal oxide nanoparticles. The MIC revealed that the Ag-CeO₂ samples have high sensitivity at the concentration of 6.25 μg/ml. Moreover, MIC results histogram of the nanomaterials against pathogens are shown in Fig. 3. This study revealed that the silver ions doped metal oxide nanoparticles exhibited the maximum sensitivity against human bacterial pathogens, since; the Ag ions induced the release of K⁺ ions from pathogens. Hence, it is observed that the bacterial plasma, which is combined with enzymes and DNA, is an essential required site of silver ions [11-12].

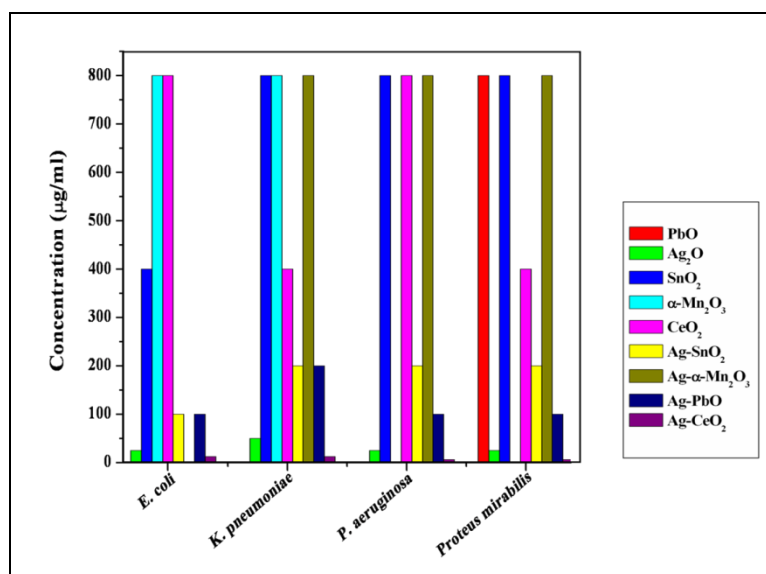


Figure 3 MIC results histogram of chosen metal oxide nanoparticles against human pathogenic bacteria

It is expected that the pathogen DNA can be damaged by reactive oxygen species (ROS). However, CeO nanoparticles are well-known to be antibacterial agents and it is demonstrated that the damage

caused to bacteria is due to the occurrence of superoxide anions on its surface [13]. Silver doped ceria nanoparticles deal with bacteria and generates electronic effects, which increase the reactivity of nanocrystalline compounds. Thus, the bactericidal activity of the Ag-doped CeO₂ is proved to be highly based on size and surface morphology due to the cause of changing interaction with the microorganism [14]. Therefore, this is observed from the current investigations that, the nanocrystalline Ag-doped CeO₂ samples can be used as antibacterial agent for urinary bacterial diseases.

4. Conclusion

In this work, the antibacterial effect of the chosen metal oxide nanoparticles such as PbO, Ag₂O, SnO₂, α-Mn₂O₃, CeO₂, Ag-SnO₂, Ag-α-Mn₂O₃, Ag-PbO and Ag-CeO₂ are observed by the Agar well diffusion and MIC methods. From this study, Ag-doped CeO₂ nanoparticles are found to possess better antibacterial activity than the Ag₂O nanoparticles. Moreover, all the chosen metal oxide nanoparticles showed sensitivity against human pathogenic bacteria, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*. Of the selected nanoparticles, the Ag-doped CeO₂ nanoparticles showed maximum sensitivity against human pathogenic bacteria. The MIC results also showed that, the Ag-CeO₂ exhibited high inhibition against pathogenic bacteria. From the results, it is suggested that the Ag-doped CeO₂ nanoparticles can be used as effective growth inhibitors in various microorganisms.

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