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ANTIBACTERIAL ACTIVITY OF Cd-DOPED ZnO NANOPARTICLES AGAINST GRAM- POSITIVE AND GRAM-NEGATIVE BACTERIA

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Abstract:
The antimicrobial activity of Cd doped ZnO nanoparticles against gram-positiveand gram-negative bacteria are discuss in this paper. The effects of particle size and concentration of Cd doped ZnO nanoparticles for antibacterial activity is studied using bacteriological test such as disc and well diffusion agar methods. Cd doped ZnO nanoparticle for antimicrobial activities is prepared via Sol-gel process. The crystalline structure, morphology and size of nanoparticles were characterized by transmission electron microscopy (TEM), X-ray diffraction spectra (XRD). Echerichia coli (E. coli), salmonella thiphy, pseudomonas fluorescence, proteus sp. Kelbsielia phemonia and Candida albicans were used as test microorganisms.

Key-words: *Nanoparticle, Cd doped ZnO, Gram-positive and Gram-negative, Transmission Electron Microscopy (TEM), X-ray Diffraction Spectra (XRD).*

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INTRODUCTION:

In recent years, ZnO has received increasing attention, owing to its unique optical, electrical, and chemical properties [1]. Among these properties, degradation of pollutants catalyzed by ZnO has been studied widely so far [2–4]. Furthermore, ZnO appears to strongly resist microorganisms [5, 6]. It is the most widely used nanomaterial as UV absorbers in textiles [7], hybrid solar cells [8], varistor fabrication [9], light-emitting diodes (LEDs) [10], wastewater treatment applications [11], and emission control [12]. ZnO-NPs has excellent biomedical properties to be effectively utilized as diagnosis, antimicrobial agent, bio-imaging, drug delivery, and in cancer treatment, etc. [13–15]. Compared to normal cells, ZnO-NPs exhibit a favorable capacity to destroy human cancer cells [16]. Most organic antibacterial agents are sensitive to temperature or pressure [17], while inorganic materials such as metal and metal oxide [18] have received more recognition over the past decade due to superior durability, less toxicity, greater selehgctivity, and heat resistance [19, 20]. Moreover, new approaches are needed for biomedical applications of ZnO-NPs to actively perform in antimicrobial and antibacterial activities [21, 22]. For this to happen, the properties and functionality of ZnO-NPs were needed to improve by incorporating other dopants materials that were some transition metal ions, e.g., Co2+ [23], Mn2+ [24], Ti4+ [25], La3+ [26], and Fe3+ [27], have been doped into ZnO. At present, most research focused on the influence of transition metal ions on the photocatalytic efficiency rather than the antimicrobial activity [28]. Our group has first prepared Cd-doped ZnO nanopowders by a sol-gel method.In this work, we performed the antibacterial study of Cd-doped ZnO nanoparticles on several bacteria of Gram-positive Candida albicans (fungi) and Gram-negative Echerichia coli (E.coli), salmonella thiphy, pseudomonas fluorescence, proteus sp. Kelbsielia phemonia and Candida albicans using a standard microbial method. The Cd-doped ZnO nanoparticles concentration effect on the MIC of various bacteria has been evaluated.

MATERIAL AND METHOD:

All chemicals and solvents were analytical grade and purchased from commercial sources.

Synthesis of Cd doped ZnO nanoparticles:

The Cd doped ZnO nanoparticles prepared by using sol-gel citrate method. The 2% Cadmium nitrates is added in zinc nitrate magnetically stirred with citric acid and ethanol at 80°C for 3 hrs to get homogeneous and transferring solution. The solution was further heated at about 130°C for 12 hrs in pressure vessel to form the gel precursor. The prepared product was subjected to 3hrs heat treatment at 350°C in muffle furnace and then milled to a fine powder. The dried powder then calcinated in range of 350° - 650°C in order to improve the crystalline structure of material.

Screening of antibacterial activity of Cadmium doped ZnO:

For screening of antibacterial activity of Cd-doped ZnO-NPs, all bacterial strains are sub-cultured from their pure cultures in Mueller–Hinton in Muller-Hinton Media solid agar Petri dish. The disc is 15 cm in diameter, sterilized by autoclaving for 15 min at 121 ºC, and was placed on bacterial cultured agar plate which were then incubated for 24 hrs at 37 ºC. The turbidity of bacterial culture is adjusted to freshly prepared 0.5 McFarland turbidity standard [29] equivalents to (1.5×108 CFU/mL) bacteria**.** Inhibition zone was monitored After incubation the presence of bacterial growth around the samples were observed and their diameter in millimeters was measured briefly.

Antibiotic resistance pattern of candida albicans:

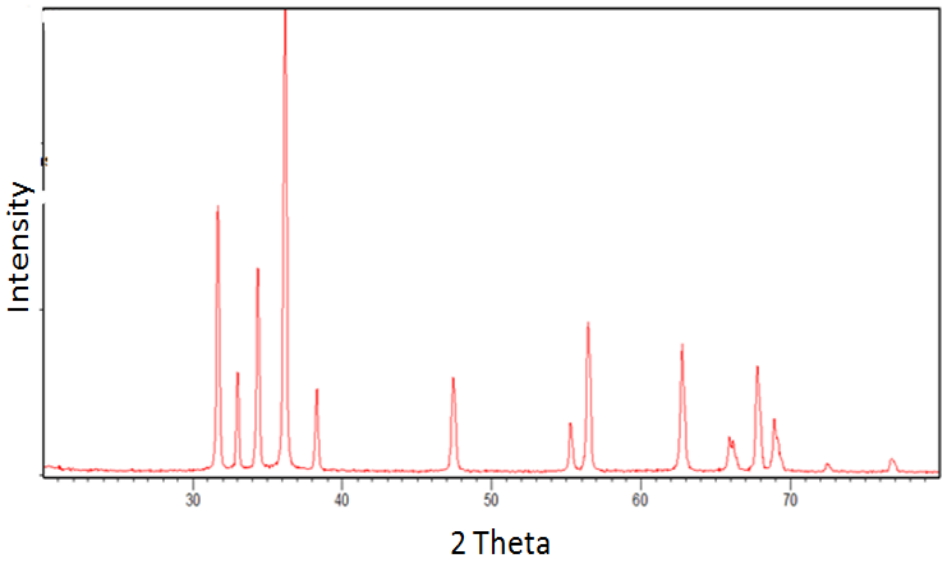
The Muller-Hinton Media used to culture the fungal and Fluconazol, Nystatin, Amphotericin B, Terbinafine HCL antibiotics were used to study for their resistance pattern according toDisk Diffusion Technique.

Anticandidal effect of the cd doped ZnO Nps in solid Media:

The fungal were cultured in Muller-Hinton Medium and Antibiogram disks of 0.01, 0.5, 1.0 and 1.5 % of cd doped ZnONps were prepared according to disk diffusion Technique. The disks were placed over the media and incubated at 37°C for 24 hr.

RESULT AND DISCUSSION:

X-Ray Diffraction: X-Ray diffraction pattern shows in Fig 1. (X-pert, PRO XRD System, Punjab) reveals crystalline nature of sample. The average crystalline size was 41nm obtained from FWHM of peak corresponding to 2Θ calculated by Debye –Scherer formula which is given by, $L = k \lambda / \beta \cos\Theta$ Where, L is the average size of crystal, K= 0.9 particle diameter, the λ (0.154 A°) is wavelength of X-Ray, β is full width at half maximum (FWHM) of the diffraction peak and 2Θ is the diffraction angle of diffraction.



Transmission Electron Microscope analysis: Transmission Electron Microscope (TEM), analysis was done using **Philips (technai 10)**. Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid were allowed to dry by putting it under incubator. In this technique, whereby a beam of electronics is transmitted through an Ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of and focused the electrons transmitted through the specimen. The image is magnified on to an imaging device. Figure-2 shows TEM images of Cd doped ZnO nanoparticles. .TEM observations revealed the formation of hexagonal shape cd doped zno nanoparticles of average particle size is 41.4, 59.0, 61 nm respectively. The results of TEM were supported by X-ray diffraction study.

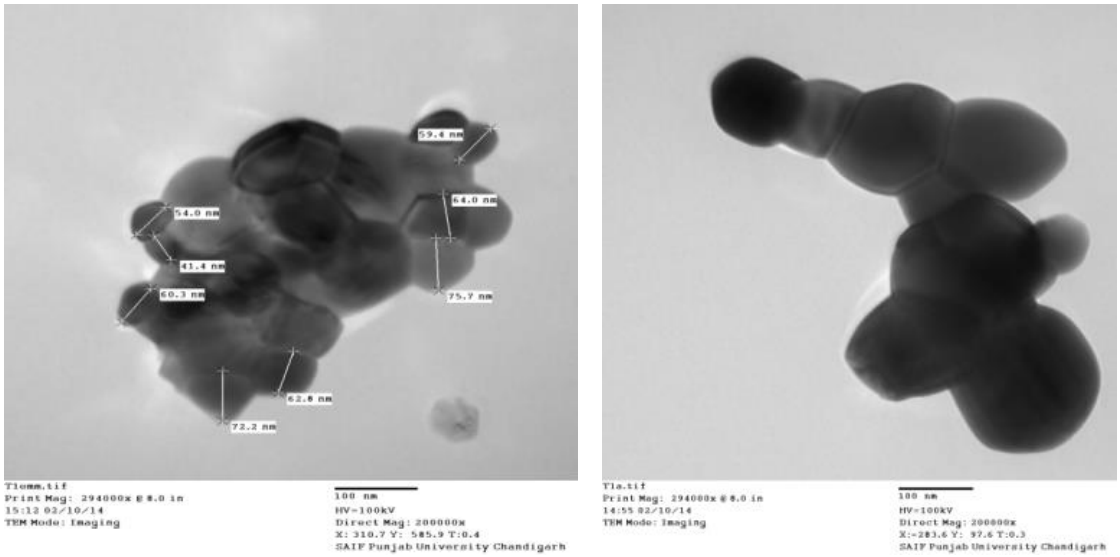
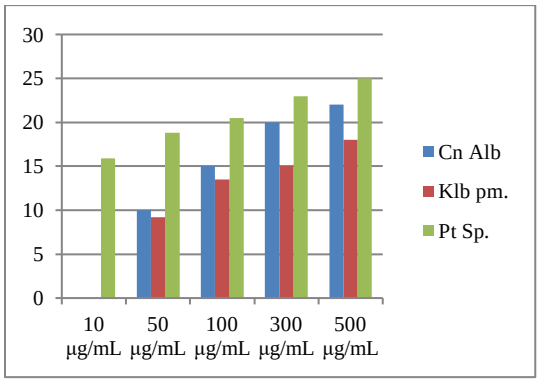


Fig 2: TEM pattern for Cd doped ZnO Calcinated at 650^0

Antifungal properties:

The antifungal activity of Cd doped ZnO *nanoparticles was* tested by the disc and well diffusion agar methods. The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles. It has been seen in this study that by increasing the concentration of Cd doped ZnO nanoparticles in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of Gram-positive and Gram-negative bacteria. The size and the concentrations of Cd doped ZnO nanoparticle was resistant to all of the antibiotics used in this study. Inhibition zone measurements show that by increasing the concentration of Cd doped ZnO the inhibition zone also increased. (6.9, 13.5, 18, 20.1, 22mm) respectively (Fig: 3). It has been known that Nanomaterials exhibit strong inhibiting effects towards a broadened spectrum of gram positive and gram-negative bacterial strain. According to several studies, it's believed that the metal oxides carry the positive charge while the microorganisms carry negative charges, this causes electromagnetic attraction between microorganisms and the metal oxides which lead to oxidization and finally death of microorganism's nanomaterials also could deactivate the cellular enzymes and DNA by coordinating to electron-donating groups. They cause pits in bacterial cell walls, leading to increased permeability and cell death. Finally, we could with this novel method, find a new way for inhibition of bacterial infections by use of Cd doped ZnO nanoparticles.



	Concentration of ZnO				
	10 µg/mL	50 µg/mL	100 µg/mL	300 µg/mL	500 µg/mL
candida Albicans	0	10	15	20	22
Kelbsielia phemonia	0	9.2	14	15	18
Proteus Sp.	16	19	21	23	25

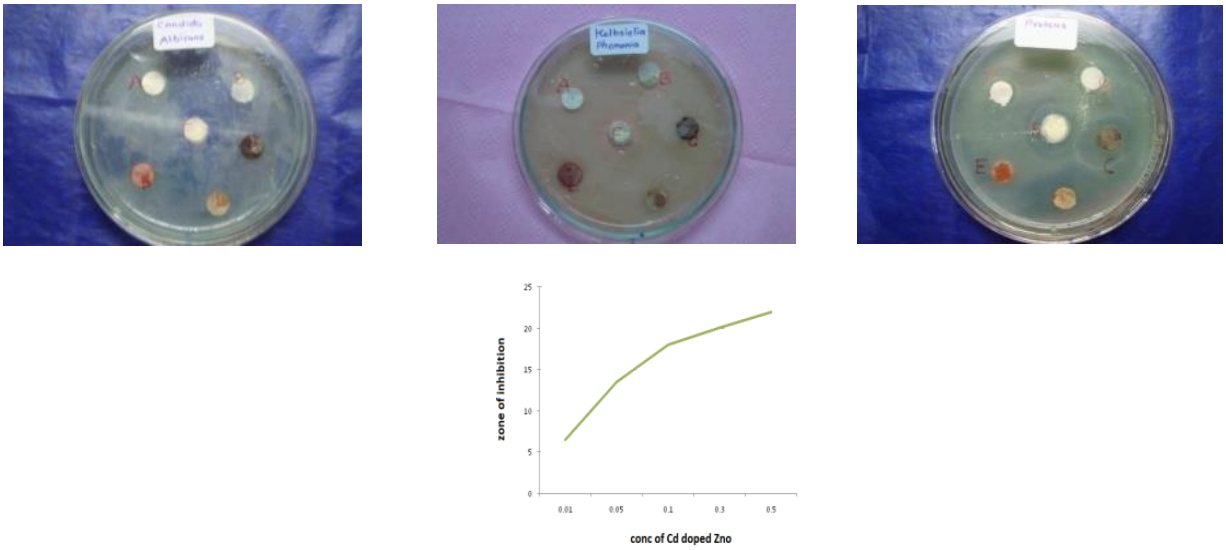
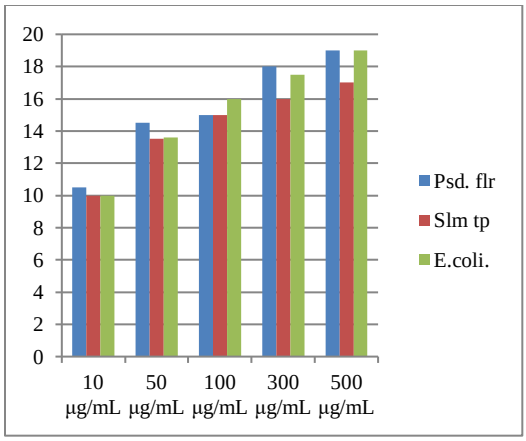


Fig 3: Antifungal activity of Cd doped ZnO against Candida albicans, Kelbsiela Phemonia and Proteus Sp.



	Concentration of ZnO				
	10 µg/mL	50 µg/mL	100 µg/mL	300 µg/mL	500 µg/mL
Pseudomonas. Furoscence	11	15	15	18	19
Salmonella thiphy	10	14	15	16	17
E. Coli.	10	14	16	18	19

Fig4: Antifungal activity of Cd doped ZnO against Pseudomonas fluroscence, Salmonella phemonia and E. Coli.

CONCLUSIONS:

Growth studies of different microbial cultures were performed in the presence of nanoparticles to observe their effect on the growth profile. This study shows that cd doped zno nanoparticles have great promise as antimicrobial agent against Gram-positive and Gram-negative bacteria. We assume that cd doped zno nanoparticles have greater affinity to surface

active groups of Gram-positive and Gram-negative bacteria, which may have led to its greater bacterial effect. Nanosized particles greatly influence the antimicrobial activity of the sample. This technique has its own advantage and is subjected to wider use in preparing nanoparticles.

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