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Synthesis, Characterization and Antimicrobial activity of Ce^{3+} doped ZrO_2 Nanoparticles

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Abstract: Pure and Ce^{3+} ions doped ZrO_2 NPs were synthesized by using sapindus trifoliatus (family Sapindaceae) solution combustion method at low temperature of 450^0C. The purity, size and crystallinity of prepared NPs were attained from PXRD studies. The surface morphology was observed from SEM, mode details with Ce^{3+} identification were obtained from Raman studies. TEM studies provide the particle size and interplanar distance. The antimicrobial activity of the nanoparticles on E.coli a Gram negative bacterium was explored for the samples by Kirby-Bauer method. The studies revealed bactericidal activity of the NPs. This activity was again confirmed by the colony forming units on LB agar.

Keywords: PXRD, SEM, TEM, E.coli, *Gram –bacilli,* LB broth, colony forming units.

INTRODUCTION

The synthesis as well as antibacterial activity of pure and doped ZrO_2 the NP is of great interest for the biomedical uses. The adaptation of bacteria and resistance to wide range of antibiotics has led to the development of various dissimilar infectious diseases. The treatment for such diseases is a difficult task. So, an effort was made that includes the synthesis of pure as well as Ce^{3+} ions doped NPs for antimicrobial activity against E. coli, a Gram –ve Bacteria. The sapindus trifoliatus was used to synthesize NPs. The Spindus trifoliatus belongs to spindaceae family. This is also called as South Indian Soap-nut which is procured from coastal regions of Karnataka.

2. MATERIALS AND METHODS

2.1 Materials

LB agar, Petri plates, Pipettes, Micropipettes, Vials, Double distilled water, E.coli culture, Bacillus culture, Cerium Nitrate hexa hydrate, Zirconium IV nitrate, sapindus trifoliatus, UV trans illuminator, Conical flask, Alcohol, Beaker, Streptomycin, Sterilized discs, Sterilized swabs, Cork borer, Incubator [37°C], EMB agar, Nutrient agar, sonicator, Laminar air flow, water bath, oven, Muffle furnace, Methanol, Sulphuric acid, Hydrochloric acid, Autoclave, Glycine, Ammonium acetate, Nitric acid Luria bertani broth, Luria bertani agar, Muller Hinton agar, Nutrient agar, Eosin Methylene Blue Agar were used. Chemicals used were of AR grade.

2.2 Method of Preparation ZrO_2: Ce^{3+} (1-9 mol %)

The nano sized pure ZrO_2 and Ce^{3+} doped of it were prepared from Zirconium IV Nitrate, sapindus trifoliatus and Cerium Nitrate hexa hydrate by solution combustion technique at low temperature of the order of 450^0C in the preheated muffle furnace. To synthesize the pure ZrO_2 NPs, the collected sapindus trifoliatus was washed thoroughly with the double distilled running water to remove the deposited dust on it. The cleaned nuts are soaked in the hot water for about two hours. By placing sapindus trifoliatus in the hot water for about two hours and then squished. The mixture of zirconium IV nitrate along with extract of sapindus trifoliatus taken in the beaker were stirred to get the uniformity by using magnetic stirrer for about 25 minutes. The beater containing stirred solution is placed in the preheated Muffle furnace maintained at temperature 450^0C. In a span of 20 minutes, the solution gets burn and foam type of material is obtained. This foam time material is grinded and calcinated by keeping it in furnace for 2H at 900^0C to remove the impurities present in the sample. This results the pure ZrO_2 NPs. The Ce^{3+} ions doped ZrO_2 was obtained by the same procedure using Cerium Nitrate hexa hydrate with proper stoichiometry. As the microbial activity is concerned, the samples were consecutively diluted in the double distilled water and filling into the wells of LB agar media and same were observed after 24H of duration.

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3. RESULTS AND DISCUSSION

3.1 PXRD Analysis

The qualitative and quantitate analysis of the prepared samples were investigated by PXRD, a non-destructive technique. The purity and crystallanity of the samples was understood from the sharp peaks shown in the Fig.(1). The crystallite size of samples calculated by Debye Scherrer formula was found to be of the order 70-85 nm. The crystallite sizes were also confirmed by WH plots. The 2θ values were corresponds to observed (h k l) planes for monoclinic are 30.2 (1 1 1), 35.2 (2 0 0), and 63.08 (2 2 2) with JCPDS card number 36-020 whereas 50.4 (2 2 0), 60.2 (3 1 1) and 74.70 (4 0 0) for tetragonal phase with JCPDS card number 17-0923. Nano materials exhibit strong inhibiting effect towards a broadened spectrum of bacterial strains. The inhibitory activity of pure ZrO_2 NPs is due to the photo-catalytic generation of the strong oxidizing power once irradiated by UV radiation wavelength of less than 385 nm for 30 mins. ZrO_2 NPs catalyze the –cidal action of bacteria on illuminator in UV radiation.

Obtaining active free hydroxyl radicals via photo exited ZrO_2 NPs was accountable for the antibacterial activity. Doped ZrO_2 NPs were better inhibitory compared to pure one. The effect of doping relatively increases the activity because the unfilled sites are occupied by Ce^{3+} ions.

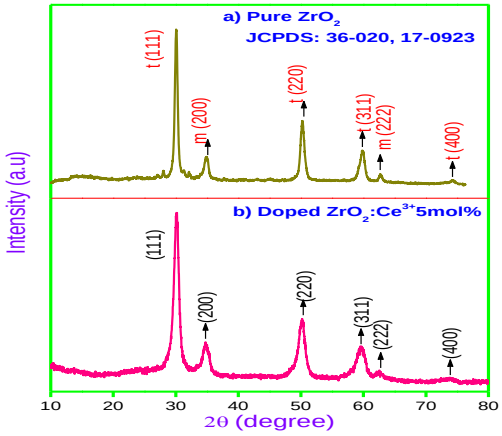

Fig. 1(a-b): PXRD of pure and Ce^{3+} doped ZrO_2

3.2 SEM studies

The SEM images are shown in the Fig 2 (a,b,c,d,e) taken at different resolutions for the pure and doped ZrO_2 NPs. The images shown reveal the morphology. SEM shows that some are irregular and cabbage morphology with huge cavities. On enlarging the images, the particles are found to be nearly spherical in shape with agglomeration as shown in the Fig 2(f). The cracks in the flake show the liberation of the gases during the process of synthesis.

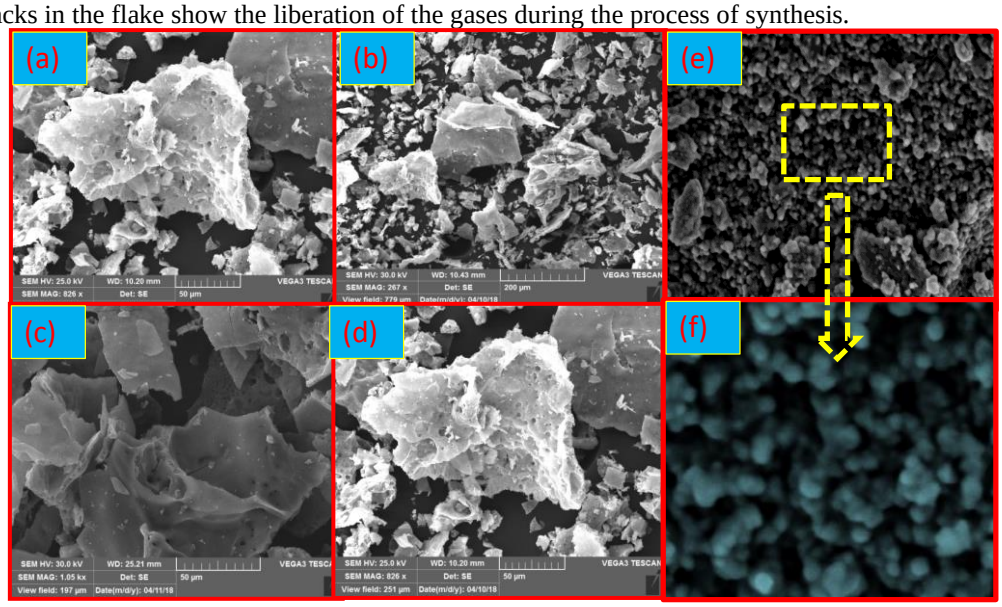

Fig. 2 (a-e): SEM images of at different resolution
Fig. 2(f): Enlarged images of the Fig 2(e) for spherical particles observation

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3.3 Raman Studies

Raman is the powerful non-destructive tool to understand purity, crystallinity, structure and oxygen defect present in the sample in addition to modes of the pure and doped prepared samples. The Raman spectra Fig 3(a) and 3(b) were observed between 90 and 800 cm^{-1}. The wave numbers obtained for the pure samples are 143 ($B1_g$), 260 (E_g), 318 ($B1_g$), 432 (E_g), 462 (E_g) and 634 cm^{-1} ($A1_g$) of the pure in Fig 3(a) whereas, 146, 262, 318, 432, 462, and 634 cm^{-1} in the Fig. 3(b) were for Ce^{3+} doped ZrO_2. On observation of the spectra for pure and doped, it was found that three is shift in the wave number of every peak and observation of extra peak at 462 cm^{-1} indicates the effect doping of the Ce^{3+} ions to the host. The shift in the peak position that is blue shift, of the doped ZrO_2 was due to modification of the structure of the ZrO_2 after doing and also due the confinement of the photons and effect of stoichiometry. Also, there was an enhancement in the intensity of the peak was attributed due to doping of RE.

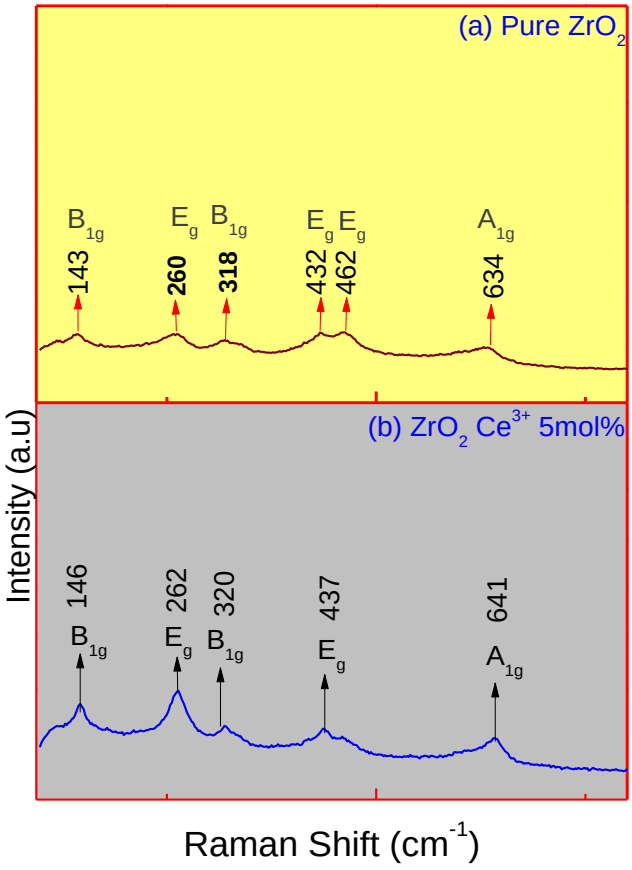

Fig. 3(a) Raman spectra for Pure ZrO_2
Fig. 3(b) Raman spectra of Ce^{3+} doped ZrO_2

The mode assignments for the peaks were also shown in the Fig.3(a) and 3(b)

3.4 TEM

TEM images of ZrO_2:Ce^{3+} images are shown in the Fig. 4(a-d). The images are taken at different resolutions from 2 to 100 nm. At 20 nm resolution, the irregular shaped particles are seen clearly with particle size from 8 to 12 nm in the Fig. 5 (a). At the resolution of 2 nm, the interplanar distances are measured and were found to be 0.32 nm as shown in the Fig 5(b).

Fig. 5(c) shows poly crystalline nature of the doped ZrO_2, the SAED pattern that helps to identify (h k l) values for various diffraction patterns. The first, second, and their diffraction rings correspond to the (h k l) values as (111), (200) and (210) respectively as shown in the Fig.5(c). The calculated interplanar distance from XRD is further confirmed by SAED pattern.

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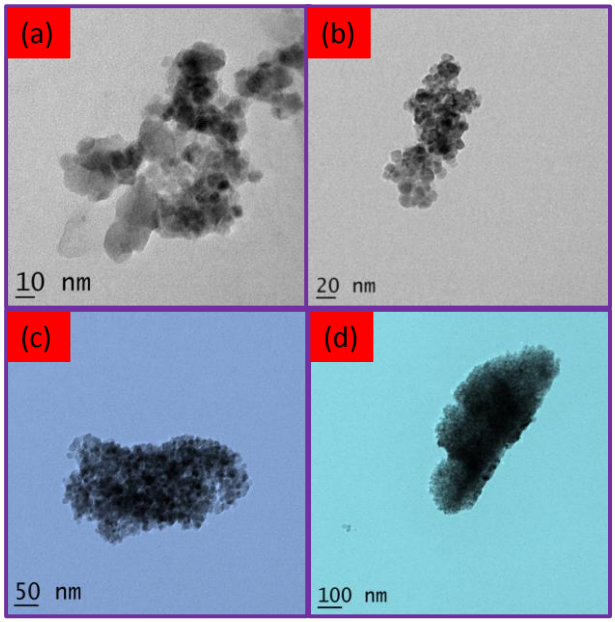

Fig. 4(a-d): TEM images of the Ce^{3+} doped ZrO_2

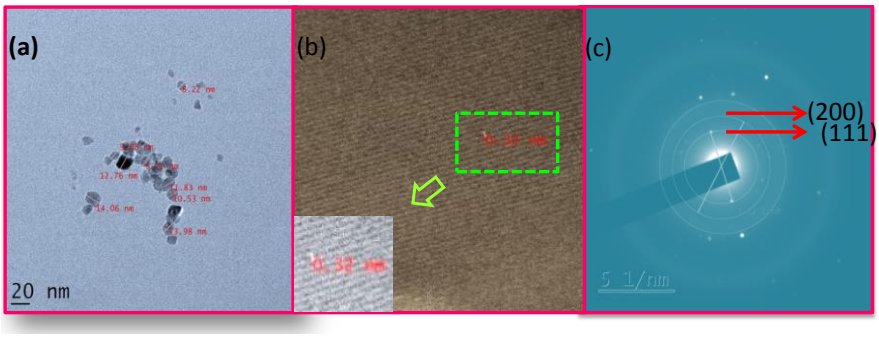

Fig. 5(a): Particle size measured in the TEM
Fig. 5(b): Interplanar distance measured in TEM
Fig. 5(c): SAED pattern observed in TEM with (hkl) values

4. PREPARATION OF STOCK SOLUTION

Pure and doped ZrO_2:Ce^{3+} (5 mol %) stock solution con. of 1 µg/ml was prepared as per the table (1) and suspended in distilled water. The suspension prepared was sonicated for 5-10 minutes to acquire homogeneity of the suspension. This suspension was exposed to Ultraviolet radiations for 30 minutes for activation of nanoparticles.

Table 1: preparation of stock solution

Stock (µg/ml)	0.2	0.4	0.6	0.8	1.0
Distilled water(µg/ml)	0.8	0.6	0.4	0.2	0.0

4.1 Kirby Bauer test

It is an Agar diffusion method used for the evaluation of the antimicrobial activity against the bacteria, indicated as clear zones. Greater the inhibition zone observed, effective is the antimicrobial. The antibacterial effect of Pure and Ce^{3+} ions doped ZrO_2 NPs was performed. Seeded agar of *E.coli* was prepared. Poured into the sterile petri plates and allowed to set. 5 Wells of 10 mm diameter were bored into the agar with a sterile cork borer. These wells were filled with pure ZrO_2 NPs of concentration 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml in duplicates. To the other plate, doped ZrO_2 nanoparticles of concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml were dispensed into each of the wells, in duplicates. Plates of Streptomycin with the same concentrations served as control. These plates were incubated at 37^0 C

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temperatures for 24H and observed. Zone of inhibitions were seen both in the un-doped and doped Ce^{3+} doped ZrO_2 with a range of 0.5-1.6 cm, and a concentration of 1µg/ml. The results are noticed in the Fig 6(a) and 6(b).

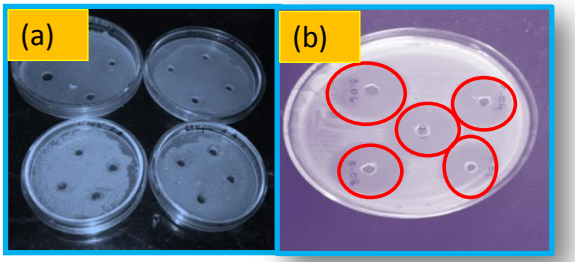

Fig 6: Results of Kirby-Bauer method (A) Doped ZrO_2 (B) Pure ZrO_2

4.2 Colony forming unit [CFU]

11 petri plates were plated with varying concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml of doped and un-doped NPs. To these petri plates 100 µl of 12H culture of *E.coli* was inoculated by spread plate technique. These plates were incubated at 37^0C for 24H. Developed colonies counted. Results shown in the Fig.7(a, b) indicated that with the rise in concentration of dopant, the growth of the colony decreased considerably. The numbers of colonies indicate the effectiveness of the antimicrobial. More number of colonies, the lower is the antimicrobial activity. The high number of colonies better is the antimicrobial activity of the metal oxide as shown in the Table 2.

Table 2: colonies of doped and un-doped ZrO2 samples with concentration

Con. of ZrO_2 in mg/ml	Ce^{3+} doped ZrO_2	Pure ZrO_2
0.2	212	257
0.4	196	218
0.6	132	198
0.8	96	174
1.0	78	122
Control	261	295

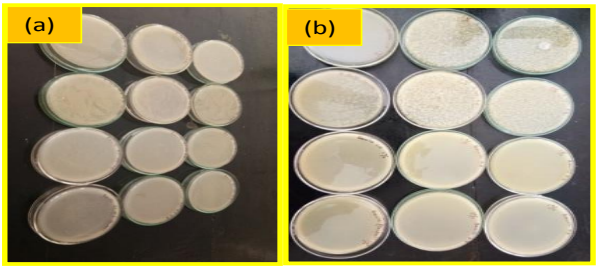

Fig 7: Colony forming Units in (A) Doped ZrO_2 (B) Pure ZrO_2

4.3 Growth curve

Sterile Luria broth was taken in 6 different conical flasks. 100 µl of 18hrs young culture of *E.coli* was inoculated. 20, 40, 60, 80, 100 µg/ml concentrations of doped ZrO_2 was added. The flask without ZrO_2 serves as control. Flasks were incubated in a shaker incubator at 37ºC. Optical density at 600 nm noted every hour for 6H. The growth curve is as shown in the Fig. 8.

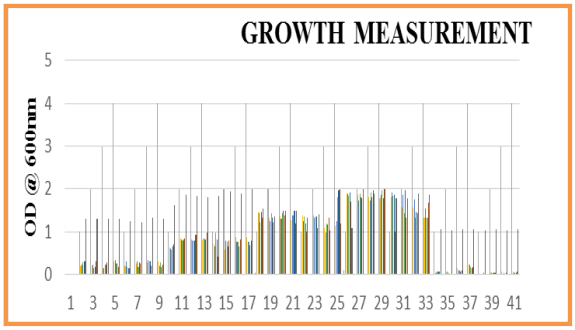

Fig. 8 Growth curve

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4.4 Effect of ZrO_2 in Liquid Media

It was shown in the Fig 9, 8 conical flasks containing 60 ml of Luria broth were autoclaved. To these flasks, varying concentrations of doped ZrO_2 were added. 100µl of 18H young culture of *E.coli* was aseptically transferred into each of the flasks. The flasks were incubated in a shaker incubator at 37^0 C for 18 hours. One flask without ZrO_2 served as control. The optical density of each of the flasks was noted for every hour for 6H duration.

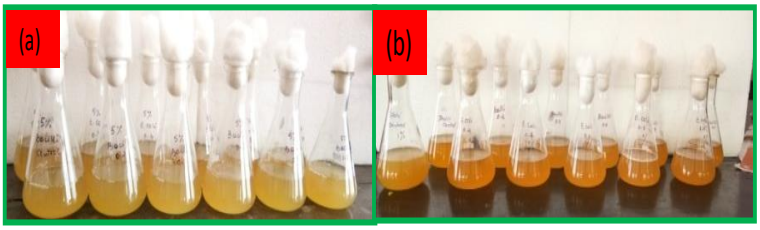

Fig 9: Results of growth measurement (A) Doped ZrO_2 (B) Pure ZrO_2

The growth was directly proportional to the turbidity of broth medium, Ce^{3+} doped ZrO_2 shows decreased turbidity with increasing concentration and hence more bactericidal activity as depicted in the Fig.10.

Fig.10 turbidity variation with concentration

CONCLUSION

The prepared samples were characterized by different tools to confirm the nano size (1-100 nm). Naturally occurring soap nut was used for the preparation instead of environment polluting chemicals. The Ce^{3+} ions doped ZrO_2 is showing better incubation than the pure ZrO_2. This was confirmed by the formation of colonies. Ce^{3+} ions doped ZrO_2 shows bactericidal activity against the bacteria. The effectiveness of these nanoparticles can be enhanced by combining it with the relevant antibiotics minimizing the antibiotic resistance amongst the bacteria. The high number of colonies better is the antimicrobial activity of the metal oxide is proved. Thus, providing a better scope in future days for combating and treating various diseases.

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