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A Study on Zinc Oxide Nanoparticles: Synthesis and Antibacterial Applications

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Abstract: In the present work, we describe a low cost and simple procedure for the synthesis of zinc oxide nanoparticles (ZnO NPs) using Corriandrum sativum leaf extract as eco-friendly reducing and caping agent. Techniques like TEM, XRD, FTIR were performed to ascertain the formation and characterization of ZnO NPs. XRD confirmed the crystalline nature of the nanoparticles and TEM showed the morphology of the nanoparticles to be spherical with an average size of 40nm. The antibacterial and antifungal activity ended with corresponding well diffusion and minimum inhibitory concentration. The maximum zone of inhibition was observed in ZnO NPs (10µg/ml) against Escherichia coli (6 ± 2.4 mm) and Staphylococcus aureus (6±1.3mm). Bacteria-mediated ZnO NPs were synthesized and proved to be a novel antimicrobial material.
Keywords: Coriandrum sativum;Z inc acetate; Zinc oxide nanoparticles; XRD; FTIR; Antimicrobial activity

I. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level(1). Recent advance in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticles of any size and shape, have led to the development of new biocidal agents. Nanomaterials are called "a wonder of modern medicine". Nanotechnology is a multidisciplinary scientific field undergoing explosive development(2). Nanometer-sized particles offer novel structural, optical and electronic properties that are not attainable with individual molecules or bulk solids. The characters of metal and metal oxide nanoparticles have been of great interest due to their distinctive feature such as catalytic activity, optical, magnetic and electrical properties. Nanoparticles interaction with biological materials and established a series of nanoparticle / biological interfaces that depend on colloidal forces as well as dynamic biophysicochemical interactions. These interactions lead to the formation of new nanomaterial with control size shape, surface chemistry, roughness and surface coatings(3). The use of plants for the synthesis of nanoparticles provides a cost-effective and environmenlly friendly alternative to chemical and physical synthesis. In addition, the use of plants can be easily scaled up for large-scale synthesis without the use of toxic chemicals or the need for high pressures, energy and temperatures. Nanoparticles present a higher surface area to volume ratio with decrease in size, distribution and morphology of the particles.

Among the metal oxide nanoparticles, zinc oxide is interesting because it has vast applications in various areas such as optical, piezoelectric, magnetic and gas sensing(4-5). Besides these properties, ZnO nanostructure exhibits high catalytic efficiency, strong adsorption ability and are used more and more frequently in the manufacture of sunscreens, ceramics and rubber processing, waste water treatment and as a fungicide(6). Furthermore, zinc oxide nanoparticles also act as good antimicrobial agent as they show antimicrobial activity against many pathogenic organisms like *Eschericia Coli, Staphylococcus Aureus, Pseudomones Aeruginosa* etc(7-8). When compared with other nanoparticles, zinc oxide nanoparticles are less toxic and safe so they find increased applications in industries like food where they are used in food packaging and processing of meat and vegetables(9).

II. MATERIALS USED

Zinc Acetate (AR) was purchased from Fischer Scientific. *Coriandrum Sativum* (Dhania) leaves were collected from local sources.
All glass ware (Conical flasks, measuring cylinders, beakers petri plates and test tubes) were purchased from Borosil, India.

A. Preparation of Coriander Leaf Extract

Fresh coriander leaves were collected from the local sources and then thoroughly washed with distilled water several times at room temperature and excess water was decanted from the leaves. To superficially remove the extra moisture present in the leaves , they were placed over filter paper for some time. And then 30 g of coriander leaves were weighed and crushed finely by using mortar and pestle and boiled in 200 ml of distilled water for 5-10 min. The extract was then filtered using Whatman filter paper no.1 for conducting studies and the filtrate was stored for further use. However the extract was always prepared fresh.

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B. Preparation of Zinc Oxide Nanoparticles

For the green synthesis of zinc oxide nanoparticles, 0.25 ml of coriander extract was mixed to 50 ml aqueous solution of .02N zinc acetate under constant stirring. To the same, 2.0M NaOH was added to attain pH 11 resulting in a pale white aqueous solution. The pale white precipitate were separated and washed twice with distilled water followed by ethanol to free it from impurities. The pale white powder of ZnO NPs was obtained after drying at 60^0 C in vaccum oven for about 8 hours.

C. Agar Disc Diffusion Method for Antimicrobial Activity

The antibacterial activity was tested against gram positive *S.Aureus* and gram negative *E.Coli* strain. The bacterial isolates were obtained from clinical samples. The antimicrobial test was carried out primilarly by the agar well diffusion method. Bacterial pathogenic inculumns were prepared from 18 h grown cultures. Petri dishes containing the bacterial inculumns on nutrient agar was used for the study. Using a cork borer (6 mm diameter), wells were made in the culture plates and different concentrations of the freshly synthesized nanoparticles were loaded. The plates were then incubated at 37^0 C for 24 h. After incubation, the zone of inhibition (ZOI) was measured.

III. RESULTS AND DISCUSSION

A. X-Ray Diffraction of ZnO nanoparticles

The structure and size of the zinc oxide nanoparticles was analyzed with the help of X ray diffraction technique. The XRD-diffractogram is shown in figure 1.

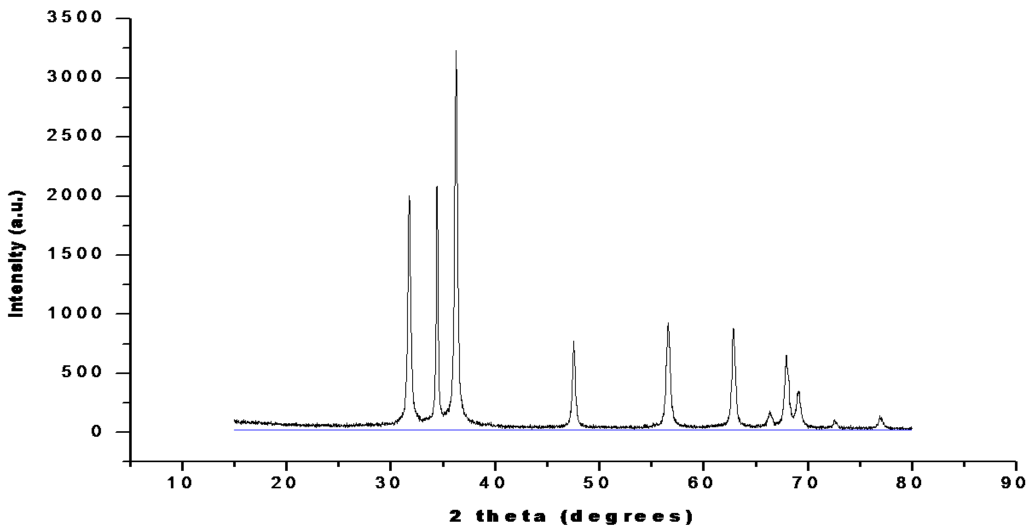

Figure 1: XRD pattern of ZnO nanoparticles

A definite line broadening of the XRD peaks indicates that the prepared material consist of particles in nanoscale range. The strong and narrow diffraction peaks indicated that the product has good crystallinity. Major diffraction peaks were seen at 31.73, 34.39, 36.24, 47.51, 56.52, 62.88, 67.94, 72.54 and 76.89, which can be assigned to diffraction from (100), (002), (101), (102), (110) and (103) planes, respectively. This revealed that the resultant nanoparticles were of pure zinc oxide with a hexagonal structure[11]. The synthesized nanopowder does not contain any characteristic XRD peaks other than ZnO peaks and suggests that it is free of impurities.

The diameter of synthesized ZnO nanoparticle was determined using Debye-Scherrer formula and was found to be 68.61 nm.

$$D = \frac{.89\lambda}{\beta cos\theta} \quad (1)$$

where, 0.89 is Scherrer's constant, λ is the X-ray wave length equal to 1.54 □, the full width at half maximum and θ is half diffraction angle. The average particle size of the sample was found to be 68.61nm which was derived from the FWHM (0.2676) of more intense peak corresponding to 101 plane located at 36.24.

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B. Transmission Electron Microscopy Analysis (TEM)

The size and the morphology of the zinc oxide nanoparticles synthesized using coriander extract was studied by Transmission Electron Microscopy (TEM).

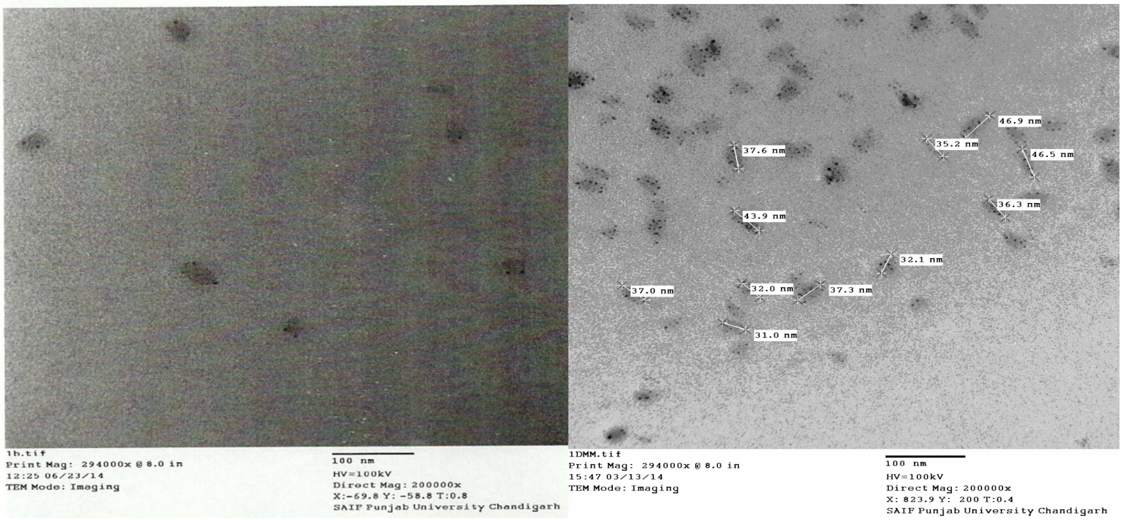

Figure 2(a) & (b) TEM images of zinc oxide nanoparticles

As it can be seen from the images zinc oxide nanoparticles are well dispersed with their dimensions ranging from 30 nm to 50 nm with the average size of 40 nm.

Most of the particles are observed to be nearly spherical.

C. FTIR spectra of ZnO nanoparticles

FTIR studies revealed the presence of ZnO nanoparticles which was confirmed from the absorption peak at 430 –480 cm^{-1}. In the IR spectrum , the band at 3394 cm^{-1} is due to stretching vibrations of O–H groups in water, alcohol and phenols and N–H stretching in amines. The C–H stretch in alkanes and O–H stretch in carboxylic acid appear at 2926 and 2864 cm^{-1} respectively. The strong band at 1627 cm^{-1} is attributed to the C=C stretch in aromatic ring and C=O stretch in polyphenols. The C–N stretch of amide-I in protein gives the band at 1396 cm^{-1}. The C–O–C stretching in polysaccharides gives a band at 1141 cm^{-1} and C–O stretching in amino acid causes a band at 1037 cm^{-1}. Finally the weak band at 819 cm^{-1} is the result of C–H out of plane bending. Thus from the IR spectrum it can be observed that coriander sample is rich in polyphenols, carboxylic acid, polysaccharide, amino acid and proteins. In addition to the absorption bands of these biomolecules, two new peaks appearing at 682 and 457 cm^{-1} in the IR spectrum of the ZnO NPs are the characteristic peaks of ZnO molecules. It may be concluded that the presence of higher percentage of phenolic group of molecules are responsible for the reduction process and the amino acids and amide linkages in protein are responsible for the stabilization of the ZnO NPs.

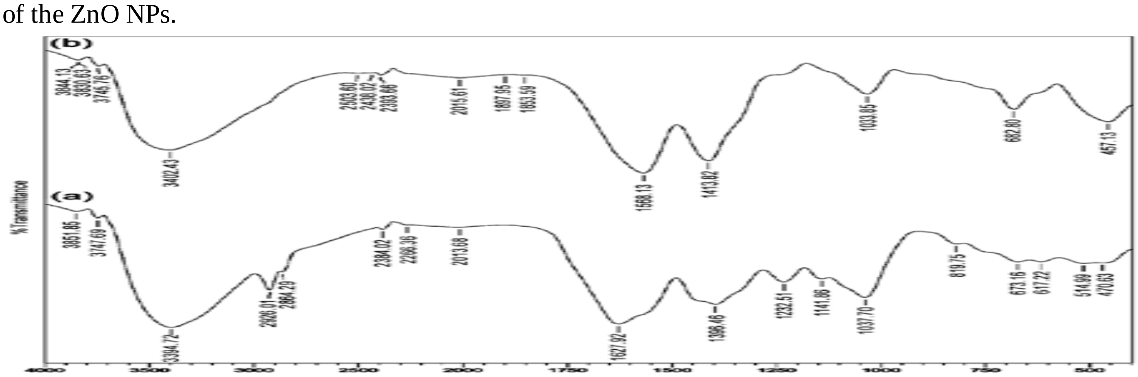

Figure 3 (a) & (b) FTIR of Coriander extract and FTIR of ZnO NPs with coriander extract

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D. Specific Surface Area of ZnO Nanoparticles

The Brunauer - Emmett – Teller (BET) equation has been used to calculate specific surface areas of the ZnO powders from nitrogen adsorption isotherms measured at 77 K and relative pressure [p/P] ranging from 0.05 to 0.2. Before measurement, samples were out-gassed at 120 degree C for over 24 h.

The BET equation is as:

$$\frac{1}{v\left[(p_0/p)-1\right]} = \frac{c-1}{v_m c}\left(\frac{p}{p_0}\right) + \frac{1}{v_m c}, \qquad (1)$$

where p and p_0 are the equilibrium and saturation pressure of adsorbates at the temperature of adsorption, v is the adsorbed gas quantity and v_m is the monolayer sorbed gas quantity. c is the *BET constant*,

Results : The specific surface area of the synthesized ZnO NPs was determined to be 22.977 sm^2/g with particle avg. size 40nm.

Reference : J. of Supercritical Fluids 52 (2010) 76–83

Surface Area	Particle diameter
17.6	60
29	36.5

E. Antimicrobial Activity against E. Coli and S. Aureus

The antimicrobial activity of the zinc oxide nanoparticles was determined using agar disc diffusion method.

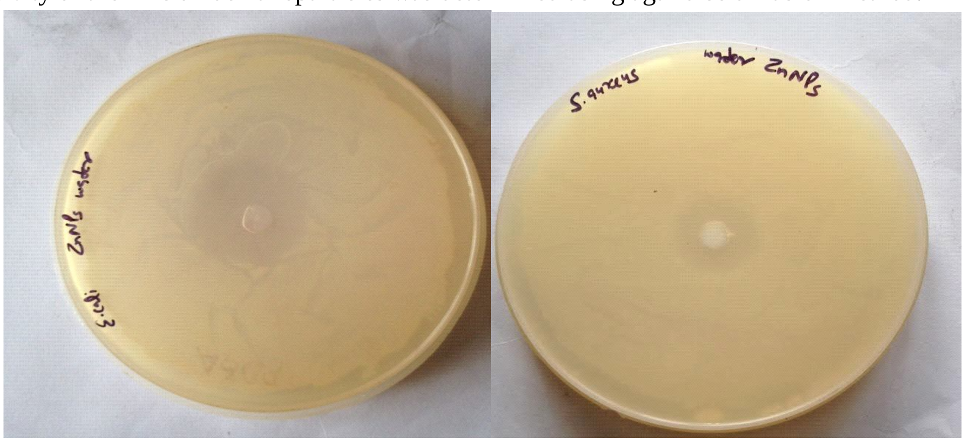

Figure 4(a) & (b) Antimicrobial activity of ZnO nanoparticles against *E.Coli* and *S.Aureus*

The effects of particle size and concentration on the antibacterial activity of ZnO nanoparticles was studied using disc and well diffusion agar method. The results shows that ZnO nanoparticles have antibacterial inhibition zone of 8.4 mm and 7.3mm at the concentration of 10 µl against *E.Coli* and *S. Aureus*, respectively. Gram negative bacteria seemed to be more resistant to ZnO nanoparticles than Gram positive bacteria.

IV. CONCLUSION

This green synthesis approach shows that the environmentally begin and renewable leaf extract of coriander can be used as an effective stabilizing as well as reducing agent for the synthesis of zinc oxide nanoparticles. ZnO nanoparticles synthesized are of avg. size 40 nm , confirmed by TEM. Antibacterial efficacy of the prepared nano particles against the pathogenic bacteria *E. coli* and *S.aureus* reveals that the ZnO nanoparticles have considerably higher antibacterial potential and is possibly due to a combination of events. Besides, the outcomes of the synthesis certainly contribute to the developing a better understanding of simple, low-cost, green and nontoxic synthesis method and growing the knowledge base needed to design a suitable antibacterial materials and framework for advanced and wise applications.

V. ACKNOWLEDGEMENT

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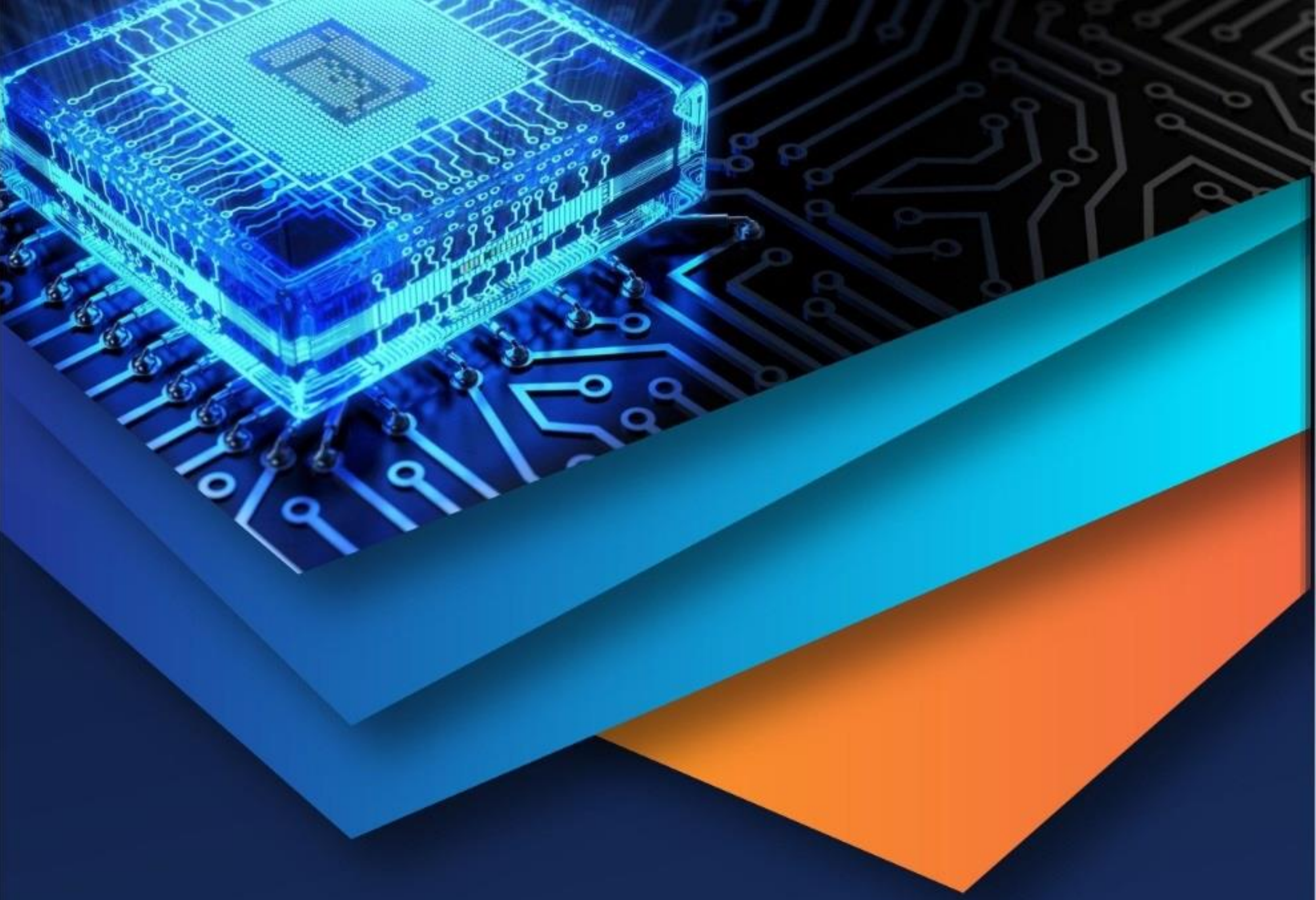

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