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**Research Article**

# GREEN SYNTHESIS OF *Duranta erecta* MEDIATED ZnO NANOPARTICLES AND THEIR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

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**ABSTRACT**

Here in this study Zinc Oxide nanoparticles (ZnO NPs) were synthesized through eco-friendly and nontoxic green synthesis method using fresh aqueous leaves extract of *Duranta erecta* (*D. erecta*) plant. The prepared ZnO NPs were characterized from UV-visible spectroscopy, X-rays diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) techniques. The formation of nanoparticles was confirmed due the specific absorption at 350 nm in the UV-visible spectra. The synthesized ZnO NPs was very effective against *Escherichia coli* (*E. coli*) and Staphylococcusaureus (S. aureus). Our ZnO NPs show better antioxidant 1-1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity and was more successful as compared to the plant precursor.



## INTRODUCTION

Nanotechnology is a research hot spot in modern material science this technology is capable of providing important application that range from innovative fabric compound, food processing, and agricultural production sophisticated medicinal techniques. It is considered in the synthesis, characterization, and exploration of material in Nano region (1-100 nm) [1-4]. In this technology, the pertinent materials are those whose structure exhibit new and considerably enhanced physicochemical and biological properties as well as distinct phenomena and functionalities as a result of the Nano scale size. This Nano scale size generally confers larger surface areas to nanoparticles compared with macro size particles [5-7]. Nanoparticles are known as particles at the atomic level (1-100 nm). They show size related properties significantly given their small size, nanoparticles have larger structure in comparison with their counter parts [8]. This distinct property allows there possible applications in many field such as biosensors Nano medicines, and biotechnology. Reducing the size at nano scale can modify their chemical, mechanical, electrical, structural, morphological and optical properties. These modified features allow the nanoparticles to interact in a unique manner with cell biomolecules and thus facilitate the physical transfer of nanoparticles in to the inner cellular structures [9-11]. Nanostructures have larger surface area due to which it has high surface reactivity. In recent year, the synthesis of oxide nanoparticles using green parts of plants such as leaves, root, latex and bark has received much attention by researcher. It is clean nontoxic ecofriendly, free from unwanted products and nonhazardosus [12-14]. For the synthesis of nanoparticles many methods have been developed among them green synthesis is very effective methods and ecofriendly [15]. Inorganic material such as metal and metal oxide due to their stability is more advantageous in many aspects than organic compound [16]. Among the metal oxide, ZnO nanoparticles have received a special attention as in anti-cancer, anti-bacterial and antifungal material. ZnO nanoparticles exhibit improved properties as a compared to bulk material and these novel properties are attributed to change in specific characteristic such as morphology and size of particles. ZnO nanoparticles have a wide range of application in solar cell, catalyst, gas sensor, luminescent devices and antibacterial activity. Now days, ZnO nanoparticles gained also significant attention due to their implication for cancer therapy [17, 18]. ZnO nanoparticles exhibit antibacterial and anti-fungal activity.

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They can decrease the viability and attachment of microbes on bio-medical surface. ZnO nanoparticles can be synthesized by different methods such as spray pyrolysis, hydrothermal treatment, sol gel process co-precipitation, combustion, sono chemical etc [19, 20]. Generally the chemical used in the synthesis and stabilization are toxic and lead to by product which are non-ecofriendly and causes danger to human being and environment. The generation of toxic by product can be avoided using green chemistry approach, for instance, using plant for ZnO nanoparticles.

In this study, ZnO nanoparticles were prepared using fresh aqueous extract of *D. erecta* fruit. The prepared nanoparticles were used for the antibacterial and antioxidant activities and characterized by UV-visible, SEM, EDX and XRD technique.

## MATERIAL AND METHODS

### *Preparation of plant extract*

The leaves of *D. erecta* were collected to prepare plant extract and washed two times with distilled water and dried at room temperature and grind into fine powder. 20 gram of this fine powder were boiled in 100 ml of double distilled water to prepare crude solution and then filtered using filter paper and stored in refrigerator for furthered use [21].

### *Preparation ZnO nanoparticles*

For the synthesis of ZnO nanoparticles, 10 ml of plant extract solution was added to the 80 ml of 20 mM of $Zn(NO_3)_2$ solutions. The reaction mixture were stirred using magnetic stirrer at 50 $^0$C for 2 hours. After two hour, the formation of white precipitate started which indicate the formation ZnO nanoparticles. The formation of ZnO NPs was confirmed by UV-visible spectroscopy. The precipitate was collected by centrifugation at 4000 rpm for 20 minutes. After centrifugation the collected pellets of ZnO washed three times with distilled water and dried for 24 hours at 75 $^0$C. The ZnO NPs were collected and used for analysis.

### *Characterizations*

The synthesized ZnO NPs were characterized by SEM and EDS from the University of Karachi. XRD analysis was carried out at Centralized Resource Laboratory (CRL), University of Peshawar. UV-visible study was carried out at the Department of Pharmacy, Kohat University of Science and Technology, Kohat, using UV-visible spectrophotometer Shimadzu-1800 Japan.

### *Antibacterial Activity*

The disc diffusion test was used to analyzed antibacterial activity of synthesized ZnO-NPs at 40 mg/mL and 20 mg/mL concentrations on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). A volume of 10 µL of the suspended ZnO NPs was taken in a micropipette and drop in hole on agar plate. The same volume of plant extract was also used. The bacterial plates were incubated at 35 ºC for 12 to 24 h. The zones of inhibition in (mm) after the incubation periods were measured.

### *DPPH free radical assay*

The aqueous leaf extract of *D. erecta* and biosynthesized ZnO NPs were tested for their antioxidant scavenging effect on radical of DPPH, as reported in our previous reports. The different concentrations 10, 15, 20, 50, and100 µg/ml of test sample solution (*D. erecta* extract and ZnO NPs) were added, to the equal volume to methanolic DPPH (0.1mM) solution. The reaction was incubated for at room temperature for 50 min. Ascorbic acid was used as positive control. The activity percentage of DPPH radical scavenging activity (RSA) of the sample was evaluated using the following equation.

$$\%\mathbf{RSA} = \left[\frac{(\mathbf{A_{DPPH}} - \mathbf{A_{solution}})}{\mathbf{A_{DPPH}}}\right] \mathbf{x\ 100}$$

where $A_{DPPH}$ indicate the absorptions of DPPH at 517 nm before and $A_{solution}$ indicate the absorptions of DPPH after addition of ZnO NPs

## RESULT AND DISCUSSION

### *UV visible study of ZnO nanoparticles*

The successful synthesis of ZnO nanoparticles was confirmed using UV-Visible spectrophotometer. UV visible spectroscopy is a powerful tool to determine the optical properties of nanoparticles. Our prepared ZnO NPs show absorption at 355 nm as shown in the Figure 1. The formation of sharp absorption peak near 355 nm showed the successful formation of ZnO nanoparticles [10, 22]. On the spectrum there are two small peaks at 270 and 290 nm, which represent the attachment of biomolecules on the surface of ZnO NPs.

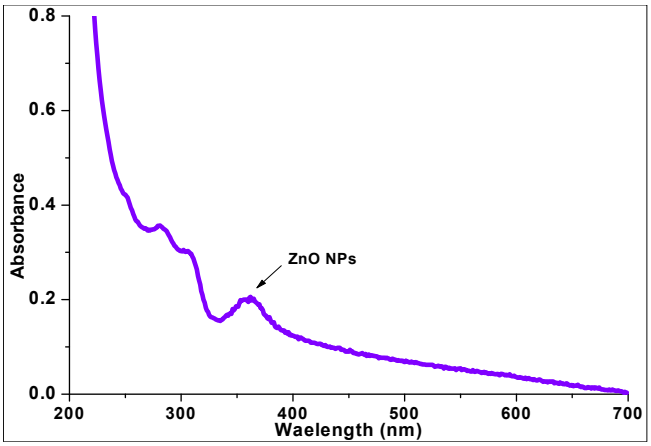



**Figure 1** The UV-visible study of ZnO nanoparticles which show a strong absorption peak at 355 nm.

### *XRD analysis of ZnO nanoparticles*

Figure 2 represent the XRD pattern of ZnO nanoparticles. XRD peaks can be indexed to a crystalline structure. The peak broadening clearly indicates the formation of ZnO nanoparticles of nano range. The major peaks observed for ZnO NPs at a 2Θ value of 31.23$^0$, 34.42$^0$, 36.12$^0$, 48.00$^0$ and 68.33$^0$ (as show in Figure 2), which corresponds to the Bragg's peaks of (100), (002), (101), (102), and (112). The Bragg's peaks of ZnO are close in agreement with our recent data of green synthesized ZnO NPs [10]. The diffraction peaks indexed as hexagonal wurtzite phase of ZnO. The average crystalline size of the ZnO NPs was calculated by using Scherrer equation. The size of ZnO nanoparticles calculated from XRD analysis was 28 (±7 nm).

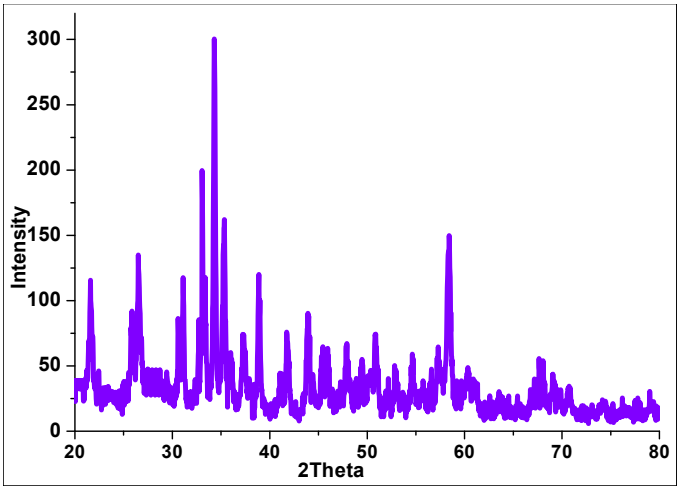
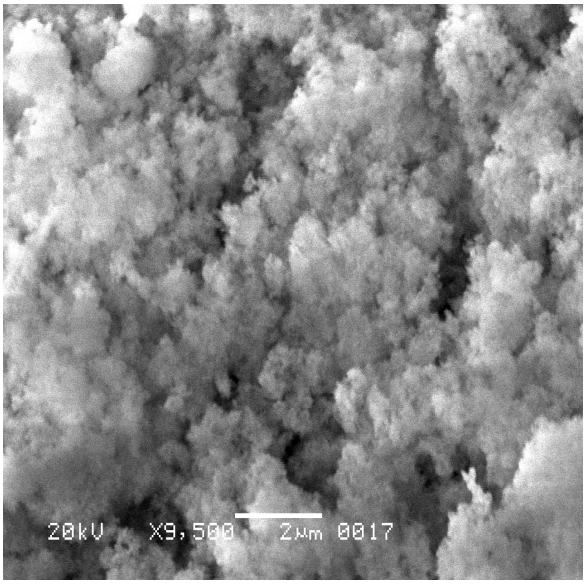



**Fig 2** The XRD pattern of ZnO nanoparticles

### *SEM and EDX Analyses of ZnO nanoparticles*

Figure 3 shows the SEM images of ZnO nanoparticles. Figure 3 represent SEM at different magnification and these images confirm the formation of crystalline ZnO nanoparticles. These picture shows that ZnO has spherical with some irregular shape. From the picture also calculate the average size of the nanoparticles is 75 nm. Nanoparticles at some position show aggregations.

For the confirmation of ZnO NPs in the composite host material, the prepared NPs were subjected to EDX investigation. The successful formation of ZnO NPs was confirmed by EDX analysis. EDX spectrum of ZnO NPs is shown in Figure 4. EDX spectrum of ZnO show strong peak for Zn at 1 keV. While the signal for oxygen also present in the spectrum. Thus from the EDX analysis, the successful formation of ZnO NPs were confirmed.



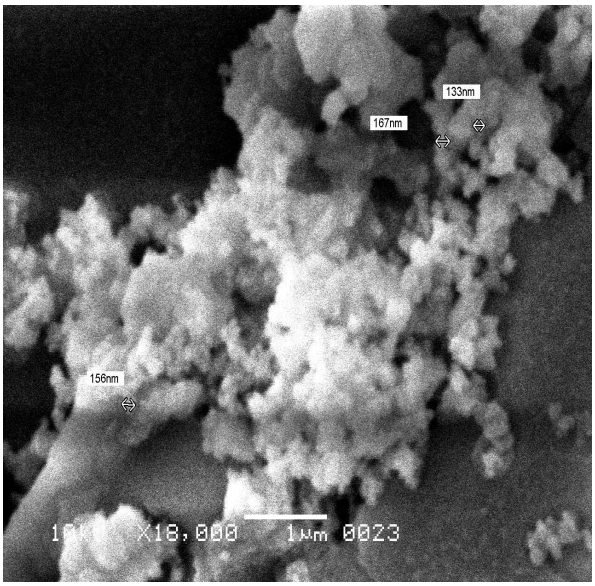




**Figure 3** SEM analysis of ZnO nanoparticles at different magnification

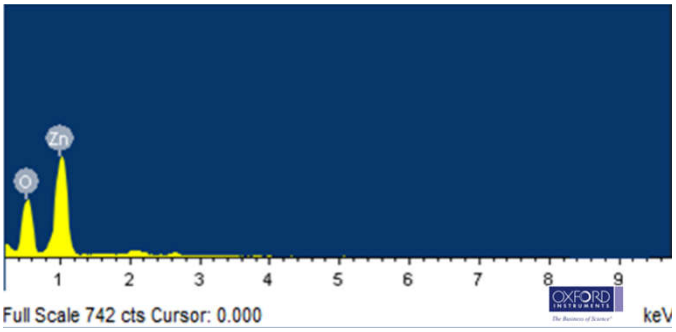



**Figure 4** EDX analysis of ZnO nanoparticles

### *Antibacterial Activity of ZnO nanoparticles*

Bacteria are generally characterized by a cell membrane, cell wall and cytoplasm. The cell wall lies outside the cell membrane and is composed mostly of homogeneous peptidoglycan layer. The cell walls maintain the osmotic pressure of the cytoplasm as well as the characteristic cell shape. Gram positive bacteria have one cytoplasmic membrane with multilayer of peptidoglycan polymer, thicker cell wall (20-80nm).Whereas gram-negative bacteria wall is composed of two membranes, an outer membrane and a plasma membrane with a thin layer of peptidoglycan with a thickness of 7-8 nm, NPs size within such ranges can pass through the peptidoglycan and hence are highly susceptible to damage. The cytoplasm, a sticky fluid that present in a cell, involves all the cellular components except the nucleus. The function of this organelle includes growth, metabolism and replication. Consequently, the cytoplasm containproteins, carbohydrates, nucleic acid, salt, ions, and water (80%). This composition contributes in the electrical conductivity of the cellular structure. The overall charge of bacterial cell walls is negative. Antibacterial activity is known according to the American Heritage Medical Dictionary 2007, as the action by which bacterial growth is destroyed or inhibited. It is also describe as function of the surface area in contact with the microorganism [23]. While antibacterial agents are selective drugs and have the ability to damage or inhibit bacterial growth and they are not harmful to the host. This can act as chemo-therapeutic agents for the treatment or prevention of bacterial infection. An antibacterial is considered as bacterial if it kills bacteria or as

bacteriostatic if it inhibits their growth. Different methods have been developed for the assessment and investigation of anti-bacterial activity in vitro. These methods include disk diffusion, broth dilution, agar diffusion methods. The most commonly methods used is broth dilution methods, followed by colony count, through plating serial culture broth dilution which contained ZnO NPs and the targeted bacteria in agar medium and incubated. The agar diffusion methods is the most frequently used methods and has been standardized as an official methods for detecting bacteriostatic activity by other direct test methods. The antibacterial of the ZnO nanoparticles was evaluated against Gram negative *E. coli*, In Agar well diffusion method the ZnO nanoparticles show significant antibacterial property against *E. coli*. Some reports are available for the mechanism behind the antimicrobial activities in the present case *E. coli* show significant zone of inhibition by ZnO nanoparticles. The presence of ammine and carboxyl groups on cell surface has high affinity of zinc towards these groups [24, 25]. The zinc ions release immediately and attached to the DNA molecules and leads to the disordering of the helical structure by cross-linking within and between the nucleic acid strands. The zincions also disrupt the biochemical process [26]. The $Zn^{2+}$ were also studied that it disrupts the bacterial cell membrane and gain entry in ordered to disrupt the enzyme activity. Another mechanism that the zinc ion will be release from the nanoparticles that may attached to the negatively charged bacterial cell wall and rupture it, there by leading to protein denaturation and causes cell death. Our ZnO NPs exhibited a zone of inhibition against the tested microbes as presented in Figure 5. Prepared ZnO NPs show zone of inhibition with 4 mm and 7 mm with 20 and 40 mg/mL respectively, after 24 h against *E. coli*. While against *S. aureus* it show 5 mm and 7.2 mm with 20 and 40 mg/mL respectively, after 24 h. Similarly plant extracts show 1 and 1.3 mm with 20 and 40 mg/mL respectively, after 24 h.

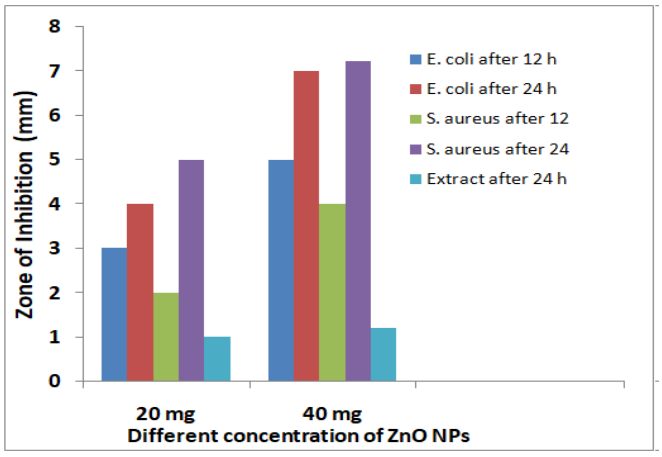



**Figure 5** Antibacterial affect of ZnO nanoparticles against *E. coli* and *S. aureus* after 12 and 24 hours (zone of inhibition)

### *Free Radical Activity*

The antioxidant free radical scavenging activity of *D. erecta* extract and biosynthesized ZnO NPs were elevated by DPPH assay. The free radical scavenging activity of prepared ZnO NPs is presented in **Figure 6**. Our synthesized ZnO NPs show good free radical DPPH inhibitory activity, which show the ZnO as a source of an antioxidant. For the free radical antioxidant activity, the different concentrations used were 10, 15, 20 50, and 100 µg/mL of the *D. erecta* extract synthesized ZnO NPs and positive control ascorbic acid was utilized to react with the 5mM solution of DPPH of equimolar concentrations. Though the radical scavenging abilities of ascorbic acid was higher than ZnO and *D. erecta* extract which showed that prepared ZnO act an antioxidant and could serve as free radical inhibitors or scavengers.

The antioxidant activity of our synthesized ZnO NPs show higher activity then *D. erecta* extract and in comparison to control ascorbic acid showed lower free radical activity. Most importantly, our ZnO NPs showed good activity with 45% scavenging capability of DPPH (**Figure 6**), which are lower than our recently reported synthesized AgNPs [27, 28]. Our ZnO NPs showed higher antioxidant activity than the *D. erecta* extract at all concentrations. The presents activity was apparent not only due to the capping agents of *D. erecta* extract but also due to the ZnO NPs and may demonstrate useful to treat several kind of diseases due to oxidative stress and to deal with various infections.

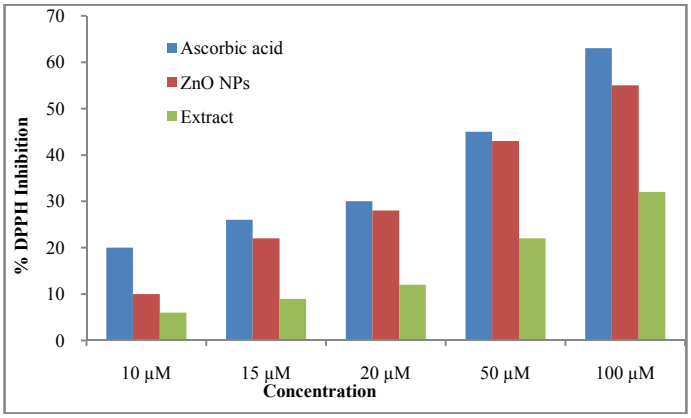



**Figure 6** Free radical scavenging activity of *D. erecta* synthesized ZnO nanoparticles

## CONCLUSION

ZnO nanoparticles have been prepared using green synthesis method. This method is less costly and synthesis process did not require any chemical reducing agent. These nanoparticles have the ability to serve as potential antibacterial agent for the infection caused by *E. coli* and *S. aureus*. This study suggests that ZnO nanoparticles can be prepared by using natural reducing agent present in the leaves of *D. erecta*. The prepared nanoparticles were characterized by SEM, EDS, XRD, and UV-visible spectroscopy. The SEM and XRD study confirm the crystalline nanostructure of ZnO nanoparticles. From UV-visible spectroscopy the formation of ZnO nanoparticles was confirmed and gives maximum absorption peak at 255 nm. The prepared ZnO NPs showed excellent DPPH free radical antioxidant and antibacterial activities. As evidenced by antioxidant and antibacterial activities, ZnO NPs were more active than their precursor and this is a simple way to be used as a nanomedicine in controlling different human and veterinary infections and also for the treatment of different diseases due to oxidative stress.

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