RESEARCH ARTICLE

Green Synthesis, Characterization, and Biological Activity of Zinc Oxide Nanoparticles using Aqueous Extract of *Beta Vulgaris* and the Seed of *Abrus precatorius*

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Received: 27th April, 2021; Revised: 18th May, 2021; Accepted: 7th August, 2021; Available Online: 25th September, 2021

ABSTRACT

Development of improved methods for the synthesis of metal oxide nanoparticles are of high priority for the advancement of material science and technology. Herein, the biosynthesis of ZnO using hydrahelix of beta vulgaris and the seed of abrus precatorius as an aqueaus extracts adduced respectivily as stablizer and reductant reagent. The support are characterized by spectroscopic methods (Fourier-transform infrared (FTIR), Ultraviolet–visible spectroscopy (UV-vis)). The FTIR confirmed the presence of ZnO band. The Uv-visible showed absorption peak at corresponds to the ZnO nanostructures. X-ray diffraction (XRD), scaning electron microscopy (SEM), dispersive X-ray spectroscopy (EDX) techniques are taken to investigation the size, structure and composition of synthesised ZnO nanocrystals. The XRD pattern matching that of (JCPDS-36-1451) card for ZnO confirmed the presence of pure ZnO NPs. SEM analysis displayed the shape of NPs to be hexagonal. The EDX revealed the composition of ZnO and a good peaks intensity are due to zinc and oxygen which indicated the formation of ZnO. The aqueous extract of beta vulgaris and the seed of abrus precatorius mediated ZnO showed various antimicrobial activity against (G-) negative of *Escherichia Coli* and (G+) positive *Staphylococcus aureus*. The antifungal activity was also tested against Candida albicans fungi with all of these clinical pathogens compared to the standard drug, suggesting that the plant based synthesis of NPs can be an excellent strategy to develop versatite and eco- friendly biomedical product.

Keywords: Antimicrobial, Biosynthesis, *Beta vulgaris* plant, Seed of *Abrus precatorius*, ZnO NPs, Nanopowder.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.56

How to cite this article: Mohammed MJ, Mahdi WK, Musa FH. Green Synthesis, Characterization, and Biological Activity of Zinc Oxide Nanoparticles using Aqueous Extract of *Beta Vulgaris* and the Seed of *Abrus precatorius*. International Journal of Drug Delivery Technology. 2021;11(3):983-987.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In recent years, the development of efficient green chemistry methods for synthesis of metal oxide nanoparticles has become a major focus of researhers. Biomolecules presents in plant extracts can be used to obtain metal oxide nanoparticales in a single step green synthesis process. This method is easily scaled up.[1] Synthesis mediated by plant extract is environmentally benign. The plant extract involved the various water soluble plant metabolites (e.g alkaloids, phenolic compounds, terpenoids) and co-enzymes. In addition to plant extracts, live plants can be used for the synthesis. Here we review the methods of making Nps using plant extracts. Methods of particle characterization are reviewed, and potential applications of the particle in medicine are discussed.[2,3] In 2012, Talamm. S. *et al.* synthesized ZnO nanoparticles by precipitation method from zinc nitrate. The powder was diagnosed by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), selected region electron diffraction (SRED), UV-visible absorption spectroscopy, and photoluminescence.[4] In 2013, Vishwakarma. K *et al.* used an aqueous solution of *Abrus precatorius* seed extract and zinc acetate salt by Green synthesis method. The color change was observed, proving the formation of nanoparticles and the particles identified by vis-UV, DLS, FTIR, XRD, and SEM spectrophotometers.[5] Ritika. C. *et. al*, biosynthesis of silver and zinc oxide using pichia fermentans and their antimicrobial property. ZnO NPs were also smooth and elongated in shape. The antimicrobial activity of extracellular biosynthesized Ag and ZnO NPs was evaluated agains various pathogenic bacteria and fungi. The strong inhibitory activity of ZnO NPs was manifested against *Psedomonas Aeruginosa* and *Candida tropicalis*, Fusarium.[6] Prasanta Sutradhar & Mitali Saha, green synthesis of zinc oxide NPs using tomato extract and its photovoltaic application. The average size of the smaller particles (20 nm) at low power of 360 w increased upto 70 nm at 540 w with spherical shap.[7] Snehal. Y., *et al.,* synthesized zinc oxide NPs using Ixora Coccinea leaf extract. Highly

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stable and spherical zinc oxide nanoparticles were produced.[8] James. D. Onubun *et al.* were reported a green synthesis of ZnO catalysis by using $Zn(NO_3)_2$ and aqueous extract of plantain peel ash (ZnO–PTH of passing Hydrogen gas) and (ZnO-PT control experiment).[9] In 2019 Huzaifa. U. *et al.* studied the biocompatibility and stability of synthesized zinc oxide nanoparticles (ZnO NPs) and plants due to their wide applications in the fields of biomedicine, industrial, cellular imaging, and biological sensors. The antimicrobial, antioxidant, cytotoxic, and antiproliferative activities of NPs synthesized on human breast cancer cell lines were evaluated using various assays. The diagnosis of the synthesized ZnO NPs was performed using different spectroscopy and microscopic techniques. The antimicrobial activity was also evaluated. Assay of cytotoxic activity on MDA-MB 231 and MCF-7 using trypan blue dye exclusion and MTT assay. U.v-Vis showed an absorption peak in the range of 370 nm. The lebbeck bioactive compounds in ZnO NPs were confirmed by X-ray diffraction and Fourier infrared analysis. Zeta studies showed an average size of 66.25 nm with a polyvalent index of 0.262. SEM and EDX results revealed the irregular spherical morphology and presence of Zn, C, O, Na, P, and K, respectively. Biosynthetic ZnO NPs showed strong antimicrobial potential against pathogenic gram-negative and gram-positive bacteria. Moreover, the biosynthetic ZnO NPs showed cytotoxic effects on MDA-MB. 231 and MCF-7 breast cancer cell lines in a concentration-dependent manner.[10] In 2020 Bin. C., *et al.*, reported the green route to synthesize of zinc oxide NPs using leaf extracts of Cassia Fistula and Melia Azadarach, and their antibacterial, ZnO NPs were spherical, size range from 3 to 68 nm. The C. Fistula and M. Azadarach mediated ZnO NPs showed strong antimicrobial activity against clinical pathogens compared to standard drugs, suggesting that plant-based synthesis of NPs can be an excellent strategy to develop versatile and eco-friendly biomedical products.[11] In 2020, Liu.D et, al used radish roots (*Raphanus sativus* L.) where the root was dried, ground, and then suspended in distilled water and boiled for 30 minutes. The TEM images showed that the zinc oxide particles are spherical with a 25–40nm diameter and are collected in chains.[12] Irshad. S. *et al.,* used the extract of leaves, stems, and flowers of basil (L Ocimum basilicum) in the manufacture of zinc particles, where the plant parts were dried in the shade to prepare a powder. After cooling, filtration, and centrifugation of the plant extract, zinc diacetate solution was added to 100 mL of the plant extract at room temperature (25°C). The change of mixture color was indicated of the formation of zinc oxide particles, and the structure of the resulting particles was crystalline with a size of 30–40 nm. Inhibiting the growth of the fungus *Aspergillus niger*.[13] Abdullah FH, *et al*. study the, using of banana peels (*Musa acuminate* L.) in the manufacture of zinc oxide particles. The resulting particles were of different shapes, and their size ranged between 30 and 80 nanometers.[14] In 2020, Tu. UD *et. al.,* used a new method to prepare the green synthesis of ZnONPs nanoparticles, which reduces the use of toxic chemicals used to prepare the materials and enhance the activity of these particles against bacteria. An aqueous extract of orange fruit peel was used as a biological reducing agent, as well as the use of zinc acetate dihyrate). The study showed that the size and shape of zinc nanoparticles ZnONPs depend largely on physical and chemical data such as the degree of annealing temperature and pH through the preparation process. These particles also showed strong activity against two types of bacteria, *Escherichia coli* and *Staphylococcus aureus*.[15]

EXPERIMENTAL

MATERIALS AND METHODS

Collection of Samples: *Beta Vulgaris* and the seed of *Abrus Precatorius* were collected from the local source marked.

Chemicals Materials and Their Supplier Company: Zinc sulphate. hepta hydrate $ZnSO_4.7H_2O$ (Merk Germany),Sodium hydroxide NaOH (Alpha CHEMIKA india), EtOH (RBL spain). All these chemicals above were purchased and used without further purification.

Methods: Synthesis and characterization of ZnO NPs were carried out using various spectroscopic and microscopic techniques: UV-Vis type, Shimadzu UV-160 spectroscopy, and FTIR type Shimadzu (FTIR)-8500S are recorded at the University of Baghdad, College of science. The XRD type (Xpert Phillips Holland) was examined in the CAC Center laboratories for examinations at Baghdad. Scanning Electron Microscope with field emission (SEM) (FE) type Ziess sigma Hv 300) Germany, EDX device (Appendix). Transmission electron microscope (TEM). Type EM10C, 100Kv, Germany, were tested at Kashan University in Islamic Republic of Iran. Antimicrobial activity study for the synthesized ZnO NPs against two reference bacterial strains (Gram-negative *E. coli* and Gram-positive *S. aureus* and *C. albicans* fungal), were performed by using disc diffusion method in a nutrient medium (jellose medium) type muller hinton agar and the same method is applied for antifungal activity while used with the nutrient medium potato dextrose PDA (agar).

Preparation of ZnONPs Nanoparticles Using (*Beta Vulgaris*) *:*

To prepare fresh (*Beta vulgaris*) extract beta vulgaris is washed with tap water to remove any impurities, then it is dried for one day at room temperature of 37°C. About 20g of Beta vulgaris was mixed in 200 mL of deionized water was added and stirred well, and heated at a temperature of 60°C for 30 minutes. The color of the extracted solution changed to a red-purple color, and then it was left to cool to room temperature. The mixture was filtered by Whatman filter paper No 1. The resultant solution was transferred to a 1.5 mL centrifuge tube at 1200 rpm for 30 minutes, then repeated the washing step to ensure contamination.

The 0.1 M (2.87 g) of zinc sulfate. hepta hydrate solution was prepared in 100 mL distilled deionized water. To synthesize ZnO NPs, 100 mL of (*Beta vulgaris*) extract was added to it, and the mixture was stirred at 70°C, then added a drop wise of 1 M NaOH solution until PH will be 12. The mixture was left for 1 hour then left for one day to form a brown precipitate. The mixture was transferred to a centrifuge, then filtered and washed several times by distilled water and hot EtOH to remove

any imputities. The product was dried at 250°C at an electric oven for 5 hours to obtain a white powder.

Preparation of ZnONPs Nanoparticles Using (Abrus precatorius Seed)

To prepare fresh (*Abrus precatorius* seed) extract, the seed of abrus precantorius is washed with tap water to remove any impurities, then it is dried for one day at room temperature of 37ºC, and then grinding with an electric mill into a fine powder. About 20 g of abrus precantorius powder was mixed in 200 mL of deionized water, was added and stirred well, and heated at a temperature of 60ºC for 30 minutes. The color of the extracted solution changed to a violet color, and then it was left to cool to room temperature. The mixture was filtered by Whatman filter paper No 1. The resultant solution was transferred to a 1.5 mL centrifuge tube at 1200 rpm for 30 minutes, then repeated the washing step to ensure contamination.

The 0.1 M (2.87 g) of zinc sulfate. Hepta-hydrate solution was prepared in 100ml distilled deionized water. To synthesize ZnO NPs, 100 mL of (*Abrus precantorius* seed) extract was added to it, and the mixture was stirred at 70ºC, then added a dropwise of 1 M NaOH solution until PH will be 12. The mixture was left for 1-hour then left for one day to form a reddish-purple precipitate. The mixture was transferred to a centrifuge, filtered, and washed several times by distilled water and hot EtOH to remove any impurities. The product was dried at 250ºC at the electric oven for 5hr to obtain a taupe powder

RESULTS AND DISCUSSION

Optical analysis of ZnO NPs formation was done by adding zinc sulfate. Hepta-hydrate in extracts of *B. vulgaris* or *A. precatorius* seed leads to physical and chemical changes in the aqueous solution with PH = 12. The most prominent of which is a change in the color of the reaction mixture that can be observed within few minutes, This was considered as an initial signature to the formation of NPs. UV-visible, FTIR spectroscopy further examined the synthesis of ZnO NPs. Figure 1 the UV peaks recorded by the spectrophotometer.

The maximum absorption peak for ZnO NPs synthesized via *B. vulgaris*, or *A. precatorius* seed was recorded at 245 nm, and 275 nm indicated that further verified the formation of ZnO NPs.,[16] therefore the energy gap for ZnO is 4.5 eV. The energy gap or band gap was calculated using the following equation Eg = 1239.83/275 nm, Eg is the bulk band expressed in eV lambada (λ) is peak absorbance.[17] The FTIR spectrum Figures 2 and 3, shows disappear peaks in the range (950–1100) cm^{-1} are due to sulphate and appear peaks in the range (500–650) cm^{-1} are due to ZnO stretching vibration.[18]

Surface morphology of ZnO NPs.The presence of nanoparticles and examination of their structural properties are confirmed by XRD for *B. vulgaris* extract showed peaks with 2 values identified at (31.6°, 34.2°, 47.6°, 56.7°, 62.9°, 67.1° and 69.18°), which are index as (hkI) = (100), 002, 101, 102, 110, 103 and 112 planes as shown in Figures (4 and 5),[19] while XRD for the seed of abrus precatorius extract due to ZnO, 2 values showed (34.2°, 37.77°, 46.3°, 56.17°, 63.45°, 67.04° and 72.038°), these peaks are in accordance with those of data card (JCPDS-36-1451).

XRD pattern of ZnO NPs. A definite line broadening of the XRD peaks indicates that the prepared ZnO consist of particles in nanoscale range. From this XRD paterns analysis we determined peak intensity. position and width, FWHM data.The average crystal size for synthesized ZnO nanoparticle in diameter for both *B vulgaris* and the seed of *A. precatorius* extracts were calculated using Debye-Scherrers equation formula.[20] For ZnO NPs, d = 0.62 λ/B cos where K = 0.62 is Sc herrer constant, λ is the wave length of X-ray,Bragg diffraction angle, and B is the full width at half – maximum (FWHM)[21] (Dp for the top three peaks 2 = (31.6°, 34.2°, 36,1°), D = (27.5, 34.08, 32.4) nm, of *B. vulgaris*, while the avarage particle size of abrus precatorius seed for the top three peaks 2 = (34.2°, 48.10°, 56.17°),

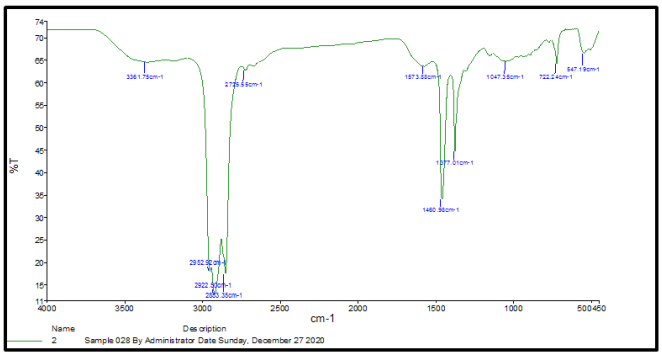
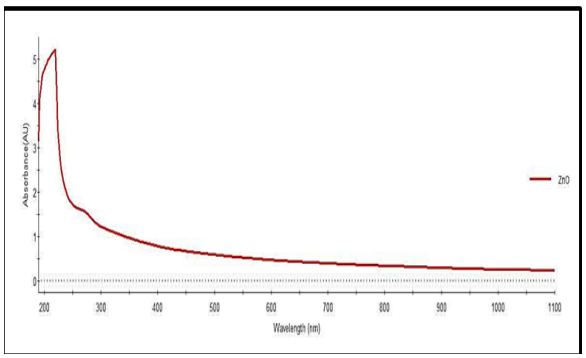


Figure 2: FTIR spectrum for prepared ZnO NPs form *B. vulgaris* extract



: U.v-Visible spectrum of ZnO NPs for *b. vulgaris* and *A. precatorius* seeds extracts

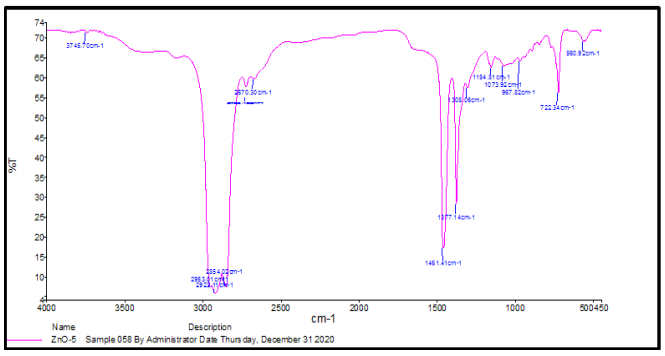


Figure 3: FTIR spectrum for prepared ZnO NPs form *A. precatorius* seed extract

D = (35.03, 23.27, 20.52) nm respectively,. These results are summarized briefly in Tables 1 and 2.

Figures 6 and 7 SEM, of ZnO NPs synthesized for *B. vulgaris* and the seed of *A. precatorius* extracts. The particles size range is 18.07–30.39 nm of beta vulgaris and the average particle size is 24.64 nm, while the particale size range of *A. precatorius* seeds is 17.84–41.94 nm with average partical size is 29.40 nm and that confirms the validity of the formation of nano-ZnONPs using both *B. vulgaris* and the seed of abrus precatorius extracts.[22]

Topographical view shows that nanoparticles are hexagonal in nature, clustered together and surface of the aggregates seem to be rough. The EDX spectrum confirms the presence of zinc and oxygen signals of zinc oxide. EDX revealed a strong signal for zinc at the energy 1.23 KeV and medium signal for oxygen at the energy 0.525 KeV and another signals due to carbon, sodium, potassium and silicon. These signals confirmed the presence of bioactive compounds of a – *B. vulgaris* or the seed of *A. precatorius* on the surface of the synthesized ZnO NPs as shown in Figure 8.[23]

The agar well diffusion and minimum inhibitory concentration experiments are conducted on ZnO NPs to perform a qualitative antimicrobial screening. The inhibition

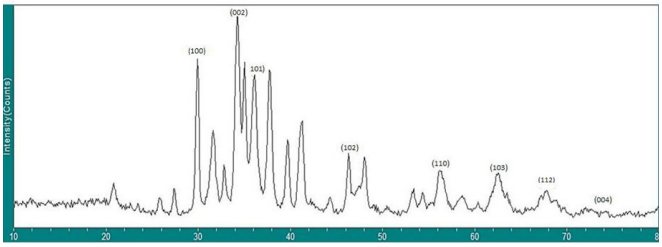


Figure 4: XRD spectrum of prepared ZnO NPs of *B. vulgaris* extract

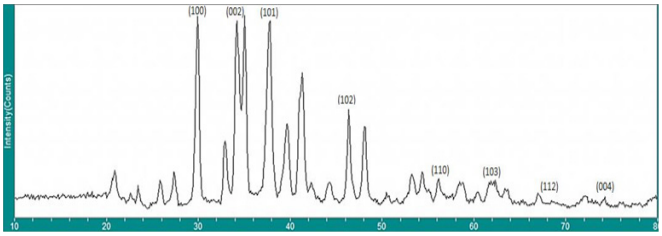


Figure 5: XRD spectrum of prepared ZnO NPs of *A. precatorius* seed extract

Table 1: Data obtained from the XRD spectrum of ZnO NPs for *beta vulgaris*

2-Theta	*d(nm)*	*BG*	*Height*	*1%*	*Area*	*1%*	*FWHM*	*XS (nm)*
31.604	0.28286	212	466	38	2672	30.9	0.487	17
34.209	0.2619	256	1225	100	8654	100	0.6	14
48.031	0.18927	185	303	24.7	1613	18.6	0.452	19
56.216	0.16349	174	224	18.3	1791	20.7	0.68	13
62.587	0.1483	176	202	16.5	1981	22.9	0.834	11
67.882	0.13796	166	87	7.1	772	8.9	0.754	12

Table 2: Data obtained from the XRD spectrum of ZnO NPs for *abrus precatorius* seed

2-Theta	*d(nm)*	*BG*	*Height*	*I%*	*Area*	*I%*	*FWHM*	*XS (nm)*
34.2	0.26196	298	1168	84.3	8484	100	0.617	13
37.773	0.23796	199	1270	91.6	8178	96.4	0.547	15
46.393	0.19556	143	657	47.4	2880	33.9	0.373	24
56.179	0.16359	147	137	9.9	547	6.4	0.339	27
63.45	0.14649	147	64	4.6	321	3.8	0.426	22
67.04	0.13949	104	76	5.5	236	2.8	0.264	39
72.038	0.13099	98	59	4.3	416	4.9	0.599	16

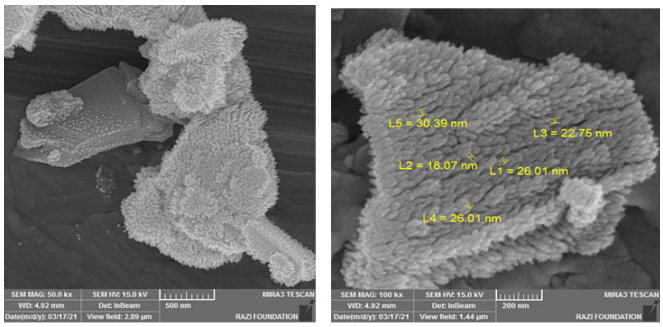


Figure 6: SEM images of ZnO NPs Synthesized using extract of *B vulgaris*

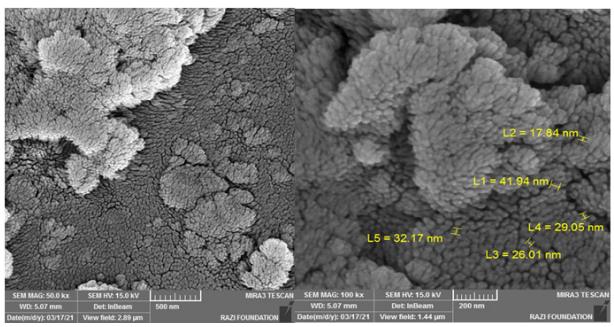


Figure 7: SEM images of ZnO NPs Synthesized using extract of *A. precatorius* seed

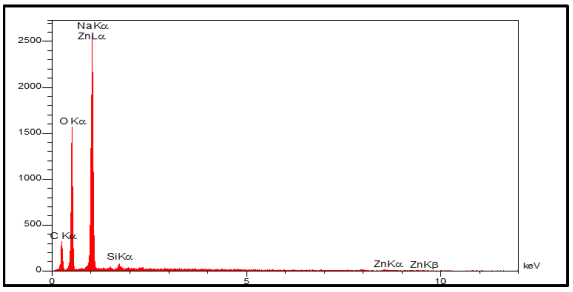


Figure 8: EDX energy dispersive X-ray spectroscopy of ZnO NPs
Antimicrobial Activity of ZnO NPs

zones at a concentration of (2 mg/mL) are obtained as *E. Coli* (20 mm), S. aures (30 mm), and *Candidia albicans* (27 mm) for ZnO NPs prepared from beta vulgaris or the seed of *A. precatorius* extracts. After this comprehensive analysis, it concluded that *S. aureus* has the highest inhibitory values with sample A1 compared with *E. coli* bacteria, and it is less inhibited with *C albicans*.[24]

CONCLUSION

The current study described a simple clean eco-freindly, safe, and inexpensive method for synthesizing ZnO NPs using two extracts of aqueous plants. The synthesized ZnO NPs,are subjected to spectroscopic investigation to ensure their nanostructures. The obtained results revealed the formation of nanoparticle size of 18.07–30.39 nm and 17.84–41.94 nm for ZnO NPs prepared using beta vulgaris and the seed of abrus precatorius extracts, respectively. These nanoparticles are screen for antimicrobial againts *E. Coli*, *S. aureus*, and *C. albicans*. It was found that nanoparticles showed improved antimicrobial.

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