Pak. j. sci. ind. res. Ser. A: phy. sci. 2023 **66A**(1) 63-73

Comparison of *Nigella sativa* Seeds Extract and Respective ZnO NPs for Antimicrobial, Antioxidant and Cytotoxic Potential

Umar Ali and Erum Dilshad*
Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences,
Capital University of Science and Technology (CUST), Islamabad-44000, Pakistan

(received July 7, 2021; revised May 11, 2022; accepted May 14, 2022)

Abstract. In the present work, ZnO NPs were synthesized by using zinc acetate salt solution and seeds extract of *Nigella sativa* L. in a ratio of 9:1, for a green chemistry approach being nontoxic and ecofriendly in nature. Colour change confirmed the formation of ZnO NPs. The synthesized nano structures characterized by UV-Vis spectrophotometer, SEM, EDX, FTIR and XRD. To evaluate the therapeutic efficacy of ZnO NPs various bioassays were performed such as antifungal, antibacterial, antioxidant and brine shrimp cytotoxicity.UV-Vis spectrophotometer generated the absorption peak at 370 nm for ZnO NPs. SEM results indicated the average size of ZnO NPs 35 nm with a hexagonal and crystalline structure which was confirmed through XRD. The active functional group of amines, methyl and OH^- were analyzed through FTIR. Zinc is the major constituent in the sample confirmed by the EDX spectrum. Biologically synthesized nanoparticles of *Nigella sativa* seeds extract showed significant antibacterial activity at 100 ppm, 80% antifungal activity at 25 ppm as compared to plant extract (15%). ZnO NPs also showed significant antioxidant potential (70%) at 100 ppm as compared to plant extract (51.72%) with EC_{50} 65.06 ppm for plant extract and 53.46 ppm for ZnO NPs. Significant cytotoxicity potential also observed against the brine shrimps with IC_{50} of ZnO NPs 147.4 ppm as compared to plant extract with IC_{50} 258.9 ppm. This is the first report stating the synthesis and antimicrobial, antioxidant and cytotoxic potential of ZnO NPs of *Nigella sativa* seed extract. These ZnO NPs can be further explored in various biomedical applications at the commercial level.

Keywords: antimicrobial, antioxidant, cytotoxic, *Nigella sativa*, zinc oxide nanoparticles

Introduction

Nanotechnology is the most advanced and emerging field in the present era. It encircles the application and production of nanoscale material in biological, chemical and physical systems (Murphy *et al*., 2008). It is also helpful in comparing cellular and molecular biology, information technology and semiconductor technology. Due to its potential, it has a positive impact on the life of humans and the treatment of multiple diseases (Ghosheh *et al*., 1999). Researchers in nano-technology have focused on the manufacturing of technically designed nano-materials, that can actively participate at the sub-cellular level of the body with high specificity as nano-medicines (Ahmad *et al*., 2008). The Zinc oxide nano-particles (ZnO NPs) have received much attention due to their astonishing properties and characteristics, these nanoparticles showed the ability of cytotoxicity in rapidly dividing cancer cells. They possess mechanical and thermal stability in chemical reactions and biological species (Zhang *et al*., 2011). This property of ZnO NPs makes them more attractive and effective in modern laser medical technology, electronics and opto-electronics.

Production of ZnO NPs by the method of green synthesis makes them bio-compatible, low toxic, and ecofriendly with increased applications in bio-medicine (Bacaksiz *et al*., 2008; Wang *et al*., 2005). The plant appears to be the leading and suitable platform for the synthesis of large-scale bio-synthesized nanoparticles (Albanese *et al*., 2012). Physical and chemical approaches are less used for the synthesis of ZnO NPs because these methods create a lot of problems with cost, bio-compatibility, particle size, size distribution and yields (Patra and Baek, 2014). Medicinal plants have played an effective role for thousands of years in human society. They are the only main source of natural pure phyto constituents. These phyto constituents are most commonly used in the treatment of various disorders, preventing epidemic diseases and also as food preservatives and flavour condiments (Silva and Fernandes Júnior, 2010).

*Author for correspondence; E-mail: dr.erum@cust.edu.pk

Nigella sativa (*N. sativa*) is one of the miracle plants that has rich historical and religious values. It is an annual herbaceous medicinal plant that belongs to the family Ranunculaceae, commonly known as ‘black cumin’ (Imran *et al*., 2022). In ancient times, *N. sativa* was most intensively used in different medical systems such as Ayurvedic, Chinese, Unani and Arabic for the curing of several disorders (Randhawa and Alghamdi, 2011). The extracts of *N. sativa* seeds actively played a therapeutic role in retarding the inflammation process and antioxidants activation, stopping the carcinogenic process, restraining the cough in patients, treating diarrhoea, abdominal pain, flatulence and polio (Al-Sheddi *et al*., 2014). The seed of *N. sativa* also contains proteins, saponin, alkaloids and essential oils (Khan *et al*., 2011). It also played an effective role in appetite stimulation, as a liver tonic and is helpful in the digestion of food, enhancing the milk production quantity in nursing mothers and to fight against the pathogens and supporting the immune system (Abel-Salam, 2012; Assayed, 2010; Abdel-Sater, 2009). The therapeutic properties of these plants are based on the main active phyto-constituents, thymoquinone (TQ) which is the main constituent of *N. sativa* seeds oil (Abdel-Zaher *et al*., 2011, Boskabady *et al*., 2010).

Materials and Methods

Synthesis of ZnO NPs. *N. sativa* seeds (20 g) were boiled in 100 mL distilled water in a 500 mL beaker for 30 min. The Whatman No.1 filter paper was used to eliminate the debris. Moreover, zinc acetate salt solution 0.25 M was prepared by adding 22.92 g zinc acetate salt in 500 mL of distilled water in the laboratory. The biosynthesis of ZnO NPs was done by mixing zinc acetate salt solution and seeds extracts of *N. sativa*in a ratio of 9:1. 5 mL seed extract was dissolved in 45 mL of zinc acetate salt manually on magnetic stirrers for 25 mins (Alaghemand *et al*., 2018). The reaction mixture was subjected to room temperature until its colour changed under sunlight. The colour change indicated the formation of the nanoparticles. All experiments were carried out in triplicate. The pellet of ZnO NPs was dried in a hot air oven at 60 °C temperature for up to 48 h (Gabriela *et al*., 2017). The ZnO NPs were obtained in the powdered form which was collected and preserved in the Eppendorf Tubes (Hashemi *et al*., 2016).

Characterization of ZnO NPs. UV-Vis spectrophotometer was used to recognize the optical properties of zinc oxide nanoparticles. The spectra were observed at 320-440 nm (Swamy *et al*., 2015; Gilani *et al*., 2004). The morphology of the synthesized ZnO NPs was identified by using Scanning Electron Microscope (JEOL-JSM-6490LATM) at the voltage of 20Kv with a frequency of 2838 cps (max) (Wang *et al*., 2005). Energy-dispersive X-ray spectroscopy was carried out for synthesized ZnO NPs to determine the elemental composition and their proportion in a sample that was coupled with Scanning Electron Microscope (SEM). Analysis of surface chemistry of ZnO NPs based on FTIR was performed. In the FTIR spectrometer, the solution was dried at the degree of 75 °C and characterization was done at the ranges of 4000-400 cm^{-1} by using the KBr pellet method (Alamdari *et al*., 2020). X-ray was bombarded on the targeted sample to analyze the structure of ZnO NPs. An X-ray diffractometer was used to generate an X-ray spectrum (Whitfield and Mitchell, 2004). The energy of the beam ranges in between 10-20 KeV which causes the X-rays to be emitted from samples. The electron beam moves across the samples and images are obtained for the synthesized ZnO NPs (Stevenson *et al*., 2005).

Antibacterial assay. The method used to analyze the antibacterial activity is a disc diffusion method described by (Ruparelia *et al*., 2008). Biologically synthesized zinc oxide nanoparticles used to determine the antibacterial activity against the gram-negative strains AT 10, *Enterobacter aerogenes, Salmonella setubal* and gram-positive strains *Bacillus subtilus, Salmonella aureus, Micrococcus letus*. The pellet (25 mg) of synthesized ZnO NPs was dissolved in the distilled water (25 mL) and the final concentration of 1000 ppm was made. During the antimicrobial assay, 10, 20, 30, 40, 50 and 100 ppm were used. Each Petri plates contain 8 discs, 6 discs contain the different concentration ZnO NPs samples in triplicates. Distilled water was used as negative control and streptomycin as a positive control. Discs were arranged on the solidified broth plates in a sequence. The Petri dishes were sealed and incubated for 24 h at 37 °C in a dark environment. After the incubation period of 24 h, the region of inhibition was observed in every disc and the zone of inhibition around every disc was measured (Huys *et al*., 2002).

Antifungal assay. The tube dilution method was used to determine the antifungal activity of biologically synthesized ZnO NPs (Singh and Nanda, 2013). There are the following strains that were used for the fungal activity *Aspergillus flavis, Aspergillus fumigates,*

Aspergillus niger, Mucor ans *Fusarium solani*, 4 mL of sabouraud dextrose agar was poured into each test tube to make a slant and cotton swabs were used to cover these test tubes. After that 100 µL of the sample (25 ppm final working concentration) was added to these test tubes. Slant was made to the 10 cm mark at room temperature inoculation lope was used for the introduction of fungal strains. The cotton plugs were used to cover. The whole experiment was performed in triplicate for each sample. Antifungal drug terbinafine was utilized as positive control and distilled water was taken as a negative control. The next step was the incubation of these tubes at 37 °C for 4 days. Negative control was taken as a reference for the determination of the fungal growth in a linear position. Reading was documented measuring the fungal growth in slanting position. The subsequent formula was utilized to calculate the proportion of fungal growth inhibition.

$$\text{Inhibition} = \frac{\text{Linear growth in negative control - linear growth in samples}}{\text{Linear growth in the negative control}} \times 100$$

Antioxidant assay. DPPH method (2,2-diphenyl-1-picrylhydrazyl-hydrate) was used to determine the antioxidant activity of biologically prepared ZnO NPs and extraction of *N. sativa* seeds that was descriptively mentioned earlier (Gyamfi *et al*., 1999). 200 µL serial solution of synthesized nanoparticles was added along with 2.8 mL of DPPH reagent. Ethanol was taken as a negative control and ascorbic acid was used as a positive control. Glass vials containing solutions were put in a dark environment for 50 min to determine the antioxidant property of ZnONPs. Ethanol blank was used as reference and absorbance of the sample was measured at 517 nm. The subsequent formula was applied to calculate the scavenging percentage of free radicals.

$$\%\ \text{Scavenging} = \frac{\text{Control absorbance - nanoparticle samples absorbance}}{\text{Absorbance of control}} \times 100$$

Brine shrimp lethality assay. The cytotoxic potential of prepared ZnONPs was determined by using brine shrimps lethality assay by following the method reported earlier (Bibi *et al*., 2011). In the first step, nanoparticles were added in glass vials at different concentrations of 25, 50 and 100 ppm with sea salt solution to make the final volume 5 mL. Distilled water was taken as a negative control. After 24 h, most of the shrimps were hatched from the eggs and young tiny shrimps were floating on the surface of sea water. They were shifted in each vails 15 in numbers. After the completion of this process, the glass vials were put at room temperature at 25 °C. After 24 h, a pasture pipette (3× magnifying glass) was used to count alive shrimps. Percentage mortality was calculated by the given formula, the whole procedure was carried out three times.

$$\%\ \text{Mortality} = \frac{\text{Number of alive shrimps in negative control - number of alive shrimps test}}{\text{Number of alive shrimps in the negative control}} \times 100$$

Results and Discussion

Synthesis and characterization. Change in the colour of the solution from colourless to light brown or yellowis the confirmatory sign of ZnONPs formation. This changed colour was observed after the 10 min when the mixing of zinc acetate salts solution was done with *N. sativa* seeds extracts (Gabriela *et al*., 2017). The extract of the plant reduced the zinc acetate salt into ZnO NPs. Furthermore, the ZnO NPs synthesis was tested for optical properties by the UV-Vis spectrophotometer. The wavelength of the UV-Vis spectrophotometer was set in between 300-500 nm. The spectra observed at 370 nm indicated ZnO NPs synthesis (Fig. 1) (Kumar *et al*., 2004). The size and morphology of synthesized ZnO NPs were observed by the SME analysis. The average size of ZnO NPs was observed to be 35 nm (Fig. 2) (Mohan and Renjanadevi, 2016). EDX (Energy dispersive X-ray spectroscopy) in operation with SEM was used to conclude the elemental composition, arrangements and chemical composition of biological synthesized ZnO NPs. The spectrum of EDX also confirmed the Zinc and oxygen as the main constituents without any contamination. The corresponding peak except for the oxygen and zinc *i.e*. carbon may be because carbon coated gird capping agents were utilized in scanning electron microscope measurements shows in (Fig. 3) (Tao *et al*., 2006). The crystalline alignment phase composition and phase identification of biologically synthesized ZnO NPs from *N. sativa* seed extract was confirmed by the XRD pattern. XRD pattern of the ZnO sample was observed by using index POWDER-X software as well as matched with standard JCPDS, 36–1451 data. The result showed that synthesized ZnO NPs have hexagonal wurtzite structure with crystalline nature with identification peaks having lattice parameter α=3.252(3) (Å), c=5.208(6) (Å). No other impurity/extra peak was detected which indicates

that obtained product is of high purity. The crystalline measurement was done by using Scherrer equation Dc=0.9 $\lambda/\beta\cos\theta$ (Fig. 4) (Alaghemand *et al*., 2018; Kavyashree *et al*., 2015). *N. sativa* seeds were actively involved in the synthesis of ZnO NPs. In the modification of Zn-O bonding, the FTIR technique has been implemented for ZnO NPs. At room temperature, the KBR method was used to perform the FTIR measurement in the wave range of 4000-400 cm^{-1} (Al-Shabib *et al*., 2016). The FTIR spectrum showed the main band near 3225 cm^{-1} which represents the O-H (covalent bond) mode and FTIR represents the methyl rock mode at the band of 1384 cm^{-1}. Another mode N–H with a hydrogen bond was observed at the band of 690 cm^{1} (Fig. 5) reported by (Manju *et al*., 2016). This can be identified as the proteins of this plant are involved in ZnO NPs synthesis.

Antibacterial assay. The antibacterial activity of biologically synthesized ZnO NPs (10, 20, 30, 40, 50 and 100 ppm) was performed against six strains

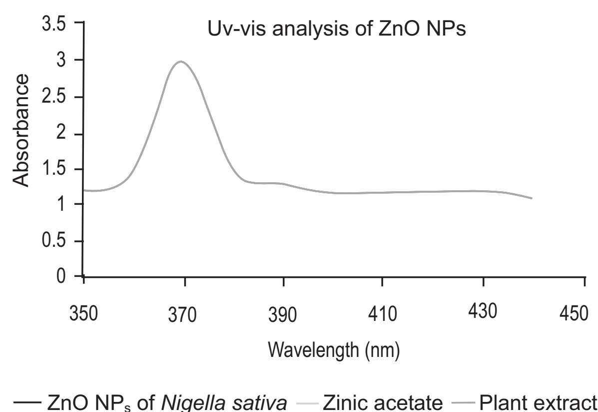

Fig. 1. UV-Vis spectroscopy. The spectra show the absorption peaks of ZnO NPs in the range of 350-450 nm.

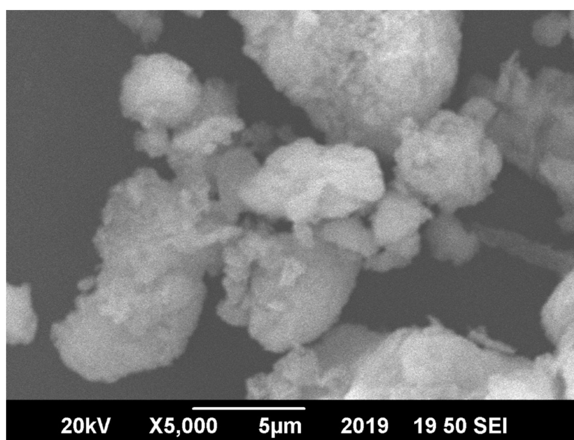

Fig. 2. Scanning electron microscopy (SEM) micrographs of ZnO NPs. The images are presenting the shape and size of the synthesized nanoparticles. Scale bar = 5 µm.

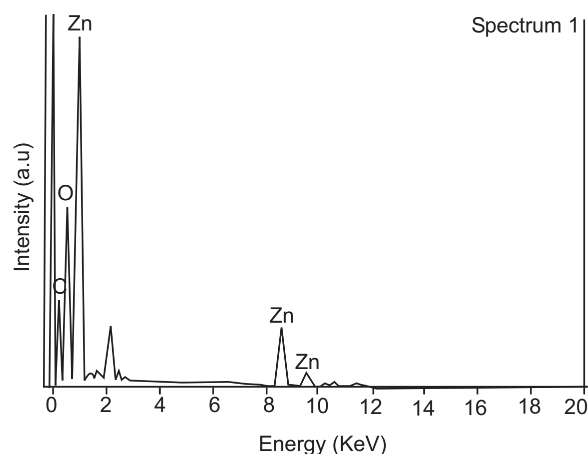

Fig. 3. Energy-dispersive X-ray spectroscopy, the EDS analysis of synthesized ZnO NPs and the spectrum shows Zn and O as the major component of nanoparticles.

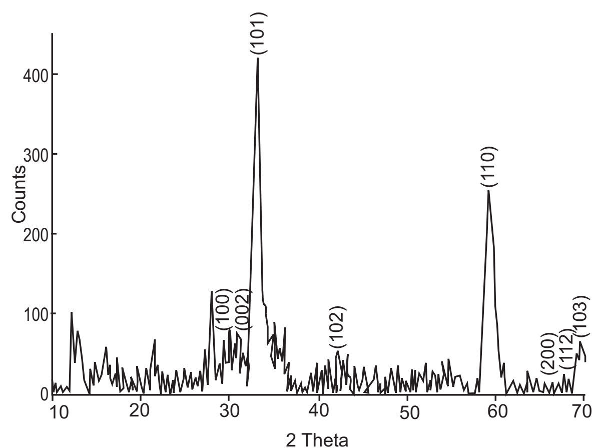

Fig. 4. X-ray diffraction (XRD) analysis of synthesized ZnO NPs. The spectrum shows hexagonal wurtzite structure with crystalline nature of ZnO NPs. The intensity on the vertical axis is measured in counts per second (CPS), and the diffraction angle (2 theta "θ") measured is taken along the horizontal axis.

Table 1. Antibacterial activity of synthesized ZnO NPs and plant seeds extracts (*Nigella sativa*)

ZnO NPs	Zone of inhibition (cm) ± S. E.											
	Gram positive strains						Gram negative strains					
Con (ppm)	*M. luteus*		*S. aureus*		*B. subtilis*		*A. tumefaciens*		*S. setubal*		*E. aerogenes*	
	ZnO NPs	P. extract	ZnO NPs	P. extract	ZnO NPs	P. extract	ZnO NPs	P. extract	ZnO NPs	P. extract	ZnO NPs	P. extract
10	1.2±0.1	-	-	-	0.7±01	-	-	-	1±0.15	-	0.8±0.1	-
20	1.4±0.1	-	-	-	1.9±0.5	-	-	-	1.2±0.1	-	1.2±0.1	-
30	1.6±0.15	-	1.5±0.1	-	1±0.1	-	-	-	1.5±0.3	-	1.7±0.1	-
40	1.9±0.1	-	1.5±0.1	-	1.5±0.1	-	-	-	1.6±0.1	-	2±2.0	-
50	2±0.1	-	1.8±0.1	-	2.5±0.1	-	-	-	1.8±0.1	-	2.5±0.3	-
100	2.3±0.1	15±0.1	2±0.1	11=0.1	3±0.05	12±0.1	1±0.15	-	2±0.1	1±0.1	2.5±0.1	11±0.1
-ve control	0	0	0	0	0	0	0	0	0	0	0	0
+ve control 100 ppm	3.1	3	2.7	2.7	3.5	3.2	1	1	3	3	3	3

ZnO NPs = zinc oxide nanoparticles; Conc = concentration; S.E = standard error; *M. luteus* = *Micrococcus luteus*; *S. aureus* = *Staphylococcus aureus*; *B. subtilis* = *Bacillus subtiles*; *A. tumefaciens* = *Agrobacterium tumefaciens*; *S. Setubal* = *Salmonella setubal*; *E. aerogenes* = *Enterobacter aerogenes*.

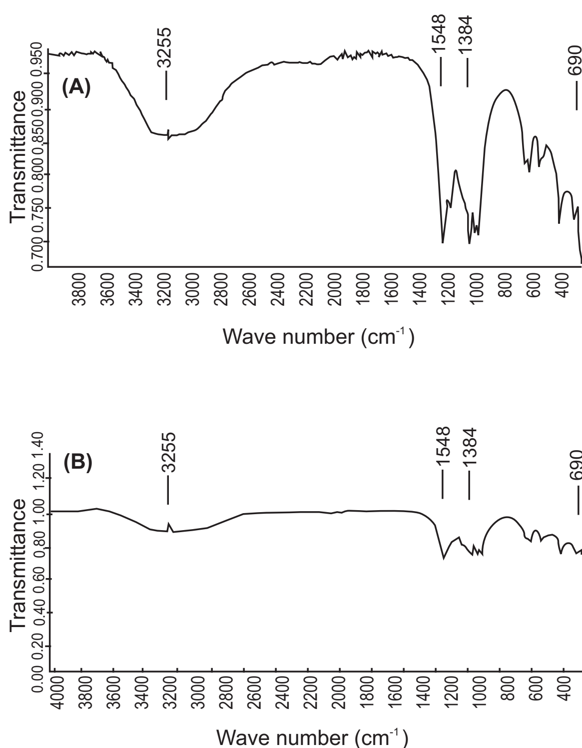

Fig. 5. FTIR analysis.FTIR analysis of plant extract **(A)** and ZnO NPs **(B)**. The FTIR spectrum showed the main band near 3225 cm^{-1} which represents the O-H (covalent bond) mode, and also represents the methyl rock mode at the band of 1384cm-1. Another mode N–H (primary and secondary amines only) with hydrogen bond was observed at the band of 690 cm^{-1}.

i.e. three-gram negatives (*A. tumefaciens, S. setubal, E. aerogenes*) and three gram-positive strains (*M. luteus, S. aureus, B. subtilis*). Results are mentioned in (Table 1). It was distinct that by increasing the concentration of ZnO NPs, a progressive increase in the inhibition of bacterial growth was observed (Wang *et al*., 2012). ZnO NPs were also tested to determine their minimum inhibitory concentration (MIC). The highest zone of inhibition was observed at 100 pm in both strains gram-positive (*M. luteus, S.aureus, B. subitils*) and gram-negative (*A. tumefaciens, S. Setubal, E. aerogenes*). Very minute inhibition activity was observed at 10 ppm of ZnO NPs in (gram-positive and gram-negative strains) (Emami-Karvani and Chehrazi, 2011). Moreover, ZnO NPs were also found more effective as compared to plant extract. It happens when the biologically synthesized ZnO NPs element interacts with the microbial cell surface (Djurišiæ *et al*., 2015). Teichoic acid in the peptidogly can layer of the gram-positive and lipoteichoic acid in gram-negative bacteria, both due to rich polyphosphate anions played as effective mediators for the entry of ZnO NPs (Xu *et al*., 2013; Hajipour *et al*., 2012). It was reported that ZnO nanoparticles exhibit antibacterial properties because of increased surface area to volume ratio and increased reactivity possibly by the mechanism of generation of reactive oxygen species. Oxidative stress produces free radical OH^- group and H_2O_2 that damage the DNA, cell membrane integrity aswell as metabolic and enzymatic functions of bacteria leading to ultimately cell death (Sirelkhatim *et al*., 2015).

Antifungal assay. The experiment was conducted against the different strains of fungi which are responsible for various diseases by using biologically prepared ZnO NPs and the *N. sativa*seeds extract. It was observed that ZnO NPs at 25 ppm showed maximum inhibitory effect against the *Fusarium solani* 80% and minimum inhibitions were observed against the *Mucor* species 20%. The ZnO NPs also showed moderate inhibition against the remaining three strains *Niger, Fumigtaus, flavis* which are 40, 40 and 50%, respectively. The results are mentioned in (Table 2). Distilled water was used as negative control and terbinafine for the positive control. The extracts of *N. sativa* seeds were also used in comparison against the ZnO NPs. Plant extract of *N. sativa* seeds only showed a significant effect against three strains of fungi except for the two strains *Mucor* and *Niger* species. In the case of seeds extract, the maximum antifungal activity was observed against *Solnia* 25%, *Flavis* 20% and *Fumigates* 15% (Table 2). *N. sativa* seeds extracts did not show any antifungal activity against Mucor 0% and Niger 0% (Kairyte *et al*., 2013).

After the comparison, it was proved that the synthesized ZnO NPs due to small size large surface to volume ratio and attachment of active secondary metabolites thymol, thymohydroqunine, saponins and alkaloids showed maximum antifungal effects as compared to just plant seeds extracts (Sharma *et al*., 2010).

Antioxidant assay (DPPH). To evaluate the antioxidant ability of synthesized ZnO NPs, the experiment was done by the DPPH (2,2 diphenyl-1-1 picrylhydrazyl hydrate) assay. The synthesized ZnO NPs showed anti-oxidant properties by donating an electron of oxygen toward the hydrogen. DPPH is one of the stable free radicals and can accept the electron from synthesized ZnO NPs and become stable diamagnetic molecules (Das *et al*., 2013). In this study, a comparison of synthesized ZnO NPs and seed extract of *N. sativa* was done. ZnO NPs revealed more antioxidant-free radical scavenging activity as compared to seeds extract. At 100 ppm concentration, the hexagonal ZnO NPs exhibited antioxidant scavenging free radicals up to 70.11%, whereas the extract of subjected seeds *N. sativa* plant at 100 ppm concentration showed 51.72% free radical scavenging activity. At 50 ppm the ZnO NPs exhibited 58.6% free radical scavenging activity and extract of *N. sativa* seeds showed 33.3% free radical scavenging activity (Hashemi *et al*., 2016). At the very lowest concentration, 25 ppm ZnO NPs pretend 49.9% antioxidant free radical scavenging activity and extract of *N. sativa* seeds showed 19.9% free radicals scavenging ability (Lee *et al*., 2010). The results were also found quite significant statistically ($P<0.0001$) (Table 3). EC50 53.46 ppm for zinc oxide nanoparticles was observed. However, plant extracts had a greater EC50 65.06 ppm which proved that ZnO NPs are more promising anti-oxidants as compared to plant extract (Fig. 6). To confirm the stability of the DPPH solution the solution was kept for 2 h without any disturbance. There was no change of colour of the solution that indicated the maximum stability of DPPH during the experiment (Das *et al*.,

Table 2. Percentage inhibition of ZnO NPs and plant seeds extracts (*Nigella sativa*) against different fungal species

Samples	Percentage inhibition against fungal species (%)				
	Mucor.sp	*F. Solani*	*A. Fumigatus*	*A. Flavis*	*A. Niger*
ZnO NPs	20	80	40	50	40
Plant extract	-	25	15	20	-
Distilled water (-ve Cont)	-	-	-	-	-
Terbinafine (+ve Cont)	100	100	100	100	100

ZnONPsz = zinc oxide nanoparticles; *Mucor*. sp = *Mucor* species; *F. Solani* = *Fusarium solani*; *A. fumigatus* = *Aspergillus fumigates*; *A. Flavis* = *Aspergillus flavis*; *A. Niger* = *Aspergillus niger*.

Table 3. Analysis of variance for factors affecting the free radical scavenging activity samples

Source of variation	Df	Sum of squares	Mean square	F-Value	P-value	Significant
Interaction	4	846.5	211.6	27.25	<0.0001	Yes
ZnO Nano particles and seeds extract	2	19110	9553	1230	<0.0001	Yes
Concentration	2	1653	826.4	106.4	<0.0001	Yes
Residual	18	139.8	7.767	_	_	_

2013). The absorption intensity was observed at 517 nm. When the ZnO NPs were added to the DPPH solution the absorption peak intensity was gradually decreased at 517 nm and the colour of the solution gradually changed from deep violet to pale yellow. The peak intensity at 517 nm of DPPH is inversely proportional to the amount of ZnO NPs. Antioxidant activity of ZnO NPs was based on the transfer of electrons located on oxygen atom toward hydrogen atom of DPPH due to this, the transition peak of DPPH at 517 nm decreased (Lewicka and Colvin, 2013). Biologically synthesized ZnO NPs and *N. sativa* seeds extract showed a significant impact as ($P<0.01$) against the free radical scavenging activity shown in (Fig. 6) (Table 3). This antioxidant activity of ZnO NPs and seed extract might be because of the bioactive compounds of *N. sativa* which were also utilized in the synthesis of ZnO NPs.

Cytotoxic assay. To evaluate the toxicity of ZnO NPs, the cytotoxic assay was performed on the brine shrimps. The different concentrations were used for ZnO NPs and plant seeds extract *i.e*. 100, 50 and 25 ppm, which showed significant toxic effects (Hameed *et al*., 2019). At the start of the cytotoxic assay to check the mortality of brine shrimps against the plant extract and ZnO NPs maximum concentration was taken. At the concentration of 100 ppm, ZnO NPs illustrated the 46% mortality of brine shrimp and *N. sativa* seeds extract showed 38%. At 50 ppm concentration, ZnO NPs confirmed the 33.3% mortality and plant seeds extract showed 26.6% mortality. At the concentration of 25 ppm, ZnO NPs proved 20% mortality and plant seeds showed 6.6% mortality. After comparison between the plant seeds extracts of *N. sativa* and synthesized ZnO NPs it was observed that ZnO NPs showed more mortality rate at 100 ppm 46% as compared to plant seeds extract at 38% (Fig. 7). It was also found that higher concentration corresponds to a higher mortality rate (Fig. 7). The result was proved significant statistically ($P<0.001$) (Table 4). After results analysis, it was observed that IC_{50} value ZnO NPs 147.4 ppm is less as compared to seeds extract of *N. sativa* IC_{50} value 258.9 ppm. ZnO NPs showed more significant results and hence can be used on cancer lines. It was suggested that a higher concentration of ZnO NPs directly related to the mortality rate of Brine shrimps. In an early study, ZnO NPs were reported to have maximum cytotoxicity due to several factors such as dose, time, small size large surface-to-volume ratio of ZnO NPs(Najim *et al*., 2014). Moreover, it was observed that biologically synthesized ZnO NPs from *N. sativa* seeds extract showed maximum anticancerous activity against the panel of human cancer cell lines (Nazrul Islam *et al*., 2004). It has also been observed ZnO NPs generated oxidative stress against the MCF-7 breast cancer cell lines and acted as an anticancerous agent (Awad, 2005). Moreover, ZnO NPs induced toxicity against the retinal ganglion cells. It was suggested that ZnO NPs inside the retinal ganglion cell lose the membrane potential of mitochondria and enhanced the over production of reactive oxygen species

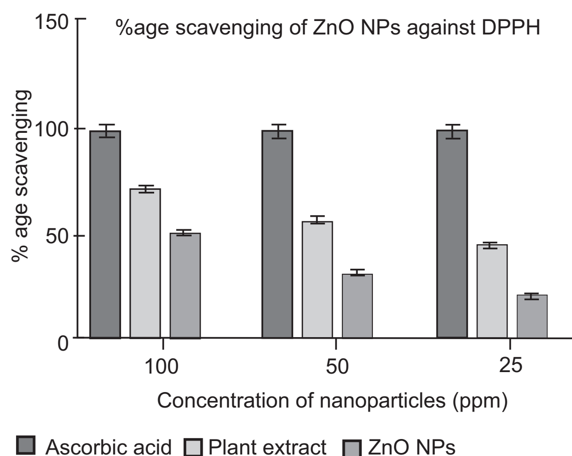

Fig. 6. Antioxidant activity of ZnO NPs and plant extract. Free radical scavenging or antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

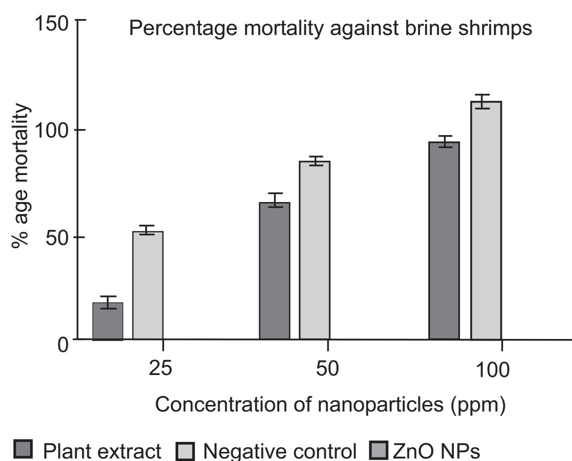

Fig. 7. Cytotoxicity of ZnO NPs and plant extract. cytotoxicity of ZnO particles against brine shrimps.

Table 4. Analysis of variance for factor affecting the mortality of brine shrimps

Source of variation	Df	Sum of squares	Mean square	F-Value	P-Value	Significant
Interaction	4	1154	288.4	7.819	<0.0001	Yes
ZnO Nano particles and seeds extract	2	21790	10890	295.3	<0.0001	Yes
Concentration	2	2339	1169	31.70	<0.0001	Yes
Residual	18	664.0	36.89	_	_	_

(ROS) and also enhanced the caspase-12 in RGC-5 cells. This oxidative stress and over disturbance triggered the endoplasmic reticulum to promote the RGC-5 damage and finally induced apoptosis or necrosis (Guo *et al*., 2013).

Conclusion

*N. sativa*is a medicinal plant with a common name (black cumin) and seeds extract was used for the biological synthesis of ZnO NPs. It was analyzed that biologically active compounds of plants played a vital role in the synthesis process and act as reducing and capping agents. Synthesized ZnO NPs were found active members in antimicrobial, antifungal, antioxidant and cytotoxic processes and can be explored further as (bioimaging, antibacterial, antifungal, anticancerous drugs). After the evaluation of synthesized ZnO NPs through Uv-Vis, SEM, XRD, FTIR, cytotoxic and antioxidant assay, antibacterial and antifungal assays it was proved that bio-activities of ZnO NPs were enhanced as compared to plant extract with less IC_{50} values. So, these stable metallic nanoparticles can also be used in nano-medicines, controlled drug treatments in cancer, and appropriate targeted drug delivery in the future.

Acknowledgment

We are grateful to the Capital University of Sciences and Technology, Islamabad Pakistan for providing us with a platform to conduct the current research work.

Conflict of Interest. The authors declear they have no conflict of interest.

References

Abdel-Sater, K.A. 2009. Gastroprotective effects of *Nigella sativa* oil on the formation of stress gastritis in hypothyroidal rats. *International Journal of Physiology, Pathophysiology and Pharmacology*, **1:** 143-149. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047244

Abdel-Zaher, A.O., Abdel-Rahman, M.S., ELwasei, F.M. 2011. Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. *Neurotoxicology*, **32:** 725-733. //www.sciencedirect.com/science/article/abs/pii/S0161813X11001306.

Abel-Salam, B.K.A. 2012. Immunomodulatory effects of black seeds and garlic on alloxan-induced diabetes in albino rat. *Allergologia et Immunopathologia*, **40:** 336-340. //www.sciencedirect.com/science/article/abs/pii/S0301054611002564

Ahmad, M.S., Hussain, M., Hanif, M., Ali, S., Qayyum, M., Mirza, B. 2008. Di-and triorganotin (IV) esters of 3,4-methylenedioxyphenylpropenoic acid: synthesis, spectroscopic characterization and biological screening for antimicrobial, cytotoxic and antitumor activities. *Chemical Biology and Drug Design*, **71:** 568-576. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1747-0285.2008.00668.x

Al-Shabib, N.A., Husain, F.M., Ahmed, F., Khan, R.A., Ahmad, I., Alsharaeh, E., Khan, M.S., Hussain, A., Rehman, M.T., Yusuf, M., Hassan, I. 2016. Biogenic synthesis of zinc oxide nanostructures from *Nigella sativa* seed: prospective role as food packaging material inhibiting broad-spectrum quorum sensing and biofilm. *Scientific Reports*, **6:**1-15. https://www.nature.com/articles/srep36761

Al-Sheddi, E.S., Farshori, N.N., Al-Oqail, M.M., Musarrat, J., Al-Khedhairy, A.A., Siddiqui, M.A. 2014. Cytotoxicity of *Nigella sativa* seed oil and extract against human lung cancer cell line. *Asian Pacific Journal of Cancer Prevention*, **15:** 983-987. https://www.koreascience.or.kr/article/JAKO201417638007739.page

Alaghemand, A., Khaghani, S., Bihamta, M.R., Gomarian, M.,Ghorbanpour, M. 2018. Green synthesis of zinc oxide nanoparticles using *Nigella sativa* l. extract: the effect on the height and number of branches. *Journal of Nanostructures*, **8:** 82-88. https://jns.kashanu.ac.ir/article_47785_0.html

Albanese, A., Tang, P.S., Chan, W.C. 2012. The effect of nanoparticle size, shape and surface chemistry on biological systems. *Annual Review of Biomedical Engineering*, **14:** 1-16. https://www.annualreviews.org/doi/abs/10.1146/annurev-bioeng-071811-150124

Alamdari, S., Sasani, G.M., Lee, C., Han, W., Park, H.H., Tafreshi, M.J., Afarideh, H., Ara, M.H.M. 2020. Preparation and characterization of zinc oxide nanoparticles using leaf extract of *Sambucus ebulus*. *Applied Sciences*, **10:** 3620-3639. https://www.mdpi.com/2076-3417/10/10/3620

Assayed, M.E. 2010. Radioprotective effects of black seed (*Nigella sativa*) oil against hemopoietic damage and immuno-suppression in gamma-irradiated rats. *Immunopharmacology and Immunotoxicology*, **32:** 284-296. https://www.tandfonline.com/doi/abs/10.3109/08923970903307552

Awad, E. 2005. *In vitro* decreases of the fibrinolytic potential of cultured human fibrosarcoma cell line, ht1080, by *Nigella sativa* oil. *Phytomedicine*, **12:** 100-107. https://www.sciencedirect.com/science/article/abs/pii/S0944711304000868

Bacaksiz, E.M.Ý.N., Parlak, M.E.H.M.E.T., Tomakin, M.U.R.A.T., Ozçelik, A., Karakýz, M., Altunbaº, M. 2008. The effects of zinc nitrate, zinc acetate and zinc chloride precursors on the investigation of structural and optical properties of zno thin films. *Journal of Alloys and Compounds*, **466:** 447-450. https://www.sciencedirect.com/science/article/abs/pii/S0925838807021998

Bibi, G., Ullah, N., Mannan, A., Mirza, B. 2011. Antitumor, cytotoxic and antioxidant potential of *Aster thomsonii* extracts. *African Journal of Pharmacy and Pharmacology*, **5:** 252-258. https://academicjournals.org/journal/AJPP/article-abstract/42B9B3629737

Boskabady, M.H., Mohsenpoor, N., Takaloo, L. 2010. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. *Phytomedicine*, **17:** 707-713. https://www.sciencedirect.com/science/article/abs/pii/S0944711310000188

Das, D., Nath, B.C., Phukon, P.,Dolui, S.K. 2013. Synthesis of ZnO NPs and evaluation of antioxidant and cytotoxic activity. *Colloids and Surfaces B: Biointerfaces*, **111:**556-560. https://www.sciencedirect.com/science/article/abs/pii/S0927776513004189

Djurišiæ, A.B., Leung, Y.H., Ng, A.M., Xu, X.Y., Lee, P.K., Degger, N., Wu, R.S.S. 2015. Toxicity of metal oxide nanoparticles: mechanisms, characterization and avoiding experimental artefacts. *Small*, **11:** 26-44. https://onlinelibrary.wiley.com/doi/abs/10.1002/smll.201303947

Emami-Karvani, Z., Chehrazi, P. 2011. Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. *African Journal of Microbiology Research*, **5:** 1368-1373. https://academicjournals.org/journal/AJMR/article-abstract/5874EE330248

Gabriela, A.M., Gabriela, M.D.O.V., Luis, A.M., Reinaldo, P.R., Michael, H.M., Rodolfo, G.P., Roberto, V.B.J. 2017. Biosynthesis of silver nano-particles using mint leaf extract (*Mentha piperita)* and their antibacterial activity. *Advanced Science, Engineering and Medicine*, **9:** 914-923. https://www.ingentaconnect.com/contentone/asp/asem/2017/00000009/00000011/art00002

Ghosheh, O.A., Houdi, A.A., Crooks, P.A. 1999. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). *Journal of Pharmaceutical and Bio-medical Analysis*, **19:** 757-762. https://www.sciencedirect.com/science/article/abs/pii/S0731708598003008

Gilani, A.U.H., Jabeen, Q., Khan, M.A.U. 2004. A review of medicinal uses and pharmacological activities of *Nigella sativa*. *Pakistan Journal of Biological Sciences*, **7:** 441-451.

Guo, D., Bi, H., Liu, B., Wu, Q., Wang, D., Cui, Y. 2013. Reactive oxygen species-induced cytotoxic effects of zinc oxide nanoparticles in rat retinal ganglion cells. *Toxicology in Vitro*, **27:** 731-738. https://www.sciencedirect.com/science/article/abs/pii/S0887233312003396

Gyamfi, M.A., Yonamine, M., Aniya, Y. 1999. Free-radical scavenging action of medicinal herbs from ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. General Pharmacology: *The Vascular System*, **32:** 661-667. https://www.sciencedirect.com/science/article/abs/pii/S0306362398002389

Hajipour, M.J., Fromm, K.M., Ashkarran, A.A., deAberasturi, D.J., deLarramendi, I.R., Rojo, T., Serpooshan, V., Parak, W.J., Mahmoudi, M. 2012. Antibacterial properties of nanoparticles. *Trends in Biotechnology*, **30:** 499-511. https://www.sciencedirect.com/science/article/abs/pii/S0167779912000959

Hameed, S., Khalil, A.T., Ali, M., Numan, M., Khamlich, S., Shinwari, Z.K., Maaza, M. 2019. Greener synthesis of ZnO and Ag–ZnO NPs using *Silybum marianum* for diverse biomedical applications. *Nanomedicine*, **14:** 655-673. https://www.futuremedicine.com/doi/full/10.2217/nnm-2018-0279

Hashemi, S., Asrar, Z., Pourseyedi, S., Nadernejad, N. 2016. Green synthesis of ZnO NPs by olive (*Olea europaea*). *IET Nanobiotechnology*, **10:** 400-404.

Huys, G., D'haene, K., Swings, J. 2002. Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. *Letters in Applied Microbiology*, **34:** 402-406. https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1046/j.1472-765X.2002.01109.x

Imran, M., Khan, S.A., Abida, Alshammari, M.K., Alkhaldi, S.M., Alshammari, F.N., Kamal, M., Alam, O., Asdaq, S.M.B., Alzahrani, A.K., Jomah, S. 2022. *Nigella sativa* L. and COVID-19: a glance at the anti-COVID-19 chemical constituents, clinical trials, inventions and patent literature. *Molecules*, **27:** 2750-2765. https://doi.org/10.3390/molecules27092750

Kairyte, K., Kadys, A., Luksiene, Z. 2013. Antibacterial and antifungal activity of photoactivated ZnO NPs in suspension. *Journal of Photochemistry and Photobiology B: Biology*, **128:** 78-84. https://www.sciencedirect.com/science/article/abs/pii/S1011134413001632

Kavyashree, D., Anandakumari, R., Nagabhushana, H., Basavaraj, R.B., Raja Naik, H., Lingaraju, K. 2015. Kalonji seed extract mediated green synthesis of ZnO nanopowders: photocatalytic, antibacterial and antioxidant activities. *Materials Science Forum*, **830:** 533-536. https://www.scientific.net/MSF.830-831.533

Khan, A., Chen, H.C., Tania, M., Zhang, D.Z. 2011. Anticancer activities of *Nigella sativa* (black cumin). *African Journal of Traditional, Complementary and Alternative Medicines*, **8:** 226-232. https://www.ajol.info/index.php/ajtcam/article/view/67964

Kumar, V.S., Nagaraja, B.M., Shashikala, V., Padmasri, A.H., Madhavendra, S.S., Raju, B.D., Rao, K.R. 2004. Highly efficient ag/c catalyst prepared by electro-chemical deposition method in controlling micro-organisms in water. *Journal of Molecular Catalysis A: Chemical*, **223:** 313-319. https://www.sciencedirect.com/science/article/abs/pii/S138111690400562X

Lee, C.W., Mahendra, S., Zodrow, K., Li, D., Tsai, Y.C., Braam, J., Alvarez, P.J. 2010. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environmental Toxicology and Chemistry: An International Journal*, **29:** 669-675. https://setac.onlinelibrary.wiley.com/doi/full/10.1002/etc.58

Lewicka, Z.A., Colvin, V.L. 2013. Nanomaterial Toxicity, Hazards and Safety. In: *Springer Hand-Book of Nanomaterials.* Vajtai, R. (eds), Springer, Dordecht, Heidelberg, 1st edition, pp. 1117-1142, New York, USA. https://link.springer.com/chapter/10.1007/978-3-642-20595-8_32

Manju, S., Malaikozhundan, B., Vijayakumar, S., Shanthi, S., Jaishabanu, A., Ekambaram, P., Vaseeharan, B. 2016. Antibacterial, antibiofilm and cytotoxic effects of *Nigella sativa* essential oil coated gold nanoparticles. *Microbial Pathogenesis*, **91:** 129-135. https://www.sciencedirect.com/science/article/abs/pii/S0882401015300991

Mohan, A.C., Renjanadevi, B. 2016. Preparation of zinc oxide nanoparticles and its characterization using scanning electron microscopy (sem) and X-ray diffraction (XRD). *Procedia Technology*, **24:** 761-766. https://www.sciencedirect.com/science/article/pii/S2212017316301621

Murphy, C.J., Gole, A.M., Stone, J.W., Sisco, P.N., Alkilany, A.M., Goldsmith, E.C., Baxter, S.C. 2008. Gold nanoparticles in biology: beyond toxicity to cellular imaging. *Accounts of Chemical Research*, **41:** 1721-1730. https://pubs.acs.org/doi/abs/10.1021/ar800035u

Najim, N., Rusdi, R., Hamzah, A.S., Shaameri, Z., Mat Zain, M., Kamarulzaman, N. 2014. Effects of the absorption behaviour of ZnO nanoparticles on cytotoxicity measurements. *Journal of Nanomaterials*, **2014:**1-10. https://doi.org/10.1155/2014/694737

Nazrul-Islam, S.K., Begum, P., Ahsan, T., Huque, S., Ahsan, M. 2004. Immuno-suppressive and cytotoxic properties of *Nigella sativa*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **18:** 395-398. https://onlinelibrary.wiley.com/doi/abs/10.1002/ptr.1449

Patra, J.K., Baek, K.H. 2014. Green nanobiotechnology: factors affecting synthesis and characterization techniques. *Journal of Nanomaterials*, **2014:** 219-231. https://dl.acm.org/doi/abs/10.1155/2014/

417305

Randhawa, M.A., Alghamdi, M.S. 2011. Anticancer activity of *Nigella sativa* (black seed)a review. *The American Journal of Chinese Medicine*, **39:** 1075-1091. https://doi.org/10.1142/S0192415X1100941X

Ruparelia, J.P., Chatterjee, A.K., Duttagupta, S.P., Mukherji, S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*, **4:** 707-716. https://www.sciencedirect.com/science/article/abs/pii/S174270610700195X

Sharma, D., Rajput, J., Kaith, B.S., Kaur, M., Sharma, S. 2010. Synthesis of ZnO NPs and study of their antibacterial and antifungal properties. *Thin Solid Films*, **519:** 1224-1229. https://www.sciencedirect.com/science/article/abs/pii/S0040609010011880

Silva, N.C.C., Fernandes, A.J. 2010. Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, **16:** 402-413. https://www.scielo.br/j/jvatitd/a/y5c3zwC7vBhWvDpFsSbSFGS/abstract/?format=html&lang=en&stop=previous

Singh, P., Nanda, A. 2013. Antimicrobial and antifungal potential of zinc oxide nanoparticles in comparison to conventional zinc oxide particles. *Journal of Chemical and Pharmaceutical Research*, **5:** 457-463.

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., Hasan, H., Mohamad, D. 2015. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-micro Letters*, **7:** 219-242. https://doi.org/10.1007/s40820-015-0040-x

Stevenson, C.B., Duran, R.L., Barrett, K.A., Colarulli, G.C. 2005. Fostering faculty collaboration in learning communities: A developmental approach. *Innovative Higher Education*, **30:** 23-36. https://link.springer.com/article/10.1007/s10755-005-3293-3

Swamy, M.K., Sudipta, K.M., Jayanta, K., Balasubramanya, S. 2015. The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. *Applied Nanoscience*, **5:** 73-81. https://link.springer.com/article/10.1007/s13204-014-0293-6

Tao, A., Sinsermsuksakul, P., Yang, P. 2006. Polyhedral silver nanocrystals with distinct scattering signatures. *Angewandte Chemie, International Edition*, **45:** 4597-4601. https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.200601277

Wang, C., Liu, L.L., Zhang, A.T., Xie, P., Lu, J.J., Zou, X.T. 2012. Antibacterial effects of zinc oxide nanoparticles on *Escherichia coli* k88. *African Journal of Biotechnology*, **11:** 10248-10254. https://www.ajol.info/index.php/ajb/article/view/128329

Wang, J., Cao, J., Fang, B., Lu, P., Deng, S. and Wang, H. 2005. Synthesis and characterization of multipod, flower-like and shuttle-like ZnO frameworks in ionic liquids. *Materials Letters*, **59:** 1405-1408. https://www.sciencedirect.com/science/article/abs/pii/S0167577X04009966

Whitfield, P., Mitchell, L. 2004. X-ray diffraction analysis of nanoparticles: recent developments, potential problems and some solutions. *International Journal of Nanoscience*, **3:** 757-763. https://www.worldscientific.com/doi/abs/10.1142/S0219581X04002620

Xu, X., Chen, D., Yi, Z., Jiang, M., Wang, L., Zhou, Z., Fan, X., Wang, Y., Hui, D. 2013. Antimicrobial mechanism based on H_2O_2 generation at oxygen vacancies in ZnO crystals. *Langmuir*, **29:** 5573-5580. https://pubs.acs.org/doi/abs/10.1021/la400378t

Zhang, H., Chen, B., Jiang, H., Wang, C., Wang, H., Wang, X. 2011. A strategy for zno nanorod mediated multi-mode cancer treatment. *Biomaterials*, **32:** 1906-1914. https://www.sciencedirect.com/science/article/abs/pii/S0142961210014663