Research Article

Nano Biomed Eng

Antimicrobial Activity of ZnO Nanoparticles Prepared Using a Green Synthesis Approach

Nadia Jasim Ghdeeb✉, Nedal Ali Hussain
Department of Physics, College of Science, Mustansyriah University, Baghdad, Iraq

✉ Corresponding author. E-mail: nadiajasim127@uomustansiriyah.edu.iq

Received: Nov. 14, 2022; **Revised:** Jan. 30, 2023; **Accepted:** Jan. 29, 2023
Citation: N.J. Ghdeeb, N.A. Hussain. Antimicrobial activity of ZnO Nanoparticles prepared using a green synthesis approach. *Nano Biomedicine and Engineering*, 2023.
http://doi.org/10.26599/NBE.2023.9290003

Abstract

Zinc oxide nanoparticles (ZnO NPs) were synthesized from cinnamon and bay leaves using chemical and environment-friendly synthesis approaches. The biosynthesized NPs were characterized using X-ray diffraction (XRD), which confirmed their crystalline nature. The morphology of the NPs was analyzed using scanning electron microscopy. The XRD analysis data showed average grain sizes of 9.968, 18.547, and 29.983 nm for the ZnO NPs. Energy-dispersive X-ray spectrum and XRD patterns showed that the synthesized ZnO NPs were unadulterated. These ZnO NPs were then used as antibacterial agents against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). The antifungal activity of the biosynthesized NPs was also tested against common unicellular fungi (*Candida albicans*). The results showed that ZnO NPs extracted from cinnamon demonstrated a higher antibacterial activity compared with those of the NPs extracted from bay leaves or the chemically prepared ones.

Keywords: zinc oxide nanoparticles (ZnO NPs); structural properties; antimicrobial properties

Introduction

Nanotechnology is the synthesis and utilization of materials that have dimensions of 100 nm or less. It is used in materials science research, agriculture, food manufacture, cosmetics, medicine, and diagnostics. Nanoscale inorganic compounds demonstrate extraordinary antibacterial activity at extremely low concentrations because of their high surface-to-volume ratios and distinctive chemical and physical properties [1]. Also, these particles are more stable at high temperature and pressure [2]. Some of these compounds are considered nonhazardous and may even contain minerals essential to human nutrition. Metallic nanoparticles (NPs) and metal oxide NPs such as silver, gold, copper, titanium oxide, and zinc oxide (ZnO), are among the most antibacterial inorganic substances [3].

ZnO NPs are one of the most important metal oxide NPs. They are employed in various fields because of their distinct characteristics. ZnO NPs were initially used in the production of elastic materials because they impart abrasion resistance to elastic compound materials and have high polymer tenacity and vigor. These NPs are used in antiaging personal care products and are also useful in industry for the manufacture of concrete and electronics, and for photocatalysis and electrotechnology, among others.

ZnO is extensively used in personal care products such as cosmetics and sunblocks [4]. Also, ZnO NPs

show exceptional antibacterial, antimicrobial, and ultraviolet (UV) light-blocking properties. In fact, in the textile industry, they are incorporated into completed fabrics to impart appealing properties such as UV and visible light resistance and antibacterial and deodorant properties [5]. Several enzymes, including carbonic anhydrase, carbopeptidase, and alcohol dehydrogenase, are inactive in the absence of Zn, which is a necessary trace element in the human body, while the other two components (cadmium and zinc) are inactive. Mercury and cadmium are hazardous metals belonging to the same set of elements with similar electronic structures. These are required by the eukaryotes as they modulate numerous physiological processes [6, 7]. Using biologically mediated processes, a green synthesis technology for NPs has been developed in recent years as an alternative to the conservative physical and chemical approaches. Unicellular and multicellular organisms such as bacteria [8, 9], fungi [10], viruses [11], and algae [12] are involved in the biosynthesis of metal and metal oxide NPs.

These approaches are inexpensive, nontoxic, and environment-friendly. Metal ions are transformed into metal NPs by bacteria, which operate as small nanofactories, with the help of yeast and other biomolecular components that are concealed or synthesized by microbes. However, at present, only a few microbial species are used for the manufacture of ZnO NPs. Thus, there is a need to find additional microorganisms for the synthesis of ZnO NPs. This study investigates the microbe-mediated synthesis of ZnO NPs, the mechanism of NP synthesis and process optimization, and their prospective application as antibacterial agents and forage supplements in animal husbandry along with their toxicological risks to animals [13].

In this study, we synthesized ZnO NPs using environment-friendly and chemical approaches, examined their structural and surface properties, determined their effects on two species each of Gram-negative and Gram-positive bacteria and one species of fungi, and compared the results obtained using both methods.

Materials and Methods

In Fig. 1, for the preparation of plant extracts, cinnamon and bay leaves were chopped, washed with distilled water, and air-dried. Then, 20 g of the chopped leaves were weighed and mixed with deionized water. The aqueous extracts were obtained after boiling the washed leaves for 15 min, after which they were allowed to cool, and then were placed in different beakers. After cooling, the extracts were filtered using a filter paper and stored at 40 °C.

Zinc acetate (0.1 mol/L) was dissolved in 50 mL of deionized water in a beaker and placed on a magnetic stirrer for 15 min for homogenization. Then, 10 mL of cinnamon and 10 mL of bay leaves were added to 10 mL of Zn $(CH_3CO_2)_2{\cdot}2H_2O$ solution in a beaker using a magnetic stirrer for 10 min at room temperature for 24 h before the experiments.

The manufactured NPs were cycled at 6 000 r/min for 10 min, rinsed with ethanol, and then dripped in water before use. The samples were then transferred to a Petri dish and dried for 4 h at 100 °C in an oven.

ZnO NPs were prepared using zinc acetate dihydrate (Zn $(CH_3CO_2)_2{\cdot}2H_2O$，99%), which was supplied by a German company. After decomposition at room temperature, a concentration of 0.1 mol/L was obtained by dissolving 2.1949 g of zinc acetate in 50 mL of deionized water [14].

The solution was mixed using a magnetic stirrer for 15 min to complete the dissolution process and then left for 1 h to obtain a homogeneous solution.

Fig. 1 Steps of the preparation process of ZnO NPs using chemical and environment-friendly methods.

Results and Discussion

X-Ray diffraction examination

Figure 2 shows the X-ray diffraction (XRD) patterns of the ZnO NPs synthesized using cinnamon and bay leaves, and following a chemical synthesis process. The XRD patterns of all ZnO NPs showed a hexagonal structure with lattice parameters of $a = b =$ 249 and c = 206, as mentioned in Refs. [15–17].

Several extensive diffraction peaks were observed at 31.90°, 34.4098°, 36.20°, 47.12°, 56.40°, 62.48°, and 67.40°, corresponding to the (100), (002), (101), (102), (110), (103), and (112) crystal planes, respectively. The strongest peak was observed at (101) and indicated a favored growth plane, demonstrating the excellent cleanliness of the ZnO NP crops. The XRD patterns of the ZnO NPs synthesized using green technology were inconsistent as already stated in Ref. [18] with the drift depicted in Fig. 2. The production of ZnO NPs was confirmed using XRD analysis. The diameters of the NPs were set using Scherrer's equation as follows:

$$D = \frac{0.9\lambda}{\beta\cos\theta} \quad (1)$$

where D is the grain size of nanoparticles, λ is the wavelength of radiation, β is the full width at half maximum in radians, and θ is the diffraction degree [16–18]. NPs produced by green synthesis approach (using cinnamon and bay leaves) and chemical approach have mean diameters of 29.983, 9.968, and 18.547 nm, respectively. Table 1 shows the average diameters of the nanoparticles produced using chemical components and cinnamon and bay leaf extracts. Compared with the preparation of ZnO NPs using the chemical methods, the average diameter of the ZnO NPs extracted from cinnamon and bay leaves was smaller, and the increase in the width of the peaks led to a smaller crystal size (Table 1).

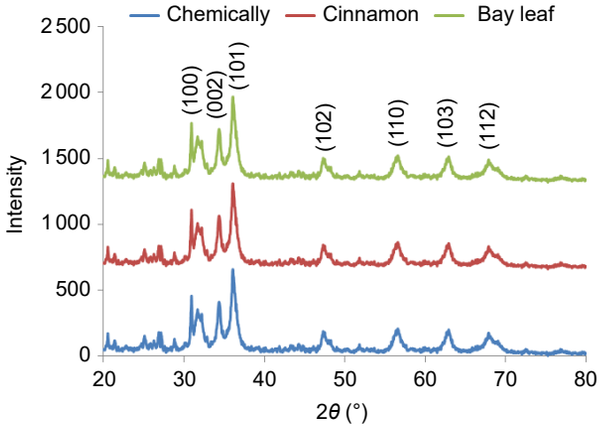

Fig. 2 XRD spectrum of ZnO NPs.

Scanning electron microscopy (SEM) analysis

Figure 3 shows the SEM morphologies of the ZnO nanoparticles generated chemically and those extracted from cinnamon and bay leaves. The shape of the spherical surfaces is shown in the SEM images. Because of their modest sizes, green synthetic structures exhibited minimal aggregation. Instead of using expensive and toxic capping chemicals to reduce agglomeration, plant extracts can function as both capping and reducing agents. Hence, this approach for producing ZnO nanoparticles is more cost-effective and ecofriendly than the other methods. The narrow-assimilation SEM images of the ZnO NPs synthesized from cinnamon and bay leaf extracts showed a hexagonal wurtzite structure with a smooth and well-defined contact surface [19, 20].

Table 1 Structural properties deduced from XRD results of ZnO nanoparticles

Sample		FWHM (rad)	2θ (°)	Diameter (nm)	Average diameter (nm)	*hkl*
ZnO chemical		0.004 647	31.701	31.230	29.983	(100)
		0.005 184	34.352	27.808		(002)
		0.005 484	36.152	26.597		(101)
		0.006 105	47.381	24.803		(102)
		0.003 489	56.145	45.603		(110)
		0.006 978	62.143	23.225		(103)
		0.005 457	67.824	30.620		(112)
ZnO green	Cinnamon	0.019 016	36.200	7.5851	9.968	(100)
		0.010 867	34.409	13.370		(002)
		0.013 730	31.906	10.624		(101)
		0.014 567	47.451	10.409		(102)
		0.018 318	56.490	8.601		(110)
		0.015 650	62.791	10.386		(103)
		0.018 981	67.861	8.809		(112)
	Bay leaf	0.009 954	31.761	14.398	18.547	(100)
		0.006 456	34.905	22.545		(002)
		0.008 466	36.140	17.331		(101)
		0.007 909	47.269	19.151		(102)
		0.011 100	56.477	14.191		(110)
		0.007 257	62.023	22.295		(103)
		0.008 374	67.422	19.921		(112)

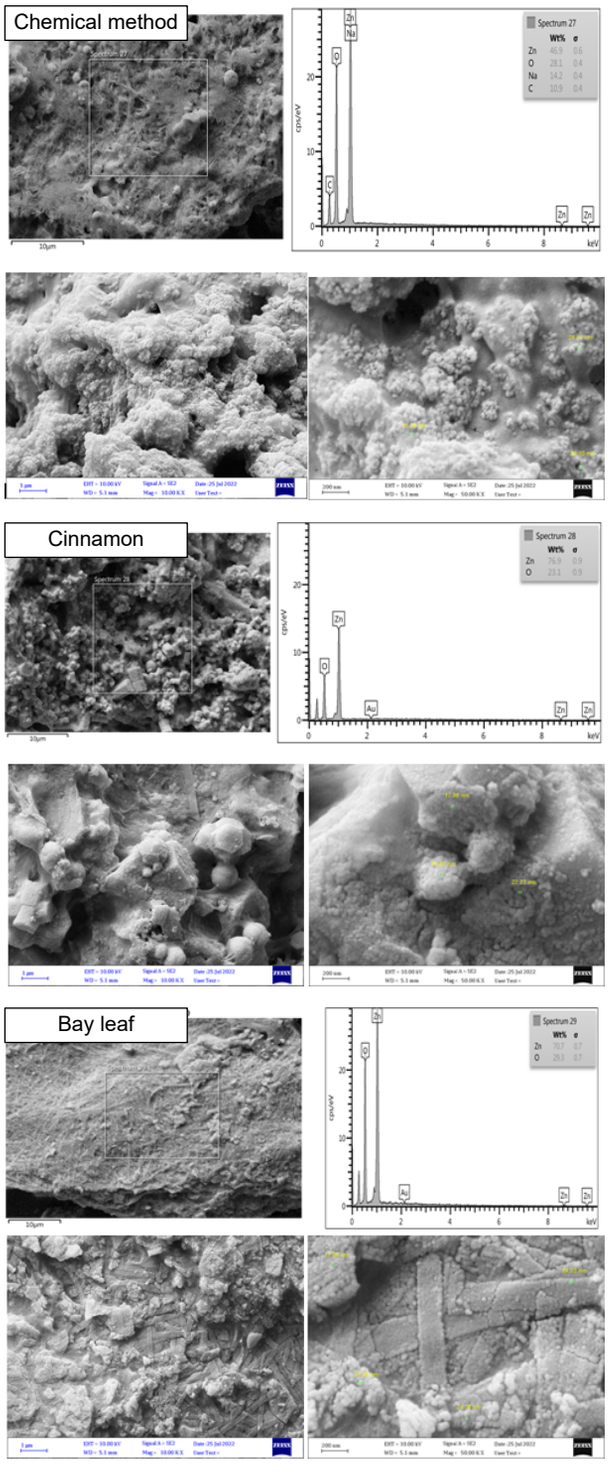

Fig. 3 SEM images of ZnO nanoparticles using chemical and green methods.

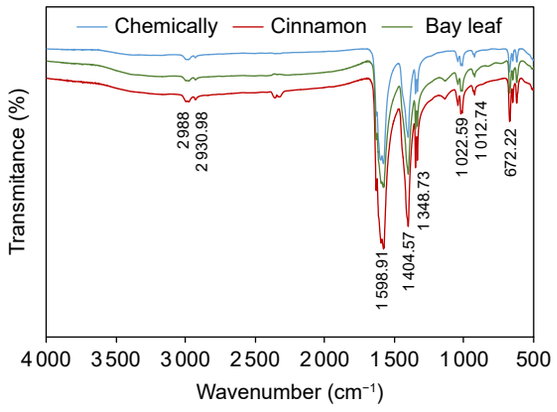

Fig. 4 FTIR spectrum of ZnO nanoparticles.

Fourier transform infrared (FTIR) analysis

Using FTIR spectroscopy, the expected functional sets of biomolecules present in a natural extract resulting from the reduction of zinc ions to ZnO NPs were discovered. Figure 4 shows the FTIR spectrum of the ZnO NPs synthesized from plant extracts. ZnO NPs produced using chemical methods and cinnamon and bay leaf extracts exhibited absorption peaks at 672.22, 1 012.47, 1 022.58, 1 348.73, 1 404.57, 1 588.91, 2 930.98, and 2 988 cm^{-1} on the FTIR spectrum.

The broad and prominent peaks between 1 500 and 2 000 cm^{-1} correspond to N—H, O—H, and C—H bonds; H-bonded phenols and alcohols; and the amide group stretching oscillations. C—O stretch vacillations are characterized by the bands in the neighborhood of 2 930.98 and 2 988 cm^{-1}. The presence of a band between 672.22 and 1 022.58 cm^{-1} in the spectrum suggests that ZnO NPs were synthesized, and these results agree with the findings of other studies [21–23].

The optical energy band gap of the ZnO NPs was calculated using the absorption edge. A very large energy gap was observed (Table 2). The increase in the band gap energies of ZnO NPs could be an indication of the quantum confinement effect because of the decrease in the structure size.

Table 2 Energy bandgap of the prepared ZnO NPs extracted from cinnamon, bay leaf, and using chemical synthesis

Sample	λ_{max} (nm)	E_g (eV)
Chemical	339.743	3.655
Cinnamon	319.044	3.892
Bay leaf	331.755	3.743

Electron transitions between the valence and conduction bands result in the intrinsic band gap absorption of ZnO. Because ZnO NPs absorb UV radiation, they can be used in sunscreen and antimicrobial ointments [24]. Band gaps in the ranges of 3.655, 3.892, and 3.743 eV were observed, which shows that ZnO NPs can be used in metal oxide semiconductor-based systems [25].

Antimicrobial activity

The antimicrobial activity of the synthesized ZnO NPs was examined against a variety of pathogenic bacteria, including Gram-positive (*Staphylococcus aureus* and *S. epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella* spp.). The antifungal activity of the synthesized NPs was also studied against a common unicellular fungus (*Candida albicans*).

The test was conducted using the agar disc technique in sterile, dishes (ϕ90 mm). Nutrition agar was prepared by dissolving 28 g of nutrient agar powder in 1 L of water. The medium was then sterilized in an autoclave at 121 °C at 1.5 MPa for approximately 15 min. The medium was then cooled to 45–50 °C and transferred to Petri dishes. Bacteria were cultured in Petri dishes using the diffusion method and dispersed uniformly onto nutritional agar plates with a sterile glass rod spreader. Wells were drilled into the bacterial and fungal media in each dish using a sterile gel drill/crook borer, proportional to the number of samples (materials), to determine the effect of these samples on the bacteria and fungi. Each well was filled with 100 µL of ZnO NPs and the plates were incubated at 37 °C for 24 h. The widths of the inhibition zones were measured after incubation. The inhibition zones were obtained for *E. coli* (10, 11, and 12 mm), *Klebsiella* spp. (13, 14, and 15 mm), *S. aureus* (11, 11, and 12 mm), *S. epidermidis* (12, 14, and 15 mm), and *C. albicans* (16, 16, and 17 mm) for ZnO NPs prepared using the chemical method and those extracted from cinnamon and bay leaves, respectively (Table 3). The inhibition zones obtained for ZnO NPs generated using the chemical approach showed antimicrobial efficacy against *E. coli* (12 mm), *Klebsiella* spp. (15 mm), *S. aureus* (12 mm), *S. epidermidis* (15 mm), and *C. albicans* (17 mm). The results suggest that the ZnO NPs generated from cinnamon exhibited superior antibacterial activity against all tested pathogens compared with the ZnO NPs synthesized from bay leaf and those synthesized using a chemical approach (Fig. 5). Additionally, the increased antibacterial action of the cinnamon-extracted ZnO NPs was characterized by modest size, shape, and nature of the biologically active compound present in cinnamon.

Conclusion

The phytoassisted synthesis of ZnO NPs using aqueous extracts of cinnamon and bay leaves is an environmentally friendly and cost-effective method for the synthesis and chemical preparation of ZnO NPs. The results of this study show that the tested pathogenic strains are susceptible to ZnO NPs, which confirm the potential efficacy of these NPs against certain bacterial strains. This makes it possible to extend the use of these NPs in the biomedical field.

Table 3 Inhibition zone of the ZnO and ZnO green nanoparticle activity against the tested pathogenic microbes

Microbial type	Organisms	Inhibition zone (mm)		
		Chemical method	Green method	
		ZnO Chemically (1)	ZnO Bay leaf (2)	ZnO Cinnamon (3)
Gram-negative	*Escherichia coli*	10	11	12
	Klebsiella spp.	13	14	15
Gram-positive	*Staphylococcus aureus*	11	11	12
	S. epidermidis	12	14	15
Fungi	*Candida albicans*	16	16	17

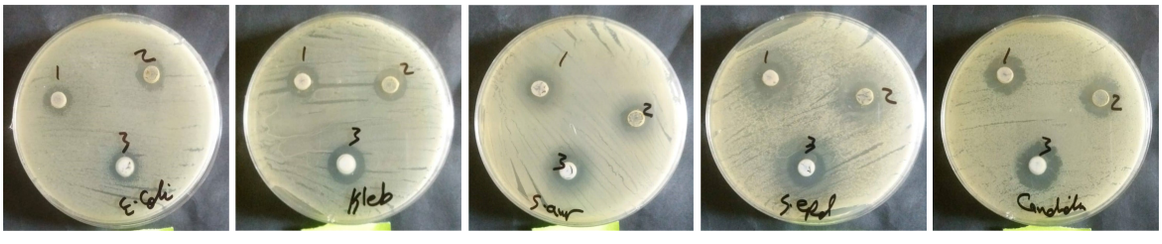

Fig. 5 Activity of ZnO nanoparticles prepared using chemical and green methods (cinnamon and bay leaf) against the tested microbial species (*S. aureus* and *S. epidermidis*, *E. coli*, *Klebsiella* spp., and *C. albicans*).

CRediT Author Statement

Nadia Jasim Ghdeeb: Conceptualization, investigation, methodology, project administration, supervision, visualization, writing (original draft), review, and editing. **Nedal Ali Hussain:** Data curation, writing, review, and editing.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

References

[1] K.S. Siddiqi, A. Ur Rahman, Tajuddin, et al. Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Research Letters*, 2018, 13(1): 141. https://doi.org/10.1186/s11671-018-2532-3

[2] K. Pradeev raj, K. Sadaiyandi, A. Kennedy, et al. Influence of Mg doping on ZnO nanoparticles for enhanced photocatalytic evaluation and antibacterial analysis. *Nanoscale Research Letters*, 2018, 13(1): 229. https://doi.org/10.1186/s11671-018-2643-x

[3] A. Kalawate, S.C. Sahoo, P.K. Khatua. Compatibility of nanobiocide as wood preservative with thermosetting resin used as adhesive for plywood manufacturing. *International Journal of Composite Materials and Matrices*, 2015, 1(1): 1−6.

[4] J.H. Jiang, J. Pi, J.Y. Cai. The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic Chemistry and Applications*, 2018, 2018: 1062562. https://doi.org/10.1155/2018/1062562

[5] A. Hatamie, A. Khan, M. Golabi, et al. Zinc oxide nanostructure-modified textile and its application to biosensing, photocatalysis, and as antibacterial material. *Langmuir*, 2015, 31(39): 10913−10921. https://doi.org/10.1021/acs.langmuir.5b02341

[6] S. Vickram, K. Rohini, S. Srinivasan, et al. Role of zinc (Zn) in human reproduction: A journey from initial spermatogenesis to childbirth. *International Journal of Molecular Sciences*, 2021, 22(4): 2188. https://doi.org/10.3390/ijms22042188

[7] K.P. Maremanda, S. Khan, G. Jena. Zinc protects cyclophosphamide-induced testicular damage in rat: Involvement of metallothionein, tesmin and Nrf2. *Biochemical and Biophysical Research Communications*, 2014, 445(3): 591−596. https://doi.org/10.1016/j.bbrc.2014.02.055

[8] D. Kundu, C. Hazra, A. Chatterjee, et al. Extracellular biosynthesis of zinc oxide nanoparticles using *Rhodococcus pyridinivorans* NT2: Multifunctional textile finishing, biosafety evaluation and *in vitro* drug delivery in colon carcinoma. *Journal of Photochemistry and Photobiology B*, *Biology*, 2014, 140: 194−204. https://doi.org/10.1016/j.jphotobiol.2014.08.001

[9] A.B. Moghaddam, M. Moniri, S. Azizi, et al. Biosynthesis of ZnO nanoparticles by a new pichia kudriavzevii yeast strain and evaluation of their antimicrobial and antioxidant activities. *Molecules*, 2017, 22(6): 872. https://doi.org/10.3390/molecules22060872 CASArticleGoogleScholar

[10] Shamsuzzaman, A. Mashrai, H. Khanam, et al. Biological synthesis of ZnO nanoparticles using C. albicans and studying their catalytic performance in the synthesis of steroidal pyrazolines. *Arabian Journal of Chemistry*, 2017, 10: S1530−S1536. https://doi.org/10.1016/j.arabjc.2013.05.004

[11] W.Q. Cao, W.Z. Wang, H.L. Shi, et al. Hierarchical three-dimensional flower-like Co_3O_4 architectures with a mesocrystal structure as high capacity anode materials for long-lived lithium-ion batteries. *Nano Research*, 2018, 11(3): 1437−1446. https://doi.org/10.1007/s12274-017-1759-0

[12] S. Azizi, M.B. Ahmad, F. Namvar, et al. Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalga *Sargassum muticum* aqueous extract. *Materials Letters*, 2014, 116: 275−277. https://doi.org/10.1016/j.matlet.2013.11.038

[13] H. Mohd Yusof, R. Mohamad, U.H. Zaidan, et al. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: A review. *Journal of Animal Science and Biotechnology*, 2019, 10: 57. https://doi.org/10.1186/s40104-019-0368-z

[14] N.K. Abdalameer, S.N. Mazhir, K.A. Aadim. The effect of ZnSe Core/shell on the properties of the window layer of the solar cell and its applications in solar energy. *Energy Reports*, 2020, 6: 447−458. https://doi.org/10.1016/j.egyr.2020.09.023

[15] Q. Tang, H. Xia, W. Liang, X. Huo, et al. Synthesis and characterization of zinc oxide nanoparticles from *Morus nigra* and its anticancer activity of AGS gastric cancer cells. *Journal of Photochemistry and Photobiology B*: *Biology*, 2020, 202: 111698. https://doi.org/10.1016/j.jphotobiol.2019.111698

[16] K. Ali, S. Dwivedi, A. Azam et al. *Aloe vera* extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. *Journal of Colloid and Interface Science*, 2016, 472: 145−156. https://doi.org/10.1016/j.jcis.2016.03.021

[17] G. Pal, P. Rai, A. Pandey. Green synthesis of nanoparticles: A greener approach for a cleaner future. In: *Green Synthesis, Characterization and Applications of Nanoparticles*, Amsterdam: Elsevier, 2019: 1–26. https://doi.org/10.1016/j.jcis.2016.03.021

[18] S. Sarkar, R. Sarkar. Synthesis, characterization and tribological study of zinc oxide nanoparticles. *Materials Today*: *Proceedings*, 2021, 44: 3606−3612. https://doi.org/10.1016/j.matpr.2020.09.595

[19] R. Verma, S. Pathak, A.K. Srivastava, et al. ZnO nanomaterials: Green synthesis, toxicity evaluation and new insights in biomedical applications. *Journal of Alloys and Compounds*, 2021, 876: 160175. https://doi.org/10.1016/j.jallcom.2021.160175

[20] M. Amde, Z.Q. Tan, R. Liu, et al. Nanofluid of zinc oxide nanoparticles in ionic liquid for single drop liquid microextraction of fungicides in environmental waters prior to high performance liquid chromatographic analysis. *Journal of Chromatography A*, 2015, 1395: 7−15. https://doi.org/10.1016/j.chroma.2015.03.049

[21] Q. Tang, H. Xia, W. Liang, X. Huo, et al. Synthesis and characterization of zinc oxide nanoparticles from *Morus nigra* and its anticancer activity of AGS gastric cancer cells. *Journal of Photochemistry and Photobiology B*: *Biology*, 2020, 202: 111698. https://doi.org/10.1016/j.jphotobiol.2019.111698

[22] D. Rehana, D. Mahendiran, R.S. Kumar, et al. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. *Biomedicine & Pharmacotherapy*, 2017, 89: 1067−1077. https://doi.org/10.1016/j.biopha.2017.02.101

[23] K. Ali, S. Dwivedi, A. Azam et al. *Aloe vera* extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. *Journal of Colloid and Interface Science*, 2016, 472: 145−156. https://doi.org/10.1016/j.jcis.2016.03.021

[24] M. Amde, Z.Q. Tan, R. Liu, et al. Nanofluid of zinc oxide nanoparticles in ionic liquid for single drop liquid microextraction of fungicides in environmental waters prior to high performance liquid chromatographic analysis. *Journal of Chromatography A*, 2015, 1395: 7−15. https://doi.org/10.1016/j.chroma.2015.03.049

[25] Alamdari, S., Sasani Ghamsari, M., Lee, C., et al. Preparation and characterization of zinc oxide nanoparticles using leaf extract of sambucus ebulus. *Applied Sciences*, 2020, 10(10): 3620. https://doi.org/10.3390/app10103620

© The author(s) 2023. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY) (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.