# ZnO Nanoparticles Synthesis using Potato Peel Waste and their Antifungal Activity

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Nanotechnology has considerably improved, or even revolutionized, many technology and industry sectors, e.g., information technology, energy, environmental science, medicine, homeland security, food safety, and transportation. Nanotechnology today uses recent advancements in chemistry, physics, materials science, and biotechnology to generate innovative materials with specific features even though their structures are defined at the nanoscale level. Nanoparticles are categorised as particles with sizes between 1 and 100 nm. Particles can act differently at smaller size scales than their bulk counterparts. "Green" synthesis has attracted a lot of interest in the field of material research as it is a trustworthy, durable, and environmentally friendly process for producing a variety of materials and nanoparticles, including hybrid materials, bioinspired materials, and metal/metal oxide nanomaterials. In order to investigate the synthesis, characterization, antioxidant and antifungal properties of zinc oxide nanoparticles (ZnO NPs), potato peel extract was used to synthesise zinc oxide nanoparticles for the current study. These NPs were also subjected to stability testing, visual examination, UV-visible spectroscopy, FTIR, XRD and other characterisation methods. Antifungal activity was tested against *Rosellinia necatrix*, *Sclerotinia sclerotiorum,* and *Fusarium spp*. The diameter of the zone of inhibition (ZIH) observed with ZnO NPs were: *Rosellinia necatrix* - 37.5%, *Fusarium spp* - 55.2%, and *Sclerotinia sclerotiorum* - 60.5%. This research has established the ZnO NP complex as a unique natural preservative with potential applications in agriculture.

**Keywords**: Nanoparticles; synthesis; antifungal activity; antioxidant activity; ZnO NPs

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Nanotechnology, the manipulation of nanoparticles for a variety of reasons, is crucial to the food and agricultural industries. Nanoparticles are evolving into cutting-edge materials that will change modern agriculture. Numerous formulations based on nanoparticles, including nano-sized insecticides, herbicides, fungicides, fertilisers and sensors, have been studied for managing plant health and enhancing soil. These have improved crop yields, raised food quality and safety standards, and enhanced human health [1]. Physical, chemical and biological approaches have been used in the production of NP. Physical methods may reduce solvent contamination, but the energy requirements for particle condensation and evaporation are substantial. The interest in biological NP synthesis has been due to its ecofriendly and efficient methods, as opposed to chemical and physical synthesis of NP. The reducing agent used in green synthesis - which may be bacteria, fungi, or plants and their extracts - changes the biological process that is employed [2]. The use of biological materials to make metallic nanoparticles in an environmentally friendly way has received a lot of attention [3]. Due to their distinctive physical, chemical and biological properties, high surface-to-volume ratio, and altered solubility and toxicity when compared to their macro scale counterparts, engineered nanometre-sized particles have attracted much attention in the fields of medicine, agro-food and sewage water treatment, among others. Because of their potential antibacterial properties, carbon nanoparticles, along with silver (Ag), gold (Au), zinc oxide (ZnO) and titanium dioxide ($TiO_2$), are currently produced 10 times more frequently than other nanomaterials. Home furnishings like air filters, food storage containers, deodorants, bandages, toothpaste and paint employ these components [4]. Top-down and bottom-up are the two main methods for creating nanoparticles. Whereas the bottom-up method begins with atoms, the top-down method starts with macro-sized materials and creates nanoparticles [5]. As the top-down strategy predominantly uses synthesis pathways from the simultaneous method and sequential method categories, the bottom-up strategy is more well-known and more sophisticated than the latter [6]. Precursors of the necessary metals must be used in the same process for the simultaneous approach (which can be bimetallic or trimetallic alloy clusters). After that, metal ions are reduced on the surface of another metal core to produce particles [7].

Green synthesis is preferable to conventional synthesis as it is less expensive, pollutant-free, and is safer in terms of both the environment and human health [8]. There are several potential applications for green nanoparticle synthesis in the environmental and biomedical fields [9]. Dependable, sustainable, and eco-friendly synthesis processes should be created to prevent the development of undesired or hazardous by-products. To do this, the best solvent systems and organic systems from nature may be used. Metallic nanoparticles generated sustainably contain a variety of biological components (e.g., bacteria, algae, fungi and plant extracts). The use of plant extracts is a reasonably simple and easy way to create metal/ metal oxide nanoparticles on a wide scale by an environmentally friendly process of synthesis. These materials are known as "biogenic nanoparticles" [10]. The antimicrobial, solar cell, electronics, photocatalysis and electrical technology industries can all use zinc oxide nanoparticles (ZnO NPs). As the US Food and Drug Administration (FDA) has recommended them as safe for both people and animals, they can also be used as food additives [11]. Due to their distinct chemical and physical characteristics, ZnO NPs are the most significant metal oxide nanoparticles [12-13]. In the current study, we describe the characterization, antioxidant and antifungal properties of zinc oxide nanoparticles (ZnO NPs) synthesised using potato peel extract.

## MATERIALS AND METHODS

### 1. Sample Collection and Authentication

The potato samples were collected from Solan, Himachal Pradesh. After collection, the potatoes were washed thrice with distilled water. The peels were collected and washed with tap water properly to remove any dirt or debris, then washed twice with distilled water. The washed peels were dried in the sun or in a cabinet dryer for 2-3 days and then ground into a fine powder which was sealed in a pouch at room temperature until further use. Then, 10 g of the potato peel powder was dissolved in 100 mL of distilled water and boiled for 30 min at 60 °C. After boiling, the mixture was filtered using Whatman paper. The ZnO NPs were produced using the filtrate that was collected [14].

### 2. Green Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

Zinc acetate dihydrate, Zn $(CH_3COO)_2{\cdot}2H_2O$ was added to the peel extract at a 7:3 (w/v) ratio. The pH was adjusted to 10-11 using 0.1 M NaOH. The mixture was then stirred continuously for about 2 h at 50 °C with a magnetic stirrer. When incubated at room temperature for 24-48 h in the dark, the reaction began within 10 minutes and the colour changed from light brown to light yellowish at various intervals before finally becoming a creamy yellow precipitate. After centrifuging the solution at 10,000 rpm for 10 min to get rid of any extraneous biological molecules, the pellet was dispersed in sterile distilled water [15]. To guarantee a good separation of free entities from ZnO NPs, the process of centrifugation and re-dispersion in sterile distilled water was carried out three times. Finally, ethanol was used to purify the suspended pellet. The pellet was then placed in a muffle furnace for three hours at 350 ℃ [16].

### 3. Characterization of Synthesized Nanoparticles

The characterization of nanoparticles is an important step to determine their shape, size and morphology. UV-vis absorption spectroscopy (Halo DB-20 UV-vis double beam) was used to analyse the synthesized ZnO NPs. The reaction mixtures were studied at wavelengths spanning from 200 to 800 nm. The ZnO NPs' crystalline makeup was investigated using XRD (Model-D8 Advance, Germany), while FTIR analysis (Spectrum One, PerkinElmer, and Waltham, MA, USA) was used to identify their functional groups. The surface morphology of ZnO NPs was studied using SEM microscopy [17], bacterial growth [18]. Dynamic light scattering (DLS) was performed by IIT Mandi.

### 4. Antifungal Study on the Peel Extract

The fungicidal potential of the synthesized ZnO NPs was measured by observing the growth response of three fungal strains (*Rosellinia necatrix, Fusarium spp,* and *Sclerotinia sclerotiorum*). Potato dextrose agar was used for fungal growth. A slightly modified method was used for measuring fungicidal capacities [19]. Sterilized PDA was mixed with 50 mg/mL extract, and a 6 mm-diameter disc of the pathogen that was actively growing and had been in the culture for 6-7 days was positioned in the middle of the plate. The negative control was a plate without any extract, while the positive control was Hygromycin at a concentration of 5 mg/mL. Growth was evaluated and compared to the controls [20]. After 6 days of incubation at 25 °C, the antifungal activity of the NPs against *Rosellinia necatrix, Fusarium spp.* and *Sclerotinia sclerotiorum* was assessed. Circular mycelium growth was also recorded. Zones of inhibition around the discs were measured [21-22].

## RESULTS AND DISCUSSION

### 1. Green Synthesis and Characterization of Zinc Oxide Nanoparticles

#### 1.1. Visual Examination

The transformation of the solution's colour served as the initial screening process for the creation of nano-particles. A change in the solution colour from light yellow to whitish yellow during the 24-48 h reaction confirmed that ZnO NPs had been produced. Studies in the literature have shown that as the incubation time increased, the solution changed colour from clear to yellowish brown, indicating the bioreduction of zinc oxide particles. The change in colour was caused by the stimulation of surface Plasmon resonance in solution [28]. Photographs of the solutions are presented in Figure 1.

### 1.2. UV-visible Spectroscopy of Synthesized ZnO NPs

When light passes through a medium, its amount of extinguishment (scatter plus absorption) is measured using UV-Vis. UV-Vis is a valuable approach to identify, characterise, and analyse nanomaterials. Nanoparticles exhibit particular optical properties that are sensitive to size, shape, concentration, aggregation state and refractive index close to the nanoparticle surface [23]. A study of UV-Vis spectroscopy was conducted to find evidence of biogenic production of ZnO NPs. The material was dissolved in distilled water for this analysis. The UV-Vis spectrum range was 200 to 600 nm. The mixture contained ZnO NPs, as shown by the strongest signal obtained at 360 nm (Fig 2). According to a previous study, the UV-Vis absorption peak for zinc oxide nanoparticles was at 320 nm [25]. The broad absorption band that extends to longer wavelengths may be caused by the migration of the electronic cloud on the general skeleton of the ZnO NPs.


A B C D

**Figure 1.** Visual observation of A) Peel extract, (B) Zinc acetate dihydrate solution, (C) Solution of zinc acetate dihydrate and extract, and (D) Final colour of synthesized NPs.

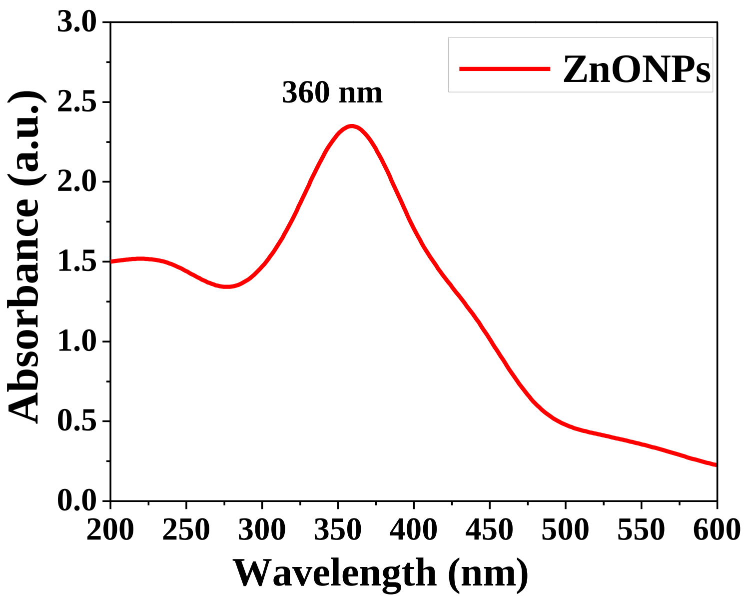



**Figure 2.** UV-visible spectrum of the synthesized ZnO NPs.

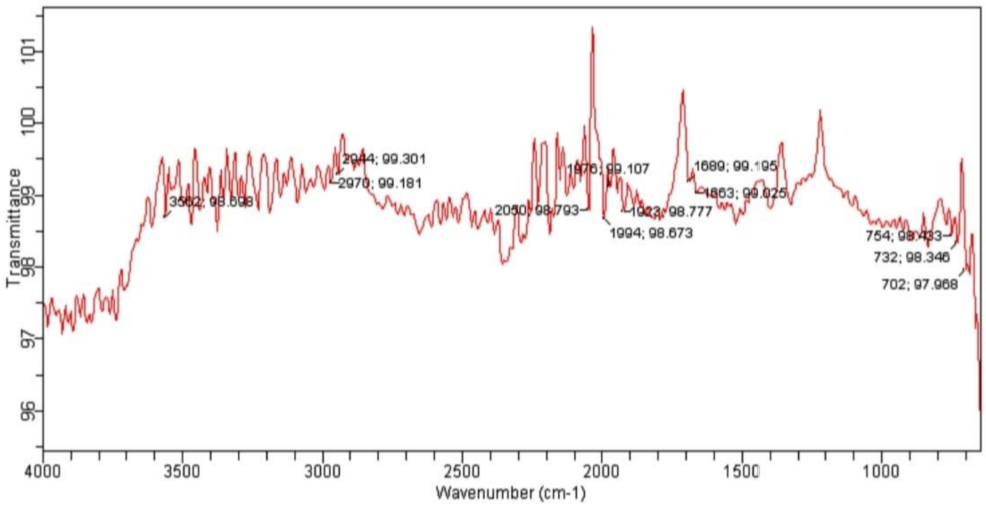



**Figure 3.** FTIR spectrum of synthesized ZnO NPs.

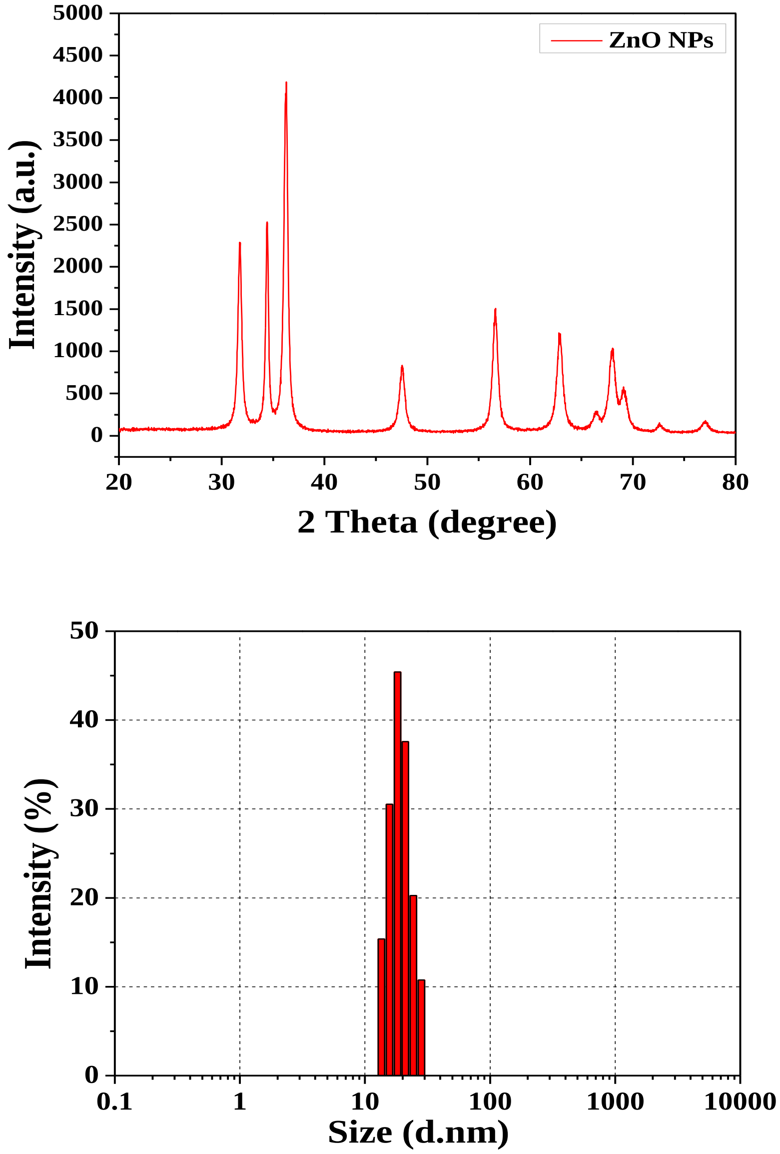



**Figure 4.** X-ray diffractogram and DLS result for the synthesized ZnO NPs.

### 1.3. Fourier Transform Infrared Spectroscopy (FTIR) of the Synthesized ZnO NPs

The conformational changes in adsorbed or immobilised proteins on the NPs were revealed by FTIR measurement. These peaks present in the FTIR spectrum (Figure 3) were connected to O-H, C-F, C-H and C=C stretching. Alcohol, aliphatic primary amine, thiol, nitrile, alkene, and isothiocyanate groups were all present. According to a previous study, peaks at 698-505 $cm^{-1}$ and 683-500 $cm^{-1}$ indicate that ZnO nanoparticles had been formed [18]. As there were no peaks in the 3500 - 2500 $cm^{-1}$ range, aldehydes were not typically OH and N-H extended. The bands between 1400 and 1000 $cm^{-1}$ relate to methylene from the proteins in solution and C-N stretching vibrations of amine, whilst the bands between 1600 and 1510 $cm^{-1}$ correlate to amide I and amide II areas as a result of carbonyl stretching in proteins. Peaks between 1460 and 1410 $cm^{-1}$ corresponded to the vibrations of alcohol, carboxylic acid, ether, and ester, whereas the bands between 94 and 769 $cm^{-1}$ represented the C-H bending vibrations of aromatic compounds.

### 1.4. X-ray Diffraction (XRD) and Dynamic Light Scattering (DLS) Analysis of ZnO NPs

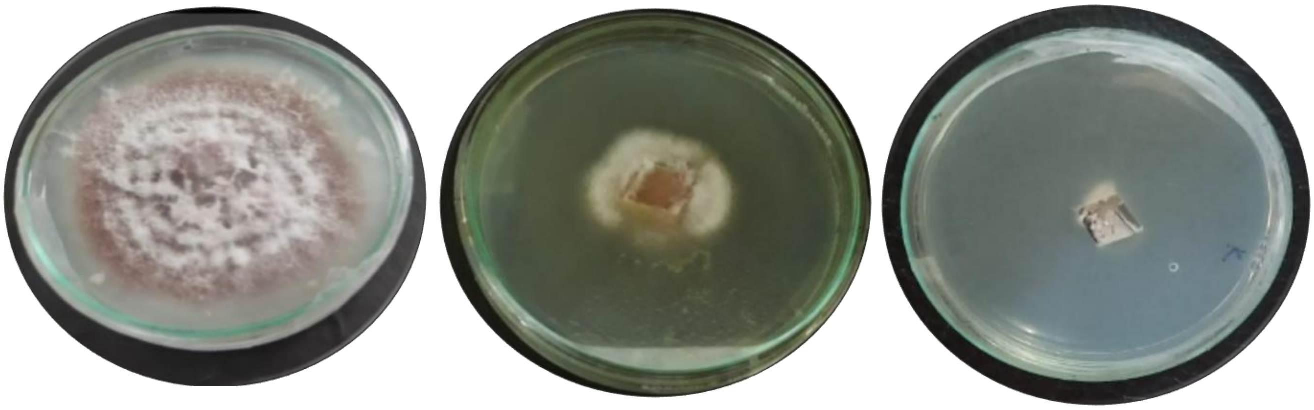
The XRD method works best with powdered samples that have been freshly created by drying the samples' respective colloidal solutions. The crystalline structure of the ZnO NPs was confirmed by XRD analysis. IIT, Mandi, HP used the analytical X'Pert PRO X-ray diffractometer for this experiment. The XRD spectra obtained were confirmed against the standard spectra of ZnO [26]. The DLS method is based on the scattering effect from the surface of the nanoparticles. To measure this, an electron, neutron, or light is often employed to bombard the colloidal solution or powder sample. The size measurement is obtained from the scattered electron, neutron, or light signal. DLS is a simple technique for determining particle size before and after shell material coating [27-28]. It is also used to measure the shell thickness over the core in an indirect manner. However, the DLS method cannot detect the homogeneity of the shell [28]. In this study, it also determined the hydrodynamic diameter of the synthesized nanoparticles. DLS analysis showed that the average particle size was 10-50 nm (Fig 4). The produced ZnO NPs had average sizes of 68.1 nm and 3.62 nm, respectively. The results show the particles produced were less than 100 nm [18].

## 2. Antifungal Activity of Green Synthesized Nanoparticles

In this investigation, we examined the effectiveness of the peel extract and manufactured nanoparticles as antifungal agents against particular fungi strains. The management of fungal infections has become a significant area of concern as a result of the emergence of fungal resistance to traditional anti-biotics. This is a result of the unrestricted and unauthorized use of antibiotics. Therefore, it is crucial for clinical purposes to generate new and powerful antifungals. Interesting research has shown that metallic nanoparticles (NPs) are a possible antibiotic substitute [29-31]. Therefore, nanoparticles have the potential to be applied as antifungal agents that may overcome the limitations in fungal infection control imposed by the developing resistance to conventional fungicides**.** The synthesized nanoparticles displayed antifungal activity against *Rosellinia necatrix* (37.5%), *Fusarium* spp (55.2%), *and Sclerotinia sclerotiorum* (60.5%). The highest zone of inhibition was observed against *S. sclerotiorum*, as shown in Table 1 and Figures 5-7.

**Negative control** **NPs** **Positive control**

**Figure 5.** Antifungal activity of synthesized nanoparticles against *Rosellinia necatrix*



**Negative control** **NPs** **Positive control**

**Figure 6.** Antifungal activity of synthesized nanoparticles against *Fusarium spp.*

**Negative control** **NPs** **Positive control**

**Figure 7.** Antifungal activity of synthesized nanoparticles against *Sclerotinia sclerotiorum*

**Table 1.** Antifungal analysis nanoparticles against *Rosellinia necatrix*, *Fusarium* spp., and *Sclerotinia sclerotiorum*

| No. | Fungus | Negative control | NPs | Positive control | Zone of inhibition |
|---|---|---|---|---|---|
| 1. | *Rosellinia necatrix* | 4 cm | 2.5 cm | ND | 37.5% |
| 2. | *Fusarium spp.* | 3.8 cm | 1.7 cm | ND | 55.2% |
| 3. | *Sclerotinia sclerotiorum* | 3.8 cm | 1.5 cm | ND | 60.5% |

## CONCLUSIONS

The "green" method of manufacturing metal and metal oxide nanoparticles has become a very alluring study area over the past ten years. Natural extracts, including bacterial, fungal, yeast, and plant extracts, have been investigated as potential sources for the creation of nanoparticles. In this study, we demonstrated that a plant extract worked incredibly well as a reducing and stabilizing agent for the creation of nanoparticles. This extract was used to create ZnO NPs in a green manner. Several characterization techniques, including visual inspection, UV-visible spectroscopy, FTIR, XRD, DLS and stability research, were performed on the produced NPs. Similarly, the in vitro radical scavenging capacity of the plant extracts and synthesized NPs was evaluated by DPPH assays. The fungi strains chosen to evaluate the antifungal activity of the NPs were *Rosellinia necatrix, Fusarium spp.*, and *Sclerotinia Sclerotiorum*. The positive control used hygromycin (5 mg/mL), whereas the negative control used plates without the plant extract. The zone of inhibition results for the ZnO NPs were: *Rosellinia necatrix* - 37.5%, *Fusarium spp* - 55.2%, and *Sclerotinia sclerotiorum* - 60.5%. Based on this study, ZnO NPs may both inhibit disease and increase agricultural output. Thus, commercialising nanoparticles for sustainable agriculture has become increasingly important as the demand for food rises while the production of staple food crops falls dramatically.

REFERENCES

1. Duncan, T. V. (2011) Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *Journal of colloid and interface science*, **363(1)**, 1-24.

2. Ramanathan, S., Gopinath, S. C., Arshad, M. M., Poopalan, P. & Perumal, V. (2021) Nanoparticle synthetic methods: strength and limitations. In *Nanoparticles in Analytical and Medical Devices, Elsevier*, 31–43.

3. Zhang, D., Ma, X. L., Gu, Y., Huang, H. & Zhang, G. W. (2020) Green synthesis of metallic nanoparticles and their potential applications to treat cancer. *Frontiers in Chemistry*, **8**, 799.

4. Nile, S. H., Baskar, V., Selvaraj, D., Nile, A., Xiao, J. & Kai, G. (2020) Nanotechnologies in food science: applications, recent trends, and future perspectives. *Nano-micro letters*, **12**, 1–34.

5. Ighalo, J. O., Rangabhashiyam, S., Dulta, K., Umeh, C. T., Iwuozor, K. O., Aniagor, C. O. & Igwegbe, C. A. (2022) Recent advances in hydrochar application for the adsorptive removal of wastewater pollutants. *Chemical Engineering Research and Design*.

6. Aman, J., Shahi, N. C., Lohani, U. C., Balodhi, D., Singh, R., Kumar, N. & Kumar, A. P. (2022) Process Optimization for Development of Guar Gum-Based Biodegradable Hydrogel Film Using Response Surface Methodology. *Bioinorganic Chemistry and Applications*.

7. Huynh, K. H., Pham, X. H., Kim, J., Lee, S. H., Chang, H., Rho, W. Y. & Jun, B. H. (2020) Synthesis, properties, and biological applications of metallic alloy nanoparticles. *International Journal of Molecular Sciences*, **21(14)**, 5174.

8. Guan, Z., Ying, S., Ofoegbu, P. C., Clubb, P., Rico, C., He, F. & Hong, J. (2022) Green synthesis of nanoparticles: Current developments and limitations. *Environmental Technology & Innovation*, **102336**.

9. Jadoun, S., Arif, R., Jangid, N. K. & Meena, R. K. (2021) Green synthesis of nanoparticles using plant extracts: A review. *Environmental Chemistry Letters*, **19**, 355–374.

10. Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P. & Kumar, P. (2018) 'Green'synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of nanobiotechnology*, **16(1)**, 1–24.

11. El-Belely, E. F., Farag, M. M., Said, H. A., Amin, A. S., Azab, E., Gobouri, A. A. & Fouda, A. (2021) Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Arthrospira platensis (Class: Cyanophyceae) and evaluation of their biomedical activities. *Nanomaterials*, **11(1)**, 95.

12. Ruszkiewicz, J. A., Pinkas, A., Ferrer, B., Peres, T. V., Tsatsakis, A. & Aschner, M. (2017) Neurotoxic effect of active ingredients in sunscreen products, a contemporary review. *Toxicology reports*, **4**, 245–259.

13. Dulta, K., Koşarsoy Ağçeli, G., Chauhan, P., Jasrotia, R. & Chauhan, P. K. (2021) A novel approach of synthesis zinc oxide nanoparticles by bergenia ciliata rhizome extract: antibacterial and anticancer potential. *Journal of Inorganic and Organometallic Polymers and Materials*, **31**, 180–190.

14. Küünal, S., Rauwel, P. & Rauwel, E. (2018) Plant extract mediated synthesis of nanoparticles. In *Emerging applications of nanoparticles and architecture nanostructures, Elsevier*, 411–446.

15. Sharma, A., Dulta, K., Nagraik, R., Dua, K., Singh, S. K., Chellappan, D. K. & Shin, D. S. (2022) Potentialities of aptasensors in cancer diagnosis. *Materials Letters*, **308**, 131240.

16. Dulta, K., Koşarsoy Ağçeli, G., Chauhan, P., Jasrotia, R. & Chauhan, P. K. (2021) Ecofriendly synthesis of zinc oxide nanoparticles by Carica papaya leaf extract and their applications. *Journal of Cluster Science*, 1–15.

17. Talam, S., Karumuri, S. R. & Gunnam, N. (2012) Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. *International Scholarly Research Notices.*

18. Naseer, M., Aslam, U., Khalid, B. & Chen, B. (2020) Green route to synthesize Zinc Oxide

Nanoparticles using leaf extracts of Cassia fistula and Melia azadarach and their antibacterial potential. *Scientific Reports*, **10(1)**, 9055.

19. Bisht, B., Gururani, P., Aman, J., Vlaskin, M. S., Anna, K., Joshi, S. & Kumar, V. (2022) A review on holistic approaches for fruits and vegetables bio-wastes valorization. *Materials Today: Proceedings.*

20. Dulta, K., Koşarsoy Ağçeli, G., Chauhan, P., Jasrotia, R., Chauhan, P. K. & Ighalo, J. O. (2022) Multifunctional CuO nanoparticles with enhanced photocatalytic dye degradation and antibacterial activity. *Sustainable Environment Research*, **32(1)**, 1–15.

21. Verma, R., Chauhan, A., Shandilya, M., Li, X., Kumar, R. & Kulshrestha, S. (2020) Antimicrobial potential of Ag-doped ZnO nanostructure synthesized by the green method using Moringa oleifera extract. *Journal of Environmental Chemical Engineering*, **8(3)**, 103730.

22. Chauhan, A., Kumari, S., Verma, R., Thakur, Z., Rana, G., Kulshreshta, S. & Kumar, R. (2022) Effect of Cu, Mg, Ag doping on structural and antimicrobial properties of synthesized ZnO using Cannabis sativa leaf extract. In *AIP Conference Proceedings, AIP Publishing LLC*, **2357**, 1, 030001.

23. Paramelle, D., Sadovoy, A., Gorelik, S., Free, P., Hobley, J. & Fernig, D. G. (2014) A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra. *Analyst*, **139(19)**, 4855–4861.

24. Jayachandran, A., Aswathy, T. R. & Nair, A. S. (2021) Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. *Biochemistry and Biophysics Reports*, **26**, 100995.

25. Gunalan, S., Sivaraj, R. & Rajendran, V. (2012) Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*, **22(6)**, 693–700.

26. He, L., Liu, Y., Mustapha, A. & Lin, M. (2011) Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. *Microbiological research*, **166(3)**, 207–215.

27. Mankad, M., Patil, G., Patel, S., Patel, D. & Patel, A. (2016) Green synthesis of zinc oxide nano-particles using Azadirachta indica A. Juss. Leaves extractand its antibacterial activity against Xanthomonas orzyae pv. Oryzae. *Annals of Phytomedicine*, **5(2)**, 76–86.

28. Ravichandran, V., Sumitha, S., Ning, C. Y., Xian, O. Y., Kiew Yu, U., Paliwal, N., & Tripathy, M. (2020). Durian waste mediated green synthesis of zinc oxide nanoparticles and evaluation of their antibacterial, antioxidant, cytotoxicity and photocatalytic activity. *Green Chemistry Letters and Reviews*, **13(2)**, 102–116.

29. Zhang, X., Lai, Z., Liu, Z., Tan, C., Huang, Y., Li, B. & Zhang, H. (2015) A facile and universal top-down method for preparation of mono-disperse transition-metal dichalcogenide nanodots. *Angewandte Chemie International Edition*, **54(18)**, 5425–5428.

30. Fraternale, D., Giamperi, L. & Ricci, D. (2003) Chemical composition and antifungal activity of essential oil obtained from in vitro plants of Thymus mastichina L. *Journal of Essential Oil Research*, **15(4)**, 278–281.

31. Dulta, K., Koşarsoy Ağçeli, G., Chauhan, P., Jasrotia, R., Chauhan, P. K. & Ighalo, J. O. (2022) Multifunctional CuO nanoparticles with enhanced photocatalytic dye degradation and antibacterial activity. *Sustainable Environment Research*, **32(1)**, 1–15.