Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized from *D. Melanogaster*

Saiqa Tabassum, Navera Rehan*, Maira Naseem, Sadia Shaikh
SZABIST Karachi, Pakistan
*E-mail: naverarehan73@gmail.com

ABSTRACT

Metallic nanoparticles have attracted significant attention in recent years due to of their unique properties and potential applications in a variety of industries. Zinc oxide nanoparticles (ZnO NPs) are promising for use in biomedical research, particularly in the treatment of cancer and against potential pathogens. The biogenic synthesis method, characterization, and antibacterial activity of ZnO NPs are demonstrated in this study. The extract of Drosophila melanogaster was employed as a reducing, stabilising, and capping agent in the synthesis of ZnO NPs from zinc nitrate hexahydrate salt. UV-Vis spectroscopy, SEM, and FTIR were used to characterise the nanoparticles. The antibacterial activity of nanoparticles against one gram-positive (methicillin-resistant S. aureus) and two gram-negative (E. coli and K. Pneumoniae) bacteria was determined by using agar-well diffusion method. The biosynthesized NPs exhibit effective antibacterial activity against these bacteria.

Keywords: Antimicrobial, Arthropods, D.melanogaster, Green synthesis, Zinc nitrate hexahydrate, Zinc oxide nanoparticles.

INTRODUCTION

The use of inorganic metal nanoparticles with diameters less than 100 nm has gained substantial attention due to their significant implications across biological, medical, and pharmaceutical applications such as medical devices, drug delivery, antimicrobial, nutrition, tumor-killing effects, skin care, and a lot more (Lee and Jun). Metal-based nanoparticles are created by reducing metals to nanometric sizes, either destructively or constructively. Sizes between 1 and 100 nm, a high surface area to volume ratio, surface charge and surface charge density, crystalline and amorphous structures, spherical or cylindrical shapes, reactivity, and sensitivity to environmental factors like as air, moisture and heat are some of their defining characteristics [Ealia&Saravanakumar].Metal nanoparticles like silver, gold, copper, iron and metal oxide nanoparticles like zinc oxide, copper oxide, titanium oxide and iron oxide nanoparticles are in current studies as antimicrobial agents. The high anti-bacterial activity of nanoparticles is due to their large surface area to volume ratio which allows binding of a large number of ligands on nanoparticle surface and hence, its complexation with receptors present on the bacterial surface [Jamkhande et al].

Depending on the process or material involved in the production process, nanoparticle synthesis techniques can be classified as physical, chemical, or biological. Physical and chemical methods can be costly, time consuming, and produce toxic compounds. These disadvantages have prompted the development of green synthesis methods that rely on environmentally friendly and biocompatible materials like plants and microorganisms. The presence of biomolecules such as proteins, coenzymes, vitamin-based intermediates, and certain other natural compounds in biological extracts can reduce metal ions to nanoparticles[Jeevanandam et al].

Furthermore, nanoscience has recently gained a foothold for animals, particularly arthropods and their metabolic products, to be employed as strong contenders for the functionalization of metal NPs. Arthropods, which include insects, arachnids, myriapods, and crustaceans make up the phylum Arthropoda. They are distinguished by hinged limbs and a cuticle formed of chitin and calcium carbonate.Arthropods, which are found worldwide, have also played a significant role in the supply of cheap and plentiful healing agents. Bee poison is one of the naturally occurring substances found in arthropods, containing a number of peptides including adolapin, mast-cell degrading peptide, melittin, as well as non-peptide compounds, and has applications in the treatment of cancer, glycemic control, neurodegenerative disorders, free radical-mediated disease, and HIV infection [Lateef et al., 2016]. Moreover, an enzyme produced by house flies known as defensin has been proved to be an effective antibacterial agent against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* [Park, S. O et al., 2010].

Nanoparticles of zinc oxide are widely used in a variety of topical products, including sunscreens, ointments, foot and skin care products and coatings (for UV protection) [Jiang et al]. The production of zinc ions and reactive oxygen species are the properties of ZnO NPs, which can cause oxidative stress and DNA damage. On the micro- and nanoscale, ZnO NPs are being researched as potential antibacterial medicinal agents. The findings demonstrate that ZnO NPs have highly effective antibacterial activities than their microparticle size [Mirzaei &Darroudi., 2017].

Objectives

The present study aimed to synthesize ZnO NPs at different concentrationsusing *D.melanogaster* extract. The specie *D. melanogaster* has never been utilized as a sourcefor synthesizing nanoparticles hence is a novel source. The biosynthesized NPs are to be characterized in order to assess their various properties and then evaluate their antibacterial activity against bacterial strains of *MRSA, E.coli* and *K.pneumoniae*.

Methodology

For this research, *D. melanogaster* were purchased from the Biotechnology Department of DOW University Ojha Campus. The sample size was 1000.

Firstly, the extract was prepared by washing and homogenizingthe sample, followed by filtration of the extract. The extract was centrifuged and thesupernatant was collected. Theobtained supernatant was dark orange in color.Secondly, various salt solution (zinc nitrate hexahydrate)concentrations were prepared.

Test Tube	Amount of salt	Concentration
T1	0.2 g	0.1 M
T2	0.9 g	0.5 M
T3	1.8 g	1.0 M
T4	3.6 g	2.0 M

The four test tubes, each containing 6 ml of the salt solution were kept in water bath at 65°C. 1 ml of the extract was added to each tube drop-wise with vigorous stirring for up to 2 minutes. A few drops of KOH solution were also added in each of the four test tubes. The tubes were left in water bath for 40 minutes after which brown precipitates were observed in each test tube. The tubes were then centrifuged at 5000 rpm for 20 minutes. The supernatant was discarded and the precipitates obtained in the pellet were washed three times with distilled water and once with ethanol to remove any impurities or futile particles.The pellet obtainedwas stored for the characterization to prove the existence of ZnO nanoparticles and evaluate their antibacterial activity. Protein estimation of the *D.melanogaster* extract was also performed by Bradford method.

Figure 1. Zinc oxide Nanoparticles (pellet)

RESULTS

Spherical-shaped zinc oxide nanoparticles with sizes ranging from 60 to 68 nm were created. They demonstrated good UV-absorption between 205 and 325 nm. The synthesised NPs were antibacterial against MRSA, E.coli, and K. pneumonaie.

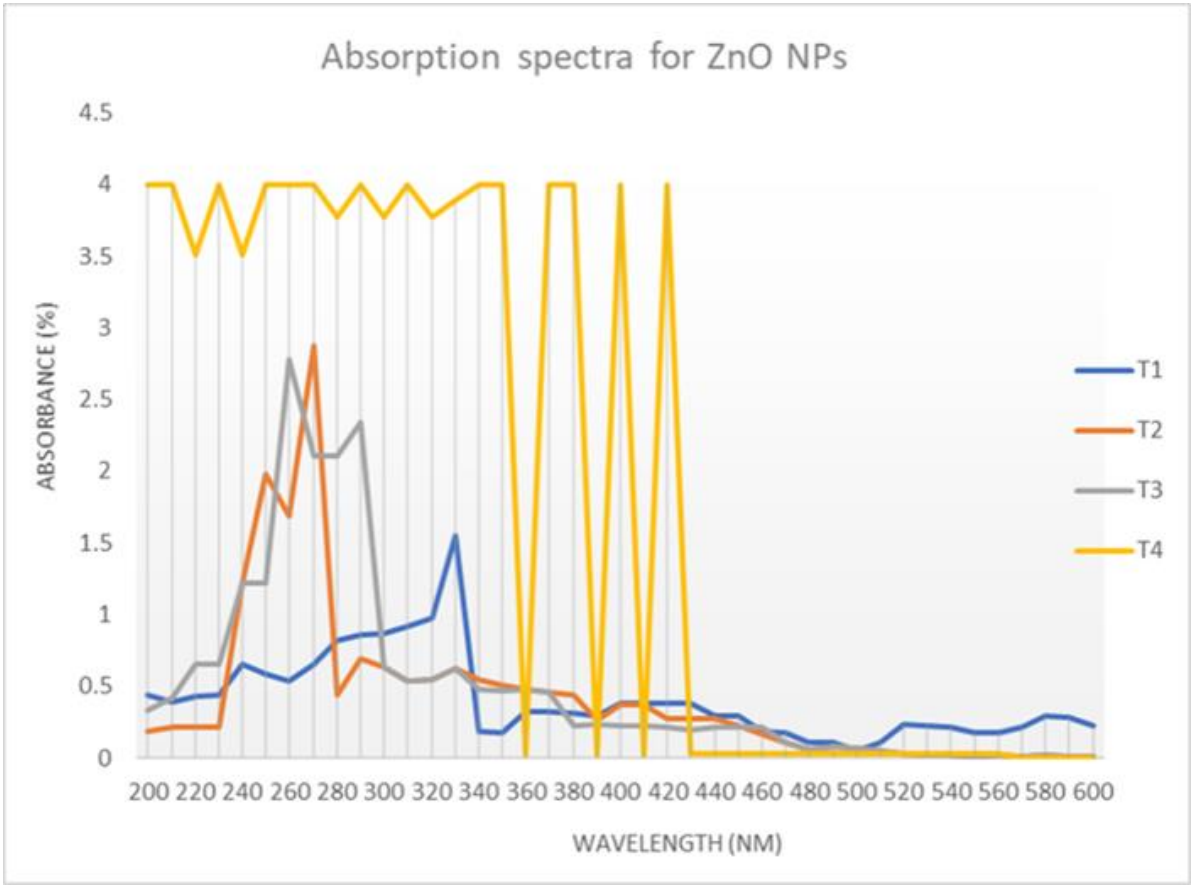

Figure 2. Absorption Spectra of ZnO NPs at four different concentrations.

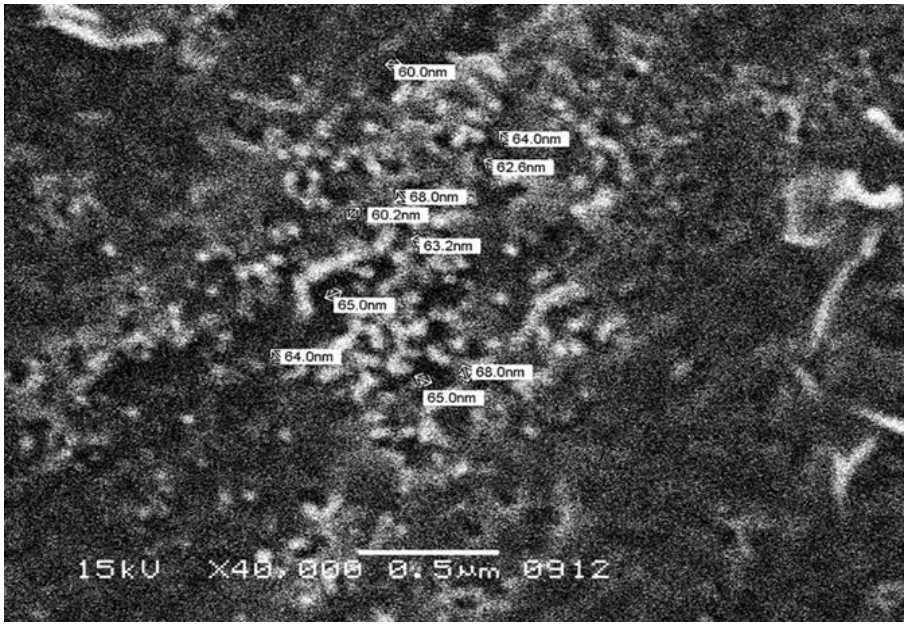

Figure 3. SEM of ZnO NPs

ACKNOWLEDGMENT

The authors would like to express appreciation for the support of the supervisor Dr. Saiqa Tabassum and co-supervisor Miss Uroosa Ejaz [Final Year Project Number = 2].

REFERENCES

1. Lee, Sang Hun, and Bong-Hyun Jun. “Silver Nanoparticles: Synthesis and Application for Nanomedicine.” International journal of molecular sciences vol. 20,4 865. 17 Feb. 2019, doi:10.3390/ijms20040865
2. Ealia, S. Anu Mary, and M. P. Saravanakumar. "A review on the classification, characterisation, synthesis of nanoparticles and their application." IOP conference series: materials science and engineering. Vol. 263. No. 3. IOP Publishing, 2017.
3. Jamkhande, Prasad Govindrao, et al. "Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications." Journal of drug delivery science and technology 53 (2019): 101174.
4. Jeevanandam, Jaison, Yen San Chan, and Michael K. Danquah. "Biosynthesis of metal and metal oxide nanoparticles." ChemBioEng Reviews 3.2 (2016): 55-67.
5. Lateef, Agbaje, Sunday A. Ojo, and Joseph A. Elegbede. "The emerging roles of arthropods and their metabolites in the green synthesis of metallic nanoparticles." Nanotechnology Reviews 5.6 (2016): 601-622.
6. Park, Sang O., et al. "Antibacterial activity of house fly-maggot extracts against MRSA (Methicillin-resistant Staphylococcus aureus) and VRE (Vancomycin-resistant enterococci)." Journal of Environmental Biology 31.5 (2010): 865.
7. Jiang, Jinhuan, Jiang Pi, and Jiye Cai. "The advancing of zinc oxide nanoparticles for biomedical applications." Bioinorganic chemistry and applications 2018 (2018).