



Antibacterial Activity of Zinc Oxide Nanoparticles Using Banana Peel Extract against Antibiotic-Resistant Bacteria

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ABSTRACT

Synthesis of zinc oxide nanoparticles on their antimicrobial activity utilizing banana peelings could be a potential candidate to develop nano-formulated natural products as an alternative, efficient, inexpensive, and environmentally safe method with specified properties. The study aimed to investigate the biological synthesis, characterization, and antimicrobial analysis of zinc oxide nanoparticles (ZnONPs) against clinical pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* using *Musa acuminata* × *balbisiana* peelings. Data revealed that varying concentration, temperature, and pH level can significantly influence the antimicrobial activity and plays crucial steps in the preparation of ZnONPs. Research on nanoparticles as antibacterial agents can have an essential application in the food industry as antibacterial agents in food packaging and foodborne pathogens. Since this study deals with the preliminary investigation on banana peelings locally grown in the Philippines as capping and reducing agent through green chemistry, it can promote the potential application in the Philippine food industry. By proper incorporation of nanoparticles into packaging materials, it may inhibit or cause foodborne pathogens bacterial death

Keywords: antimicrobial, nanoparticles, *Musa acuminata* × *balbisiana*.

INTRODUCTION

Nanoparticles research is an emerging field not because of its broad application in biotechnology but is also considered a viable alternative to antibiotics to solve the problem of the emergence of bacterial multidrug resistance (Abbasi et al., 2020; Mahmood et al., 2019; Srikar et al., 2016; Rai et al., 2012). It also opened new horizons in nanomedicine, such as the synthesis of candidate nanoparticles that can be assembled into complex architectures (Franci et al., 2015; Thirumavalavan et al., 2013; Natarajan et al., 2010). With its unique, peculiar morphology and environmentally friendly material desirable for bio-applications, it considerably gets much attention. However, the development of more reliable technology and the use of environmental-friendly procedures to synthesize nanoparticles must also be considered. Thus, the prospect of exploiting natural resources for metal nanoparticle synthesis has become a more competent and eco-friendly approach to explore potential plants to synthesize nanoparticles (Mubayi et al., 2012). Accordingly, plant-mediated nanoparticle synthesis is more preferred because it is more the environmentally friendly, cost-effective, single-step method, and safe for human therapeutic use (Kumar & Yadav, 2009). Different parts of plant materials such as extracts, fruit, bark, and fruit peels have been studied so far to synthesize nanoparticles showing different sizes and shapes (Iqbal et al., 2019; Iqbal et al., 2020). The factors affecting the synthesis of nanoparticles are the reaction temperature, metal ion concentration, extract contents, pH of the reaction mixture,

duration of reaction, and agitation (Srikar et al., 2016).

Banana, as one of the leading fruits grown in the Philippines and a consistent top dollar earner, its peel can represent 36% of the total weight of the fruits and does not constitute an industrial application. Banana peel is an underutilized source of phenolic compounds, a good source of functional foods and antioxidant compounds. In particular, gallocatechin and dopamine can help prevent cardiovascular and neurodegenerative diseases that can neutralize the action of free radicals in the body (Baskar et al., 2011). Utilization of banana peel as reducing and capping agents will be more advantageous over other biological processes because it is non-toxic, cost-effective, eco-friendly, that would be suitable for developing large-scale production. Capping agent plays an essential role in controlling the morphology of nanostructure because of their soft-template effect and their ability to modify the chemical kinetics, while, the reducing agent controls the particle size. The previous study shows that biosynthesis silver nanoparticles (AgNPs) using banana peel extract were characterized with uniform, spherical, crystalline, and monodispersed nanoparticles with an average particle size of 23.7 nm (Ibrahim et al., 2015). Moreover, the synthesized AgNPs have excellent antimicrobial activity

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showing the synergistic effect of the standard antibiotic levofloxacin against selected pathogenic microorganisms (Ibrahim et al., 2015; Ali et al., 2015). In this study, zinc oxide nanoparticles (ZnONps) were investigated to understand the mechanism of ZnONps using banana peel extracts as an antimicrobial agent.

Pseudomonas aeruginosa and *Staphylococcus aureus* are bacterial strains responsible for the majority of hospital-acquired infections. In the present investigation, banana peel extract was used to investigate how it can mediate a novel route for the green synthesis of ZnONps. The widespread cases of multidrug-resistant bacteria against the standard antibiotics have led researchers to incorporate ZnONps as an ingredient to boost the antibiotic effects potentially.

METHODOLOGY

Microorganisms. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were purchased from Central Mindanao University, College of Veterinary Medicine. Bacterial strains were maintained on nutrient agar slants at 4°C for further use.

Chemicals. Standard metal ion solution, zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], was purchased commercially from Aldrich prepared in distilled water. Chemicals and reagents used in this study were analytically graded and were used without any further purification.

Banana peels extract preparation. Cardava banana (*Musa acuminata* × *balbisiana*) peels were washed and boiled in distilled water for 30 min at 90°C. The peels (100g) were crushed in 100 ml distilled water, and the extracts were filtered using cheesecloth to remove the insoluble fractions and macromolecules present in the sample. An equal volume of chilled isopropyl alcohol was added, and the resultant precipitate was centrifuged at 1000 rpm for 5 min, suspended in distilled water, and stored in a refrigerator 4°C for further studies. The extract was used as a reducing as well as a stabilizing agent.

Synthesis of zinc oxide nanoparticles using banana peel extract. The reaction mixtures contained 1 ml of banana peel extract (equivalent to 6.8 mg dry weight) in 50 ml of zinc nitrate solution (1 mM). Banana peel extract, zinc nitrate solution, was used as a control, and all experiments

were carried out in triplicates. The effect of the zinc was determined by varying the ZnONps concentration from 0.25 to 2.0 mM. To evaluate the effect of banana peel extract concentration from 0.25 to 3.0 ml was used while keeping the ZnONps concentration at a level of 1.75mM. The effect of pH was also studied by adjusting the reaction mixtures (3 ml banana peel extract at 1.75 mM ZnONps) to 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, or 6.5. The effects of temperature on nanoparticle synthesis were also investigated wherein the reaction mixtures containing 3 ml banana peel extracts and 1.75 mM ZnONps at pH 4.5 were incubated at different temperatures (30, 40, 50, 60, 80, or 100°C) for 5 min. The effect of reaction time was evaluated by incubating the reaction mixtures with optimum composition for 3, 5, 10, 15, 20, 25, 30, 45 min, 1, 24, 48, 72, and 96 h.

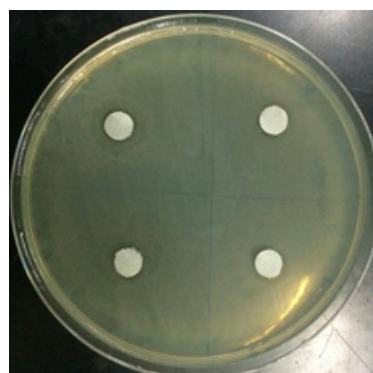
Antimicrobial activity of synthesized zinc oxide nanoparticles. The researchers estimated the antimicrobial activity of ZnONps in terms of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). For each bacterial strain, three replicates were used. For MIC determination, 96-well plates containing sterile 200 ul of nutrient broth media, supplemented with various ZnONps concentrations (0.5, 1.5, 3.0, 6.0, and 10 ug/ml) was incubated with 20ul of the microbial culture broth (0.8 OD₆₀₀) and the flask was incubated for 24 h at 37°C. Zinc oxide nanoparticles-free broth media was used as a positive control. The microbial growth index was determined using a UV-Vis spectrophotometer. MBC estimation microbial cultures grown in nutrient broth medium supplemented with ZnONps was inoculated onto nutrient agar plates free from ZnONps and incubated at 37°C for 24 h. The lowest concentration of the nanoparticles that prevent microbial growth was designated as the MBC.

RESULT & DISCUSSIONS

The study aims to investigate the potential use of synthesized zinc oxide nanoparticles (ZnONps) by green chemistry using an extract derived from banana peeling against human pathogenic microorganisms. The purpose of synthesizing nanoparticles using plant material as a potential stabilizing, reducing, and chelating agent, is to determine the therapeutic potential of green synthesis of metallic nanoparticles such as ZnONps. This study will help in environmental issues by determining potential antibacterial sourced from natural products



Staphylococcus aureus



Pseudomonas aeruginosa

Figure 1. Inhibitory effect of banana peeling concentration grown in nutrient agar medium

that are easy, efficient, and eco-friendly against common bacteria that can lead to severe diseases. Figure 1 shows the inhibitory effect of banana peeling against *S. aureus* and *P. aeruginosa* pathogenic bacteria grown in nutrient media 24 hours at 30°C. At minimal concentration, a zone of inhibition (ZOI) was observed in both representative microorganisms that may imply that extracts from banana peeling may have a potential inhibitory effect. Banana peel has an underutilized source of phenolic compounds, which can be considered a good source of functional foods and antioxidant compounds (Baskar et al., 2011). Moreover, secondary metabolites such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids were found in banana peel (Imam, 2011). The presence of these phytochemicals/secondary metabolites might be responsible for the antibacterial activity of banana peel. In the study of Ehiowemwenguan et al. (2014), when banana peel extract was prepared using isopropyl alcohol it dissolves more active compounds and had higher antibacterial activity than the aqueous solution. Therefore, in this study, isopropyl alcohol was used to dissolve more active compounds from the banana peel and might be responsible for its antimicrobial activity (Kadapa et al., 2015).

We then tried to measure the growth rate at OD₆₀₀ of *S. aureus*, and *P. aeruginosa* grew in nutrient broth for 24 hours at 30°C using a spectrophotometer with varying concentrations of ZnONPs (Figure 2). The data shows that the addition of ZnONPs at different concentrations inhibited the growth rate *S. aureus* and *P. aeruginosa*. This data implies that increasing the level of zinc nitrate is fatal to the growth of the bacterial strains. Accordingly, banana peels are mainly composed of pectin, cellulose, hemicelluloses, and some functional groups associated with these polymers, and the proteinaceous matter may be responsible for reducing the zinc salt to Zn⁰ (Kokila et al., 2015). The biological components of some plants are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles (Bar et al., 2009; Ganesh Babu and Gunasekaran, 2009; Ibrahim, 2015). The Gram-negative bacteria *P. aeruginosa* showed a maximum zone of inhibition when cultivated for six (6) hours. This may be due to the cell wall structure of gram-negative bacteria that possesses a thinner peptidoglycan

layer compared to the Gram-positive bacteria composed of a thicker peptidoglycan layer. Gram-positive bacterial peptidoglycan consists of linear polysaccharide chains cross-linked by short peptides, thus forming a more rigid structure leading to difficult penetration of the zinc nanoparticle (Shrivastava et al., 2007). The high antimicrobial activity is certainly due to the zinc cations released from ZnONPs that act as reservoirs for the Zn⁺ bactericidal agent. The same result in silver nanoparticles was changed in the membrane structure of bacteria resulted from the interaction with silver cations leading to the increased membrane permeability of the bacteria (Dibrov et al. 2002; Iqbal et al., 2019).

To test if the pH level can affect the inhibitory effect of ZnONPs, we have tested different pH levels to further produced a larger mass of ZnONPs (Figure 3). We have chosen at pH 5 (medium acidic), pH 7 (neutral) and pH 12 (basic). Data shows that at pH 5, higher antibacterial activity was observed at acidic pH levels with the maximum toxicity at pH 5 for *S. aureus* and *P. aeruginosa*, respectively. However, at pH 12, when there are more ZnONPs produced, it also showed a greater zone of inhibition effect as compared to acidic with pH 5 or at a neutral level with pH 7. Accordingly, analysis of the results demonstrated that exposure media of ZnONPs and cultural factors play a role in their cytotoxic effects (Prasad et al., 2012). It could also be attributed to the principal mechanism at different reaction conditions. The results showed that the overall influence of pH on the antibacterial activity of ZnONPs against both bacteria was similar. As the pH increased from acidic to neutral, the percentage of the growth inhibition of both microorganisms decreased. It may suggest a pH-dependent mechanism for ZnONPs antimicrobial properties. Synthesized ZnONPs size can be tuned with pH variation, and small-sized nanoparticles exhibited higher antibacterial activity. According to the study of Ariz et al. (2019), as the pH increases from 7, particle size starts to increase to a micrometer, and at lower pH like pH4, the particle size is in nanometers. And when keeping the pH low and increasing reaction time, it also gives bigger size particles, but at higher pH by decreasing reaction time, particle size almost remains the same (Ariz et al., 2019). Previous studies also showed that with pH increment, the particle size was observed to be decreased due to increased

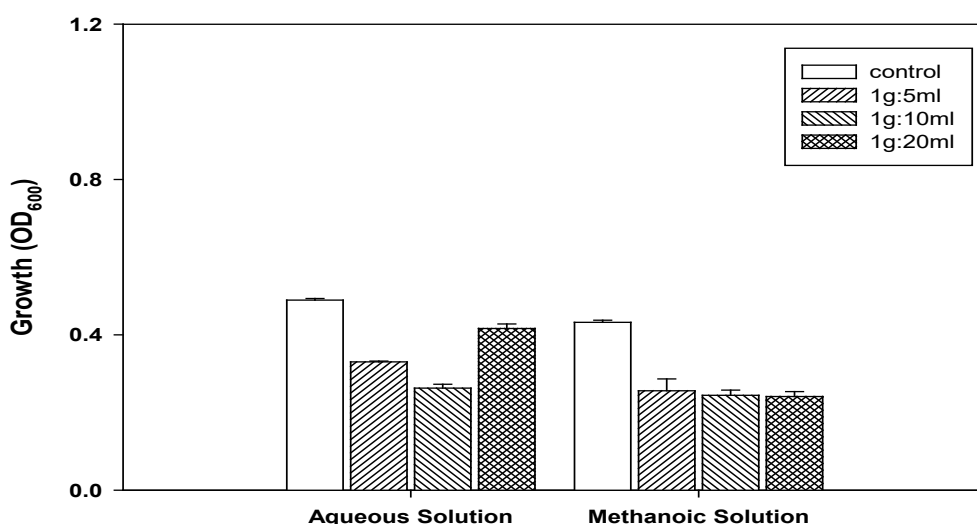


Figure 2. ZnONPs at the different concentration tested against in *S. aureus* and *P. aeruginosa*

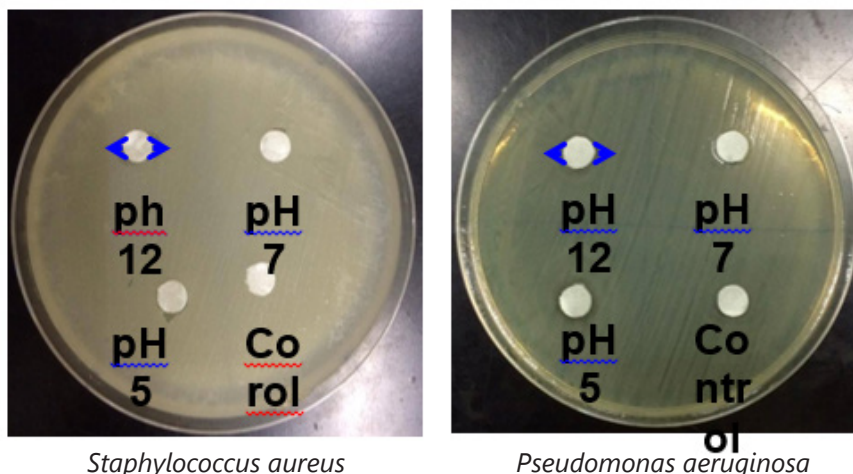


Figure 3. Inhibitory effect of zinc oxide nanoparticles (ZnONPs) at a different pH level

reactivity (Rajesh et al., 2016). At pH values 2.0–4.0, a white precipitate was obtained, and no color change was observed. The varying shades of reddish-brown color were observed at pH values between 4.5–6.5; thus, we used at pH 5 for an antimicrobial test. The highest color intensity was obtained at pH 4.5. In agreement, Roopan et al. (2013) reported that at pH 2.0, no reaction occurred, while at pH 11, highly monodispersed nanoparticles were obtained with an average size of 23 ± 2 nm accordingly. A variety of biomolecules are postulated to be involved in biological nanoparticle synthesis, and such biomolecules are likely to be inactivated under extremely acidic conditions (pH 2.0). The differences in the colors obtained over the range of pH could be ascribed to a variation in the dissociation constants (pKa) of functional groups on the biomass involved (Pimprikar et al., 2009).

The temperature also affected the process of zinc reduction ((Figure 4). Reaction mixtures that were incubated at 30, 40, and 50 °C showed a light reddish-brown color. At higher incubation temperature (60, 80, and 100 °C), the filtrate showed a dark reddish brown color. At room temperature (30 °C), the color change took 10-20 min to develop. Heating the reaction mixtures at 40–100 °C, the reduction process was faster, and the reddish-brown color was developed within 5 min. The incubation time of the reaction mixture was directly proportional to the intensity of the color change. Interestingly, when *S. aureus* and *P. aeruginosa* were grown in NB medium supplemented with

ZnONPs at 1.75 mM at pH 5, the *S. aureus* has significantly decreased as compared with *P. aeruginosa*.

CONCLUSION

In this study, banana peels were successfully utilized for the consistent and quick synthesis of zinc nanoparticles. The biosynthesized ZnONPs using banana peel extracts revealed good antimicrobial activity against the selected pathogenic microorganisms. This green synthesis approach showed to be a cost-effective, non-toxic, and eco-friendly alternative to a conventional microbiological application. It would be suitable for developing a biological process for large-scale production. The ZnONPs were produced by using of zinc nitrate with peel extract in aqueous media without any external human-made chemicals. The reaction pathway satisfies all the conditions of a 100 % green chemical process. These zinc nanoparticles may be used in the effluent treatment process for reducing the microbial load.

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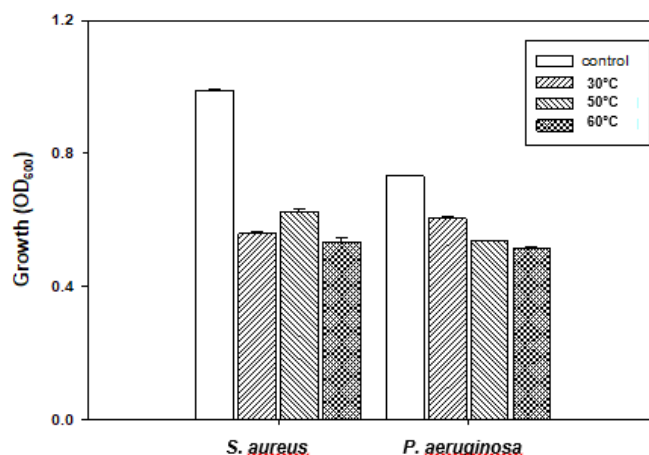


Figure 4. Growth performance of *S. aureus* and *P. aeruginosa* in ZnONPs prepared at different temperature

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