ANTIBACTERIAL ACTIVITY OF Fe-DOPED ZnO NANOPARTICLES WITH *Citrus hystrix* EXTRACT SYNTHESISED BY USING A LOW-COST METHOD

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Abstract: In this work, the structural and optical properties of Fe-doped zinc oxide were investigated by using X-ray diffraction and Raman spectroscopy. The sample was successfully grown by the low method using the sol-gel method from *Citrus hystrix* extract. For the doping process, ferum powder was used to make a doping for ZnO nanoparticles through the chemical process. Based on the previous work, there was no finding reported on the Fe-doped ZnO from *Citrus hystrix* extracts. The ZnO nanoparticles were produced by a biosynthesis process, and there are no hazardous chemicals used in this process. After adopting the doping process, the sample yielded significant structural and antibacterial findings. XRD measurement shows the ZnO peak and hexagonal property of the samples, confirming the existence of ZnO nanoparticles. For the antibacterial analysis, two types of bacteria were used to study the effectiveness of Fe-doped ZnO on this treatment.

Keywords: *Zinc oxide, ZnO, Citrus hystrix, ferum, antibacterials*

INTRODUCTION

Many modern applications and optoelectronic devices use zinc oxide (ZnO) extensively due to the promising optical and structural properties of the sample. ZnO is commonly used for breaking down organic pollutants (Abdullah et al., 2022), fighting bacteria (Mendes et al., 2022), in optoelectronics (Shahzad et al., 2021), and in healthcare products (Verma et al., 2021) because it is cheap and good for the environment. However, a higher recombination rate of excess carriers reduces photocatalytic process (Ramos et al., 2022). One of the potential efforts is by adding transition metals into ZnO NPs for improving the photocatalytic process of ZnO NPs (Gopalakrishnan & Ashokkumar, 2024). Currently, many researchers reported the progress of doping transition metal ions into ZnO for reducing the recombination rate and improving antibacterial activity (Okeke et al., 2022). The objectives of this work are to synthesise ZnO nanoparticles (NPs) by using citrus hystrix extracts, and the process was continued with the doping process using ferrum metal as a dopant. Ferrum element is used to improve the stability and the effectiveness of Fe-doped ZnO nanoparticles in combating the bacterial species. For the antibacterial analysis, two types of bacteria were used to study the effectiveness of Fe-doped ZnO on this treatment.

MATERIALS AND METHODS

Kaffir lime, or *Citrus hystrix*, was used in the biosynthesis of zinc oxide nanoparticles because it was easily found in the nearby market and exhibited excellent behaviour as a bio-reduction in the treatment. The fresh kaffir lime fruits were rinsed with deionised water, and they will dry at room temperature. The juice from the fruits was manually extracted and filtered using muslin cloth to remove unnecessary particles. 50 mL of deionised water was mixed with 0.1 M zinc acetate dihydrate, and the mixture was stirred continuously for one hour using a magnetic stirrer. 25 mL of the filtered juice was added to the mixture, followed by titration with NaOH until the pH reached 12. The mixture was continuously stirred at 500 rpm for the next three hours. One can see that the final colour of the ZnO NPs sample is brownish yellow, indicating the completion of ZnO NPs using *Citrus hystrix* extracts. For the doping process, 0.029 g $FeCl_3$ and 1 g of ZnO were mixed in 100 ml DI water, and it was stirred for 30 minutes, as shown in Figure 1. The mixture was then transferred to a round-bottom flask and heated in the reflux system at 80°C for 2 hours. The precipitates were filtered and rinsed with DI water to remove unnecessary particles. The samples were transferred to a furnace and dried at 80°C for four hours and calcined at 400°C for one hour. For the antibacterial test, the disc diffusion method using Muller Hint on agar was used. The bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were used for the antibacterial test.

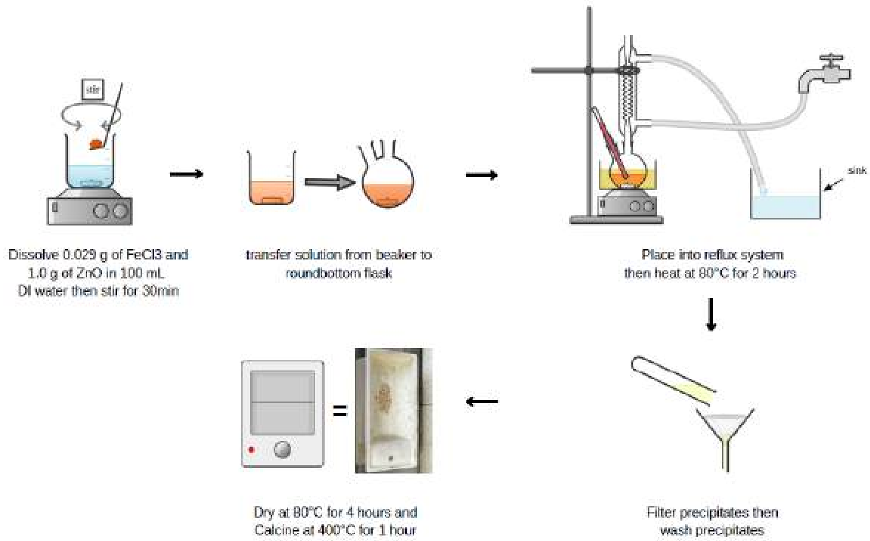

Figure 1. A schematic representation for the synthesis of Fe-doped ZnO NPs

RESULTS AND DISCUSSION

The effectiveness doped ZnO of antibacterial reaction are depends on several factor such as physio-chemical, type and concentration of ion dopant, dye structure and bacterial type (Musat et al., 2017). Table 1 shows the antibacterial activity of Fe-doped ZnO NPs samples at 1 mg/ml against *E. coli*. We found that the Fe-doped ZnO in 10% DMSO solution showed significant antibacterial activity with a mean inhibition zone of 21 ± 0.6 mm. However, when the Fe-doped ZnO NPs samples were mixed with 10% methanol solution, there was no activity observed. Meanwhile, Fe-doped ZnO was mixed with 10% acetic acid, and a 10 ± 0.6 mm inhibition zone was created, showing the moderate reaction. On the other hand, positive control of ciprofloxacin shows a significant antibacterial reaction with an inhibition zone of 38 mm. While, for the negative control samples, there is no activity detectable except for the 10 % acetic acid solution.

Table 2 shows the antibacterial activity of Fe-doped ZnO NPs samples at 1 mg/ml against *S. aureus*. We observed that there was a 10 ± 0.6 mm inhibition zone for the mixed Fe-doped ZnO and 10% DMSO, showing moderate activity. While when the Fe-doped ZnO was mixed with 10% methanol, the mean inhibition zone was recorded as 16 ± 1.2 mm, indicating good antibacterial activity. Moderative reaction is also reported when Fe-doped ZnO was placed in 10% acetic acid, creating a 12 ± 0.6 mm inhibition zone. Besides, positive control (ciprofloxacin) shows an inhibition zone of 25 mm, which is a higher antibacterial activity rate. While there was no activity observed for the negative control (10% DMSO and 10% methanol) except for 10% acetic acid with an inhibition zone of 17 mm. Previous work states that the antibacterial activity increased when applying to both gram negative and positive bacterial species, compared without doping (Musat et al., 2017). Besides, same finding also discovered by (Busila et al., 2015; Lin et al., 2009, Manna, 2012), when they investigated the impact of different metals doping on the antibacterial mechanism of ZnO nanoparticles.

Table 1. Antibacterial activity of nanoparticle samples at 1 mg/ml against *E. coli*

Compound	Solvent	Inhibition Zone (mm)			
		1	2	3	Mean ± SD
Fe doped-ZnO	10% DMSO	21	21	20	21 ± 0.6
	10% Methanol	NA	NA	NA	NA
	10% Acetic Acid	11	10	10	10 ± 0.6
PC	1 mg/ml ciprofloxacin	38			
NC	10% DMSO	NA			
NC	10% Methanol	NA			
NC	10% Acetic Acid	20			

Table 2. Antibacterial activity of Fe doped ZnO nanoparticle samples at 1 mg/ml against *S. aureus*

Compound	Solvent	Inhibition Zone (mm)			
		1	2	3	Mean ± SD
Fe doped-ZnO	10% DMSO	10	9	10	10 ± 0.6
	10% Methanol	16	16	18	16 ± 1.2
	10% Acetic Acid	12	12	13	12 ± 0.6
PC	1 mg/ml ciprofloxacin	25			
NC	10% DMSO	NA			
NC	10% Methanol	NA			
NC	10% Acetic Acid	17			

CONCLUSIONS

As a conclusion, we have successfully fabricated Fe-doped ZnO nanoparticles using *Citrus hystrix* extracts for the antibacterial applications. For the antibacterial activity of Fe-doped ZnO NP samples at 1 mg/ml against *E. coli*. We found that the Fe-doped ZnO in 10% DMSO solution showed significant antibacterial activity with a mean inhibition zone of 21 ± 0.6 mm. The result from the antibacterial activity of Fe-doped ZnO NP samples at 1 mg/ml against *S. aureus* shows that there was a 10 ± 0.6 mm inhibition zone for the mixed Fe-doped ZnO and 10% DMSO, indicating moderate activity. The Fe-doped ZnO has a higher potential to be used in the antibacterial treatment.

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