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ANTIMICROBIAL ACTIVITY OF BIO-WASTE MEDIATED ZINC OXIDE NANOPARTICLES AGAINST HUMAN PATHOGENS

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ABSTRACT

Fish waste management has been one of the major problems having the greatest impact on the environment. On the other side Zinc oxide nanoparticles are one of the most increasingly utilized nanomaterials in consumer products. The study aimed at a simple method for the green synthesis of Zinc oxide nanoparticles (ZnO) using aqueous extract of fish waste as a reducing and stabilizing agent. ZnO NPs were synthesized using the aqueous extract of fish waste within 3 hrs. The green synthesized ZnO NPs were characterized for size and structure using particle size analyzer and TEM respectively. Characterization data reveals that the particles were spherical shaped with an average size of 86.84 nm. The antimicrobial activity tested against *Staphylococcus aureus* and *Escherichia coli* were significant with zone of inhibition 11.75 and 10.05 at 50(µg/ml). Thus, the study provides an alternative approach for fish waste management.

KEY WORDS : Zinc oxide nanoparticles, Fish waste, Bio-waste, Antimicrobials.

INTRODUCTION

Bio-waste is a form of biomass, capable of decomposing under anaerobic or aerobic conditions. The amount of bio-waste generated across the globe has increased tremendously, mainly due to the rapid increase in industrialization, urbanization, and population growth. Aquaculture contributes to one third of the global food fish production (FAO, 2018). Fish wastes are necessary to identify technologically and economically viable ways for reutilization of fish waste. Fish waste is a potential source of protein that is being utilized as fish meal, fish sauce, fertilizer, animal feed and fish silage (Ghaly *et al*., 2013). The problem with the under-utilization of fish waste is two-fold. Firstly, a direct financial cost is associated with the disposal of fish waste and it pollutes the environment. Secondly, the opportunity cost in terms of missed opportunity to add value to fish waste. Therefore, it is necessary to find ecologically acceptable alternatives for utilization of fish waste.

Recent studies have reported recovery of highly valuable biological compounds such as peptides, oligosaccharides, fatty acids, enzymes, watersoluble minerals and biopolymers from fish waste and aquatic invertebrates (Ross and Stanton, 2011). However, the demand for high purity in such application leads to a higher capital/ installation cost for downstream processing of these components. In this context, it is of increasing interest to study direct applications of partially purified bio compounds derived from the waste generated by fish processing units. There are many physiological and biochemical differences between fish species, which translate into differences in the composition of fish waste components (Sabtecha *et al*., 2014). Most fish waste contain 15-30% protein, 0-25% fat and 50-80% moisture (Ghaly *et al*., 2013). Recent studies have revealed that fish waste may contain several enzymes including pepsin, trysin, and chymotrypsin with unique physical, chemical and catalytic properties (Zhou *et al*., 2011). Though these approaches are in the research and development stage, they provide an optimistic outlook for reclamation of fish waste for conversion into useful products.

The ZnONPs have been used for various

applications, including cell imaging, drug delivery, and nanomedicine. TheZnONPs are also used in goods, such as personal care products and known to exhibit excellent antibacterial and UV blocking properties. *In-vitro* analyses have revealed the biocompatibility of ZnONPs. ZnONPs serve as non-toxic, biocompatible, and safe drug carriers (Mahalakshmi *et al*., 2019). Zinc is an important trace element present in all body tissues (Jiang *et al*., 2018). In biological systems, zinc has important roles in metabolic pathways such as, carbohydrate, lipid, nucleic acid, and protein syntheses (Jain *et al*., 2013). Biological synthesis of nanoparticles is more labour and time intensive and expensive than physical and chemical methods (Saptarshi *et al*., 2013).Till date, many routes were fabrications of ZnONPs like laser ablation, electrochemical depositions, sol–gel method, chemical vapour deposition, thermal decomposition, ultrasound, co-precipitation, electrophoretic deposition etc (Streckovaa *et al*., 2012). The biological system, involved in the green synthesis of NPs using plants extracts and microorganisms like bacteria, fungi, algae, yeast etc (Pavithra *et al*., 2017). ZnO NPs prepared with green route exhibits various nanostructures which influence diverse properties.

MATERIALS AND METHODS

Sample preparation

Fish wastes were cleaned to remove impurities and extract was made by using blender. Finally filtered to obtain pure extraction by using centrifuge method.

Green synthesis of zinc oxide nanoparticles

20 ml of the fish waste extract was heated at 50 °C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise to it under stirring. The reaction mixture became yellowish and cream coloured precipitate of zinc hydroxide was formed. The reaction mixture was left for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16000 rpm for 10 min at 4 °C.

Characterization of synthesised nanoparticles

Particle size

Particle size and zeta potential of ZnO NPs were measured using Nanopartica (HORIBA), Differential Scanning Calorimetry (DSC) (TAInstruments DSC Q10) in the range 50-600 °C.

TEM Analysis

Morphology and crystallite size were examined by transmission electron microscope (TEM, TECNAI F-30) respectively.

Antibacterial study

Test organisms

The bacterial strains were used throughout the investigation namely; *Staphylococcus aureus* and *Escherichia coli* were obtained from Department of Food science and technology, PSGR Krishnammal College for Women, Coimbatore.

Disc diffusion method

A total of 2 microbial pathogens Viz, *Staphylococous aureus*, and *Escherichia coli* were tested using of fish waste extract and Zinc oxide nanoparticles. In this study, were performed by (Bauer *et al*., 1996) using disk diffusion method. Nutrient broth was used to culture bacteria as well as fresh overnight culture of inoculums. The test cultures were swabbed on nutrient agar (NA) plates and permissible to dry for 10 min. Two concentrations used like as 25µg and 50µg. DMSO and chloramphenicol (30ìg/disc) were used as negative as well as positive controls. The culture plates were incubated for 12 h in an incubator at 37 ºC. The diameters of the inhibition zones were measured in millimeters (mm).

RESULTS

Yellow suspension showed the formation of Zinc oxide nanoparticles from the fish waste extract.

Particle size

Particle size analysis confirmed that the synthesized

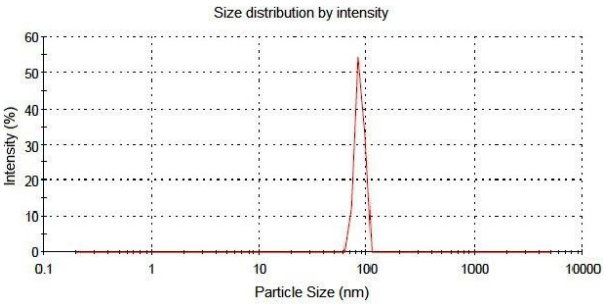

Fig. 1. Particle size Analysis

zinc oxide nanoparticles is in a nano size form. The particle size of synthesize nanoparticles is about 86.84nm (Fig. 1). This result is based on their length. In the measurement of particle size analyzer, the assumptions are based on the length of structure.

TEM Analysis

The TEM study was carried out to understand the crystalline characteristics and size of the nanoparticles. The average particle size by histogram was found to be 50–200 nm. This image reveals that most of the ZnO NPs are spherical in shape with average particles of the size of 100 nm.

Antibacterial Activity

The antibacterial activity of ZnO nanoparticles tested against *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition was determined after 12 hours of incubation. Chloramphenicol used as positive control which gives 23.25±0.3 and 22.5±0.2 zone of inhibition for the microbes *S. aureus* and *E. coli* respectively. For the same bacterial species, fish waste extract showed 7±0.1 and 7.25±0.5 zone of inhibition. ZnO nanoparticles synthesised from fish waste at two different concentrations tried against the same pathogens. At a concentration of 25(µg/ml), it provided an inhibition zone of 08.75±0.4 mm and 10.5±0.5 mm, and at a concentration of 50(µg/ml), it provided an inhibition zone of 10.5±0.7mm and 11.75±0.4 mm (Table 1).

DISCUSSION

Green synthesis of ZnO nanoparticles fromfish waste is an effective method of fish waste management. Sampath *et al.*, 2022 stated that nanoparticles synthesised from fish waste has antimicrobial property. According to that study, AgNPs synthesised from fish waste has given zone of inhibition at concentrations of 25(g/ml) and 50 (g/ml) are 1.7±0.7 and 5.7±0.2 respectively for S. aureus and 0.5±0.3 and 3.3±1.04 for *E. coli*. This study shows that synthesis of ZnONPs have greater significant than AgNPs from fish waste.

CONCLUSION

Fish waste management has been one of the problems having the extreme impact on the environment.Treated fish waste has found many applications among which the most important are animal feed, biodiesel/biogas, dietic products (chitosan), natural pigments (after extraction), food-packaging applications (chitosan), cosmetics (collagen), enzyme isolation, Chromium immobilisation, soil fertiliser and moisture maintenance in foods (hydrolysates).The results of the present study an additional application of fish wastes management through green synthesized nanoparticles. The synthesized Zinc oxide nanoparticles were analysed for size and structure used Particle size analyzer and TEM. The antimicrobial activity of the synthesized nanoparticles were also significant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal

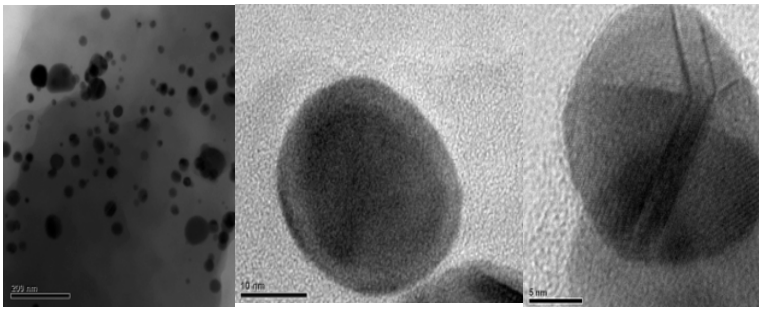

Fig. 2. TEM Analysis

Table 1. Antibacterial activity of ZnO-NPs using fish waste extract

Microorganisms	Positive control (Chloramphenicol)	Fish waste extract 25 (µg/ml)	Zinc acetate	ZnO-NPs	
				25(µg/ml)	50(µg/ml)
S. aureus	23.25±0.3	7±0.1	00.0±0.00	08.75±0.4	10.5±0.7
E. coli	21.5±0.2	7.25±0.5	00.0±0.00	10.5±0.5	11.75±0.4

relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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