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Original Article

The use of Aloe Vera Gel Functionalized Biogenic Zinc-Oxide Nanoparticles Against Fish Putative Pathogens

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ABSTRACT

Indiscriminate uses of antibiotics have resulted in the development of antibiotic-resistance among pathogens which possess a potential risk to the ecosystem, aquaculture and human health. In this study, biogenic zinc oxide nanoparticles (ZnO-NPs) were synthesized using aqueous extract of *Aloe vera* gel (AVGE) and tested against putative pathogenic bacterial strains *in-vitro*. Ultraviolet-Visible (UV-VIS) spectroscopic analysis confirmed the synthesis of AVGE-ZnO-NPs while X-ray diffraction (XRD) and Scanning Electron microscope (SEM) analysis revealed that the average size of synthesized ZnO-NPs is within the nano range. The elemental and chemical compositions of synthesized ZnO-NPs were studied using Energy-dispersive X-ray spectroscopy (EDX) and Fourier-transform infrared (FTIR) spectrometer, respectively. Two widespread bacterial strains, *Aeromonas veronii* strain ONKP1 (MN602971) and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972) that are known as emerging opportunistic pathogens in various marine and freshwater fishes as well as humans and other animals, were used as test organisms. AVGE-ZnO-NPs showed strong antibacterial activity, against the tested Gram-negative multi-drug resistant bacteria in the disc diffusion assay. The results of the present investigation could be useful for the development of new disease management strategies in the fisheries industry.

Keywords: Green nanoparticles, ZnO-NPs, Antibacterial, *Aloe vera*, Fish diseases

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INTRODUCTION

The aquaculture industry highly depends on antibiotics such as erythromycin, tetracycline and streptomycin, to control bacterial infections in the farm. However unregulated and excessive use of antibiotics leads to the emergence of antibiotic resistance in fish pathogenic bacteria. Multi-drug resistant bacteria have been isolated from fish, sediment, and water of farms (Austin & Austin, 2016; Shaalan et al., 2016). Since genetic elements can be shared between aquatic and terrestrial bacteria, human and animal pathogens can acquire such antibiotic-resistance genes from fish pathogens, arising public health issues (Swain et al., 2014; Luis et al., 2019). Thus, there is an urgent need to establish some novel strategies to combat antibiotic-resistance development and disease outbreaks in aquaculture without affecting the aquatic ecosystem. Recently, inorganic metal nanoparticles of silver, gold, titanium and zinc have generated widespread interest among aquaculture scientists as a potential alternative to antibiotics (Swain et al., 2014; Shaalan et al., 2016). They are considered as next-generation nanomedicine due to their unique pharmaceutical characteristics and novel pharmacological functions (De Villiers et al., 2008; Bharti & Singh, 2009; Das et al., 2013).

Interestingly, Zinc oxide nanoparticles (ZnO-NPs) are emerging as a most promising metal based nanodrugs due to their biocompatibility,

selectivity, and high potency (Bisht & Rayamajhi, 2016; Elshama et al., 2018; Jin & Jin, 2019). It is used in the area of drug delivery carrier, wound dressings, biosensors, bioimaging, medical devices, therapeutics, and diagnostics in medicine (Xiong, 2013; Zhu et al., 2016; Martínez-Carmona et al., 2018; Mirzaei & Darroudi, 2017). ZnO-NPs are also valued as a source of the trace element zinc (Pati & Mondal, 2019) which is required for the physiological development of animals, including fish (Watanabe et al., 1997). There are recent reports on the application of ZnO-NPs in aquaculture as an alternative of conventional zinc sources as feed additive to promote growth (Faiz et al., 2015; Wang et al., 2017; Onuegbu et al., 2018) and immunity (Anjugam et al., 2018; Awad et al., 2019). Owing to their nano size and high specific surface area, ZnO-NPs have several advantages over conventional ZnO, such as higher bioavailability, molecular dispersion, and antibacterial properties (Swain et al., 2016; Raje et al., 2018). Currently, Ag-NPs are the most widely used inorganic antimicrobial nanomaterials in water disinfection, wastewater treatment (Dimapilis et al., 2018; Shah & Mraz, 2020) and fish medicine (Shaalan et al., 2016; Khosravi-Katuli et al., 2017), but ZnO-NPs have the potential to be used as multifunctional material in aquaculture in coming years.

Green nanoparticles are mostly synthesized using plant parts like root, flower, leaves, fruit, stem, and seed extracts. Plant products are considered as safe, biocompatible, cheap, and environmentally friendly natural sources (Agarwal et al., 2017; Singh et al., 2018). ZnO-NPs have been successfully synthesized by using extracts of various plants, such as, *Vitex trifolia* (Elumalai et al., 2015), *Catharanthus roseus* (Gupta et al., 2018), *Pongamia pinnata* (Sundrarajan et al., 2015), *Citrullus colocynthis* (Azizi et al., 2017), *Borassus flabellifer* (Vimala et al., 2014) and *Aloe vera* (Sangeetha et al., 2011; Ali et al., 2016). Green ZnO nanoparticles are found to be a more potent antimicrobial agent than chemical ZnO nanoparticles on various human pathogenic strains like *Staphylococcus aureus* (Gunalan et al., 2012), *Escherichia coli* (Elumalai et al., 2015), *Bacillus cereus* (Gupta et al., 2018), *Klebsiella pneumoniae* (Mahendiran et al., 2017), *Pseudomonas aeruginosa* (Ali et al., 2016) and *Bacillus subtilis* (Chandran et al., 2018). However, studies on antibacterial activity of ZnO-NPs as well as green NPs on aquatic pathogens are limited.

Aloe vera (synonym: *Aloe barbadensis* Miller) is a popular and easily available perennial succulent plant of the Liliaceae family. It is well known for its therapeutic properties like antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and wound healing from ancient times (Sangeetha et al., 2011). *A. vera* gel extract is reported to be rich in phytochemicals like terpenoids, phenols, alkaloids, carbohydrates, flavonoids, saponins, and tannins (Parthasarathy et al., 2017; Mahendiran et al., 2017). These phytochemicals help in nucleation and stabilization of nanoparticles during synthesis (Sangeetha et al., 2011; Ali et al., 2016). In the present study, *A. vera* gel extract has been used as a capping and stabilizing agent for the synthesis of ZnO-NPs. The antibacterial activity of synthesized biogenic ZnO-NPs was evaluated against two Gram-negative fish pathogenic bacteria, *Aeromonas veronii* strain ONKP1 (MN602971) and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972). *A. veronii is* a world-wide distributed fish pathogen, causing hemorrhagic septicemia with high mortality rate (Sun et al., 2016; Tekedar et al., 2019). *S. maltophilia* is emerging as a pathogen in cultured catfishes in India (Abraham et al., 2016) and China (Geng et al., 2010). In China, it has become the most prevalent disease in cultured channel catfish, which spreads rapidly and causes mortality within a few days.

MATERIALS AND METHODS

Synthesis of biogenic zinc oxide nanoparticles

The biogenic ZnO-NPs were synthesized following the standard method of Patel et al., (2017) with slight modifications. Analytical grade zinc chloride ($ZnCl_2$, 99% purity, Himedia, India) salt and sodium hydroxide pellets (NaOH, 99% purity, Merck, India) were used as precursors. A total of 20 g gel was purified from the *Aloe vera* leaves that were collected from the university garden; boiled in double distilled water, and filtered to get the aqueous extract. Later, 50 mL aloe gel extract was added to 50 mL $ZnCl_2$ (quantity = 6.8167 g) solution with constant stirring by a magnetic stirrer. Then the pH was adjusted to 12 by adding 2 M NaOH solution and stirred for another 30 minutes. The suspension was centrifuged and the supernatant was discarded. Then, the precipitate was washed twice with double distilled water and ethanol, respectively. The white product was collected on a watch glass and dried in a hot air oven at 80 ^{0}C for 4 hours. The dried product was then crushed into powder and stored in a vacuum desiccator for future use. The possible chemical reactions were given below (Nath et al., 2018):

$$ZnCl_2 + 2NaOH \rightarrow Zn(OH)_2 + 2NaCl$$

$$Zn(OH)_2 \xrightarrow{80^\circ} ZnO$$

Elemental analysis and chemical characterization of synthesized zinc oxide nanoparticles

The synthesized ZnO-NPs have been characterized following the method of Mahendiran et al., (2017). Optical absorption spectrum for ZnO-NPs was recorded in the UV-Vis (Ultraviolet-Visible) range using a UV-Vis spectrophotometer (UV-3092, LabIndia Analytical Instrument Pvt. Ltd.). The chemical composition of ZnO-NPs was studied by using the Fourier-transform infrared (FTIR) spectrometer (Perkin Elmer L120−000A, spectral range 4000-450 cm^{-1}, KBr pellets). The elemental composition of ZnO-NPs was analyzed using the Energy-dispersive X-ray (EDX) spectroscopy (AMETEK EDAX). The morphology of the products was studied by using Scanning Electron microscope (SEM) (ZEISS Sigma 300). Phase purity and grain size were determined by X-ray diffraction (XRD) analysis (Brucker D8 Advance power-XRD, Cu-Kα-λ= 1.54 Å, range 2θ of 5–80°, scan speed = 0.2 nm, step size = 0.2°).

Determination of antibacterial activity and antibiotics susceptibility

Microorganisms

Two Gram-negative bacteria putative pathogenic strain, viz., *Aeromonas veronii* strain ONKP1 (MN602971), and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972) are used in the present investigation. They were isolated from the gastrointestinal tract of fresh tilapia fish (*Oreochromis niloticus*) which were collected from local wet markets of Kalyani, Nadia, West Bengal. The bacteria

were isolated and characterized (biochemical tests and 16S rRNA sequencing), as in previous studies (Ghosh et al., 2017).

Disc diffusion assay

The antibacterial activity of synthesized biogenic ZnO-NPs and standard antibiotics was tested against the isolates, following the disc diffusion assay (Mahendiran et al., 2017). All discs and materials were autoclaved for sterilization before the experiments. Bacterial inocula were prepared by growing a single colony overnight in 5 ml nutrient broth at 30 °C and streaked onto Mueller-Hinton agar (Himedia, India) plates. Nanoparticle suspension of different concentrations viz. 5, 10, 20, 30, 40 and 50 mg/mL were prepared in double distilled water and sonicated for the uniform suspension of nanoparticles. Then 10 µL suspension was pipetted from each stock and impregnated onto respective 6 mm diameter sterile blank antibiogram discs. Then the dried discs containing different concentrations (50, 100, 200, 300, 400 and 500 µg/disc) were placed onto Mueller-Hinton agar. Discs of streptomycin (100 µg), tetracycline (30 µg) and ciprofloxacin (5 µg) were prepared from their powder form (HiMedia, India) as described above. Commercial discs of erythromycin (15 µg), ampicillin (10 µg), penicillin-G (10 µg), chloramphenicol (30 µg) and amoxicillin (30 µg) (Himedia, India) were also used. After incubation at 37 °C for 24 h, the diameter of the inhibition zones around discs were measured with a ruler. The disc diffusion assay was performed in triplicate for nanoparticle and antibiotics. The results were expressed as means ± standard errors.

RESULTS AND DISCUSSIONS

Elemental analysis and chemical characterization findings of zinc oxide nanoparticles

UV-Vis analysis

The sample exhibits strong UV-absorption spectra with maximum absorption at 340 nm (Fig. 1). Similar observations have been reported on the green synthesis of stable ZnO-NPs (Varghese & George, 2015; Qian et al., 2015). The optical absorbance spectra of noble metal nanoparticles are known to shift to longer wavelengths (red shift) with increasing particle size and to smaller wavelengths (blue shift) with decreasing particle size, due to Surface Plasmon Resonance (SPR). Moreover, only a single SPR band is found in the absorption spectra of spherical nanoparticles, whereas anisotropic and non-spherical shaped particles could give rise to two or more SPR bands depending on symmetry (Sangeetha et al., 2011). Hence, the results of UV absorption spectra of synthesized ZnO-NPs indicate its smaller particle size and spherical shape which is further validated.

XRD analysis

The XRD pattern of AVGE-ZnO-NPs (Fig. 2) shows Bragg reflections at 2θ values of 31.42°(100), 34.01°(002), 36.10°(101), 47.26°(102), 56.22°(110), 62.50°(103), 66.18°(200), 67.38°(112), 68.77°(201), 72.26°(004) and 76.84°(202) that are in good agreement with JCPDS CARD NO: 36-1451. The plane values of XRD patterns confirm the hexagonal wurtzite structure of ZnO-NPs (Sangeetha et al., 2011; Ali et al., 2016). The sample also shows diffraction peaks of the orthorhombic form of $Zn(OH)_2$ (JCPDS CARD NO: 38-0385), which probably arose from surface hydroxylation of ZnO (Deb et al., 2013). The average particle size (D) of

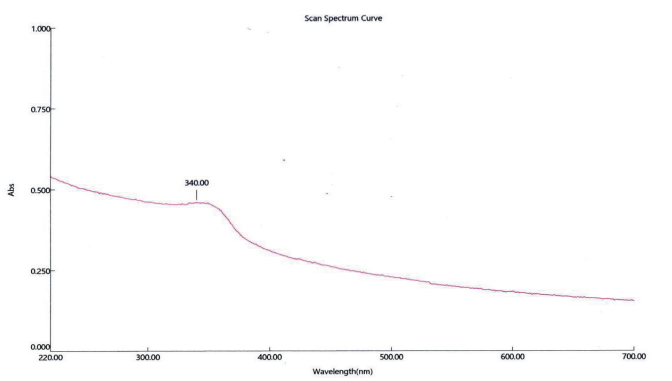

Figure 1. UV-Vis spectra of AVGE-ZnO-NPs.

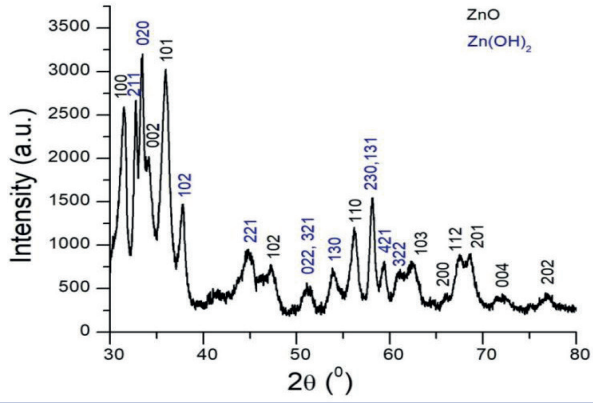

Figure 2. XRD Pattern of AVGE-ZnO-NPs.

synthesized nanoparticles is calculated using the Scherrer's formula, $D = K\lambda/(\beta \cos \theta)$, where D is the crystal size, K is a shape factor (it is a constant; approximately equal to 0.9), λ is the X-ray wavelength, θ is the Bragg's angle in radians and B the full line width at half maximum (FWHM) of the main intensity peak in radians (Mahendiran et al., 2017). The value of D is obtained as 9.72 nm by taking 101 as the main intensity peak for calculation.

SEM and EDX analysis

The SEM images (Fig. 3) show the spherical and rod shaped AVGE-ZnO-NPs. The nanoparticles are found to be agglomerated with a particle size ranging from 37.5-63.75 nm. The agglomeration could be induced by the densification and microstructural changes resulting in the narrow space between particles and also decreased pore size and diameter (Sangeetha et al., 2011). The SEM results of AVGE-ZnO-NPs are similar to previous studies on green ZnO-NPs (Vimala et al., 2014; Qian et al., 2015; Chandran et al., 2018).

The EDX analysis confirms the presence of metallic zinc (Zn) (79.21%) and oxygen (O) (20.79%) as elements in AVGE-ZnO-NPs (Fig. 4). The EDX spectra of AVGE-ZnO-NPs exhibit three characteristic emission peaks of metallic Zn and one small emission peak from O element (Ali et al., 2016). Besides Zn and O, EDX spectra shows a weak signal of Cl element which is probably from precursor $ZnCl_2$ or compounds present in aloe extract.

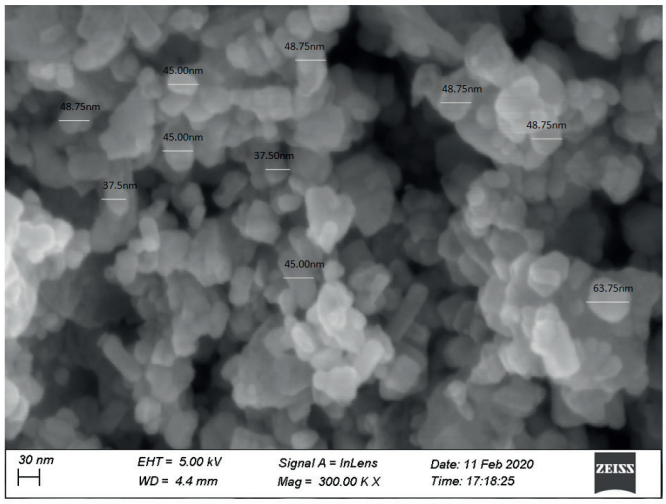

Figure 3. SEM of AVGE-ZnO-NPs (37.3-63.75 nm).

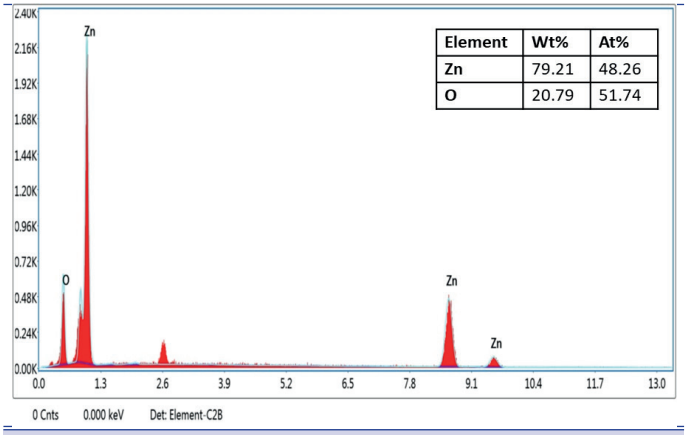

Figure 4. EDX spectrum of AVGE-ZnO-NPs.

FTIR analysis

The infra-red (IR) spectrum of AVGE-ZnO-NPs shows characteristic absorption bands at 470.83 cm^{-1} and 535.57 cm^{-1} owing to the presence of two transverse optical stretching modes of Zn-O (Elumalai et al., 2015; Patel et al., 2017) (Fig. 5). The peak in the region between 600 and 400 cm^{-1} is allotted to Zn-O bond vibrational frequencies (Sangeetha et al., 2011). The absorption band observed at 626.99 cm^{-1} and 708.76 cm^{-1} indicates the deformation of Zn-O bond (Chandran et al., 2018) and C-N stretching of amine group, respectively (Gupta *et al.*, 2018). The peaks at 897.17 cm^{-1}, 1620.40 cm^{-1} and 3460 cm^{-1} are due to C–H bond of alkene group, amide I of proteins/enzymes and stretching vibration of O–H groups in adsorbed moisture, alcohol and phenolic compounds (Mahendiran et al., 2017). 1037.14 cm^{-1} and 2927.15 cm^{-1} are assigned to stretching vibrations of C–H and C–O (Zhou et al., 2017). The peaks in the region of 2900-3700 cm^{-1} also correspond to amide linkages between amino acid residues of the proteins (Sangeetha et al., 2011). The intense band at 1476.92 cm^{-1} can be attributed to alcohols and phenolic groups, C–N groups of aliphatic and aromatic amines and –C–O–C– or –C–O– bonds of alkaloids and flavones (Patel et al., 2017). Thus, the results of FTIR spectrum suggest the role of biological molecules (alkaloid, flavonoid, phenolic compounds, proteins etc.) present in the plant extract as a capping and stabilizing agent for the synthesis of ZnO-NPs.

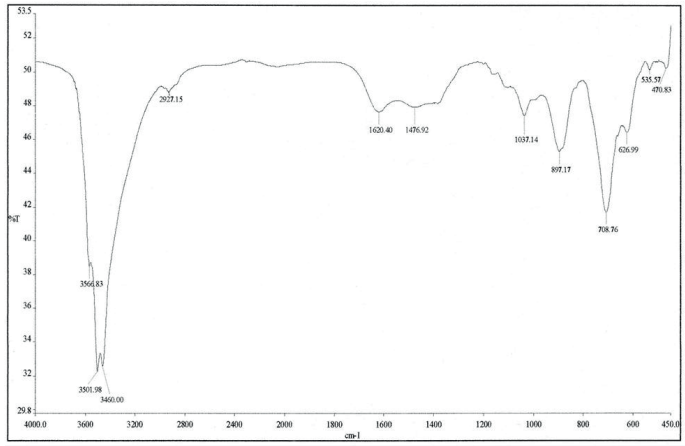

Figure 5. FTIR spectra of AVGE-ZnO-NPs.

Antibacterial activity and Antibiotics susceptibility findings

In the *in-vitro* disc diffusion assay, the presence of clear inhibition zones around the discs indicates the bactericidal activity of AVGE-ZnO-NPs as well as antibiotics (Fig. 6). The antibacterial effect of AVGE-ZnO-NPs and other antibiotics were quantitatively assessed based on the diameter of the inhibition zones which was shown in Table 1. A graphical representation of the zone of inhibition of bacterial pathogens against different antimicrobials was illustrated in Fig 7a. Whereas, In Fig 7b, a graphical representation comparing the zone of inhibition between the highest dose of nanoparticles and the antibiotics to which the bacteria showed susceptibility, was given. The results indicate that the AVGE-ZnO-NPs have good inhibitory activity at all concentrations (50-500 µg/disc) against all the bacterial strains, in comparison to standard commercially available chemotherapeutic agents. In the present study, the inhibitory effect of AVGE-ZnO-NPs increased steadily with the increasing concentration. AVGE-ZnO-NPs showed a maximum zone of inhibition (15.67±1.20 mm) at the highest concentration of 500 µg/disc or 50 mg/ml. As the concentration increases, the diffusion rate of ZnO-NPs increase in the agar medium, causing increased antibacterial activity at higher concentrations (Gunalan et al., 2012).

Both *A. veronii* and *S. maltophilia,* are known to cause serious epidemic disease outbreaks in fish farms, with dominant clinical signs of skin ulcers (Nawaz et al., 2006; Austin & Austin, 2012) and ascites (Geng et al., 2010), respectively. Several studies have isolated *S. maltophilia* from diseased fishes, for example, yellowtail (Furushita et al., 2005), giant gourami (Musa et al., 2008), channel catfish (Geng et al., 2010) and African catfish (Abraham et al., 2016). *A. veronii* is reported to be associated with infections in a number of economically important fish, including cichlid oscar (Sreedharan et al., 2011), gibel carp (Sun et al., 2016), tilapia (Hassan et al., 2017) and channel catfish (Hoai et al., 2019). They are also known as opportunistic pathogens in human and other animals (Gopalakrish-

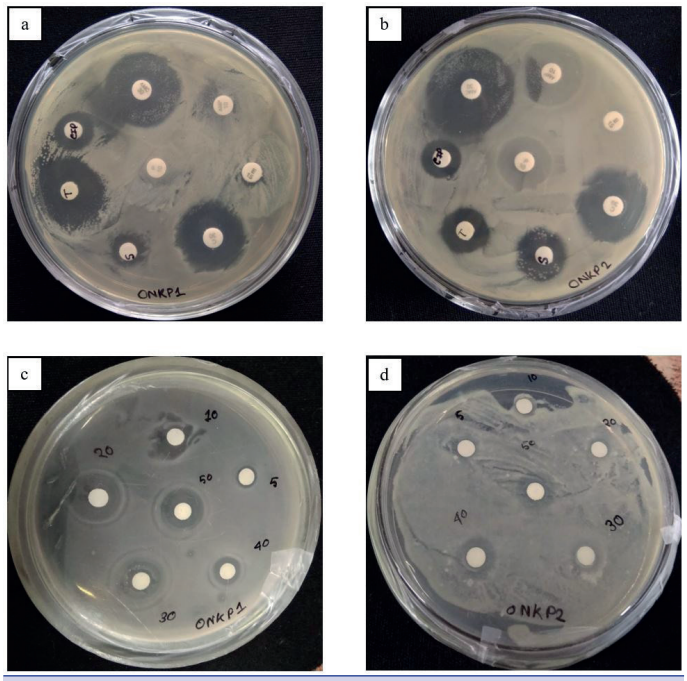

Figure 6. Antimicrobial activity of Standard antibiotics (a,b) and AVGE-ZnO-NPs (c,d) against A. veronii ONKP1 (a,c) and S. maltophilia ONKP2 (b,d). In c and d, nanoparticle concentration was written as per stock value (5-50 mg/ml) which would be 50-500 µg/disc. (E = Erythromycin; C = Chloramphenicol; AMP= Amoxicillin; AMP = Ampicillin; P = Penicillin-G; T= Tetracycline; CIP = Ciprofloxacin; S = Streptomycin).

Table 1. Antimicrobial activity of green synthesized ZnO nanoparticles (AVGE-ZnO-NPs) and standard antibiotics against putative fish pathogenic bacterial strains

Samples (µg/disc)	Bacterial Strain/Zone of inhibition[a] (Mean±SE mm)[b]	
	Aeromonas veronii ONKP1	*Stenotrophomonas maltophilia* ONKP2
AVGE-ZnO-NPs		
50 µg	8.33±0.33	7.67±0.33
100 µg	10.33±0.33	8.67±0.67
200 µg	11.33±0.67	12±1
300 µg	12.33±1.33	14.67±0.33
400 µg	13±1	14.67±0.88
500 µg	15.33±0.33	15.67±1.20
Standard Antibiotics		
Erythromycin (15 µg)	-	-
Chloramphenicol (30 µg)	21.67±0.88	21.33±0.67
Amoxicillin (30 µg)	12.67±0.33	12±0.58
Ampicillin (10 µg)	-	-
Penicillin-G (10 µg)	-	-
Tetracycline (30 µg)	14.67±0.88	15.67±0.88
Ciprofloxacin (5 µg)	12.67±0.33	11.67±0.33
Streptomycin (100 µg)	9.67±0.33	11.33±0.88

SE = standard error, - = No zone; [a] Diameter Zone of inhibition (mm) including disc diameter 6 mm; [b] Results of triplicate analysis.

nan et al., 1999; Nawaz et al., 2006; Janda & Abbott, 2010; Geng et al., 2010). Studies have found that a variety of commercially available antimicrobial agents like, β-lactams, quinolones, aminoglycosides and tetracycline are resisted by *A. veronii* (Sun et al., 2016; Nawaz et al., 2006) and *S. maltophilia* (Looney et al., 2009; Geng et al., 2010) which makes them very difficult to control. In the current study, *A. veronii* and *S. maltophilia* were also found to be resistant against erythromycin, ampicillin and penicillin-G, whereas susceptible to tetracycline, ciprofloxacin, streptomycin, chloramphenicol and amoxicillin. Moreover, *Oreochromis* spp. has been reported to carry antibiotic-resistant pathogenic bacteria such as, *Salmonella* (Budiati et al., 2013), *Aeromonas hydrophila* (Marathe et al., 2016), *Klebsiella pneumoniae* (Marathe et al., 2016; Thongkao & Sudjaroen, 2019) and *Staphylococci* (Thongkao & Sudjaroen, 2019) in their internal organs and is considered as a reservoir of zoonotic diseases. The results of our study also suggest that the marketed Nile tilapias *(O. niloticus) can carry antibiotic-resistant human pathogenic* bacteria like *A. veronii* and *S. maltophilia*, which is a concern of microbiological safety.

Previously, Swain et al., (2014) has studied various metal nanoparticles including Zn, ZnO, CuO, Ag, Al_2O_3, Ag–TiO_2 and Fe_2O_3 (both commercial and laboratory synthesized) as potential antimicrobial agents against bacterial isolates such as *A. hydrophila, Edwardsiella tarda, Pseudomonas aeruginosa, Flavobacterium branchiophilum, Vibrio* sp., *Staphylococcus aureus, Bacillus cereus* and *Citrobacter* sp. which were isolated from various diseased freshwater fish. Chemical ZnO-NPs (105-122 nm), chemical CuO-NPs and green Ag-NPs show better inhibition against all bacteria, than others. However, the authors did not include any antibiotics in the study. Shaalan et al., (2017) also used commercial ZnO-NPs (≈66 nm) and oxytetracycline against six pathogens from infected fish samples. The ZnO-NPs exhibited antibacterial activity against *A. hydrophila* (common bream), *A. salmonicida* subsp. *Salmonicida* (rainbow trout) and *Yersinia ruckeri* (rainbow trout), but fail to inhibit growth of *E. ictaluri* (channel catfish), *E. tarda* (discus) and *Francisella noatunensis* subsp. *orientalis* (Malawi cichlid). In our study, laboratory synthesized green ZnO-NP as well as eight standard antibiotics were tested against the pathogenic strain for direct comparison. Interestingly, AVGE-ZnO-NPs (37.5-63.75 nm) were found to be equally effective, even at lower doses, towards multiple antibiotic-resistant, *A. veronii* and *S. maltophilia*, in the study. There are differences in the results of antibacterial activity of nanoparticles including ZnO, based on size, dose and species (Swain et al., 2014). This is the first *in-vitro* study to demonstrate the inhibitory effects of green

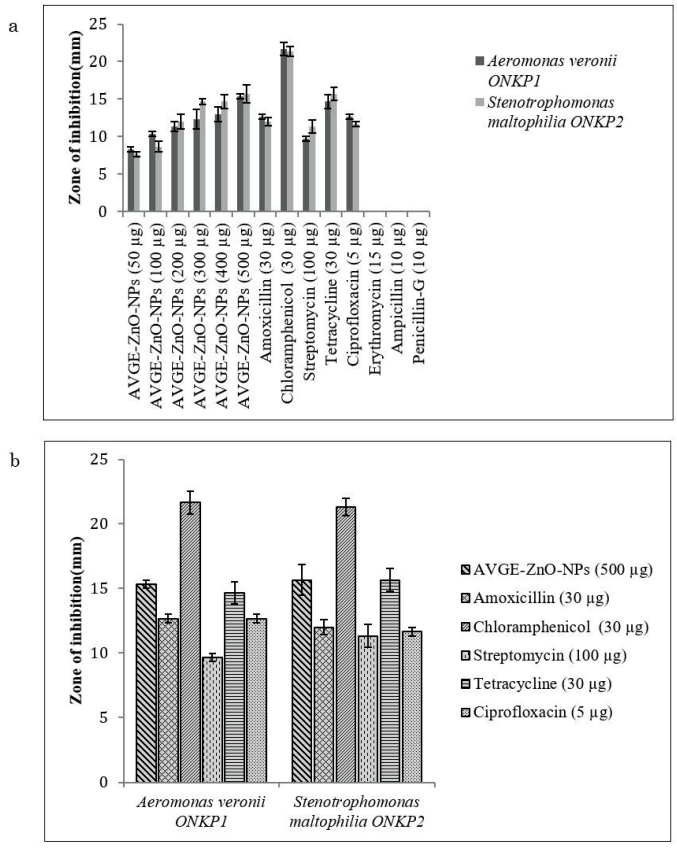

Figure 7. Graphical representation of Zone of inhibition (mm) against bacterial pathogens for antimicrobials; a) Different antimicrobials used in test; b) Comparison between AVGE-ZnO-NPs (highest dose) and the antibiotics to which the bacteria showed susceptibility (error bars represent the mean ± SE of three replicates; concentration of samples as µg/disc).

or *Aloe* functionalized ZnO-NPs against the multi antibiotic-resistant fish pathogen, *A. veronii* and *S. maltophilia*, in addition to their isolation from fresh live marketed, *O. niloticus*. However, synthesized ZnO-NPs may behave differently in live fish and to other pathogenic bacteria. It is a subject of further experimental study which can be carried out in the future.

Nanotechnology is rapidly incorporating into aquaculture by providing new nano-enabled products with novel and unique functions (Luis et al., 2019). But, nanoparticles could be a source of new contamination in the aquatic ecosystem. Studies have shown some toxic effects of ZnO-NPs on aquatic species (Connolly et al., 2016; Kaya et al., 2016; Skjolding et al., 2016; Chupani et al., 2018). The toxicity potential of ZnO-NPs is reported to depend on the exposure route, contact time, concentration, environment and target organism (Swain et al., 2016; Khosravi-Katuli et al., 2017; Elshama et al., 2018; Shah & Mraz, 2020). In freshwater, ZnO-NPs generally tend to dissolve rapidly, increasing the risk of acute toxicity for aquatic organisms (Shaalan et al., 2017).

CONCLUSION

In summary, the results of our study indicates that the green synthesis of ZnO-NPs using *Aloe vera* gel extract is simple, rapid, convenient and cost-effective. *A. vera* has been also found to be equally effective in capping and stabilization of ZnO-NPs. In this study, the synthesized biogenic ZnO-NPs showed strong inhibitory activity towards multi-drug resistant fish pathogenic bacteria, *A. veronii* and *S. maltophilia*, even at a low dose in the disc diffusion assay. Thus the synthesized biogenic ZnO-NPs may be utilized as a potential alternative for disease prevention and treatment in fish. However, detailed *in-vivo* studies accessing the efficacy as well as associated risks and safety of synthesized biogenic ZnO-NPs are needed for application as nano-antibiotics in aquaculture.

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