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Synthesis and antimicrobial activity of zinc oxide nanoparticles against foodborne pathogens *Salmonella typhimurium* and *Staphylococcus aureus*

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ABSTRACT

ZnO nanoparticles are well known multifunctional inorganic nanoparticles with predominant antimicrobial potential. In the current study, ZnO nanoparticles were synthesized and characterized by UV–Vis and Fourier transform infrared (FTIR) spectroscopy, shape and size of the nanoparticles were analyzed with the help of electron microscope. The nanoparticles were evaluated for antimicrobial potential against *Salmonella typhimurium* and *Staphylococcus aureus* by kill time analysis. Further quantification of bacterial response was evaluated by measuring minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). UV–Vis and FTIR spectra conformed the formation of ZnO nanoparticles, whereas, the transmission electron microscope (TEM) showed that the average size of the synthesized nanoparticles was in the range of 20 nm. ZnO nanoparticles showed significant ($p < 0.05$) decrease in viability of test bacteria with increase in time duration and complete elimination (0 Log CFU/mL) was achieved after 8 h for *S. typhimurium* and 12 h for *S. aureus*, indicating the bactericidal effect of nanoparticles. Whereas, for the control treatments (without nanoparticles), bacterial counts for *S. typhimurium* and *S. aureus* were 7.19 and 9.5 Log CFU/mL. Moreover, the morphological changes observed in bacterial cells after treating with nanoparticles showed that the treated bacterial cells became pitted and deformed. These changes resulted in simultaneous growth reduction of the test bacteria and eventually to cell death and decomposition. ZnO nanoparticles showed antimicrobial potential against the foodborne pathogens, indicating that ZnO nanoparticles due their safe status and cheap cost can be used for food products as a preservative and packaging material.

1. Introduction

The advancements in nanotechnology have led to the development of new antimicrobial compounds (Gunalan et al., 2012). The biocidal spectrum of nanomaterials is wider than antibiotics and nanomaterials even showed the response against different types of cells (Sungkaworn et al., 2007). Inorganic nanoparticles exhibit novel and improved biological functions due to their size and structure (Gunalan et al., 2012). Previous reports presented that metallic nanoparticles due to their toxicity induced cell death in eukaryotic cells and growth inhibition in prokaryotes (Long et al., 2006; Magrez et al., 2006). Inorganic metal oxides (CaO, MgO and ZnO) showed considerable antimicrobial activity due to generation of reactive oxygen species (Sawai and Yoshikawa, 2004; Raja et al., 2018). Inorganic metal oxides in addition to antimicrobial activities provide an additional benefit by supplying essential mineral elements to body (Padmavathy and Vijayaraghavan, 2008). Nanocrystalline metal oxides are of interest as they can by synthesized with high surface area suitable for biological applications. The inorganic antibacterial agents have an advantage over organic antibacterial compounds in terms of selectivity, specificity and less toxicity.

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Nanotechnology is based on materials with nano-scale size, exhibiting advance physicochemical and biological functions based on unique structural characteristics (Sahoo et al., 2007). Recently, nanoparticles gained attraction due to their unique characteristics compared to conventional macroscopic materials. The nanotechnology showed considerable impact in various fields of biomedical research such as drug delivery system, tumour therapy and cell imaging are predominant biomedical applications of nanotechnology (Wang et al., 2006; Seo et al., 2006). Among inorganic materials, ZnO nanoparticles are of special interest due to diverse wide range of nanostructures and biotechnological applications (Singla and Kumar, 2009; Kuo et al., 2009; Khatami et al., 2018). ZnO is documented as safe zinc compound by the United States Food and Drug Administration (21CFR182.8991) (FDA, 2011). Several studies have been attributed towards the antimicrobial potential of ZnO due to generation of free radical species on the surface of the oxide (Sawai, 2003).

The antimicrobial potential of ZnO nanoparticles have been studied previously but the exact mechanism of action is still not clear, however, the release of reactive oxygen species is thought to be one of the leading mode of antimicrobial action (Espitia et al., 2012). Food safety is a major concern for consumers and food industries due to increased incidence of food-borne illnesses in developing and even developed countries. In Unites States of America, annually 25–81 million cases of food-borne illnesses were reported with an estimation of 9000 deaths due to consumption of contaminated of food (López-Gómez et al., 2009; Jevšnik et al., 2013). The foodborne pathogens impose a considerable burden of infection in both developed and developing countries. Among the foodborne pathogens, *S. typhimurium* and *S. aureus* are the most common and frequent pathogens responsible for food poisoning and food related infections (Costa et al., 2012; Aydin et al., 2011). The application of nanomaterials can be an alternative solution to food safety challenges faced by food industries (Silvestre et al., 2011; Duffy et al., 2018).

In the current study ZnO nanoparticles were synthesized and tested against food-borne pathogens including *S. aureus* and *S. typhimurium*.

2. Materials and methods

2.1. Zinc oxide nanoparticles synthesis

Zinc oxide nanoparticles were prepared following the method of (Jaisai et al., 2012), with slight modifications. Zinc acetate dihydrates [Zn $(CH_3COO)_2{\cdot}2H_2O$] (Merck Ltd.), 4 mM solution (A) was prepared in 20 mL of absolute ethanol (Sigma-Aldrich Ltd.) under vigorous stirring at 50 °C for 1 h. The solution was further diluted by adding 20 mL of ethanol and heated for 30 min at 80 °C. Sodium hydroxide [NaOH] (Sigma-Aldrich Ltd.), 4 mM solution (B) was prepared in 20 mL ethanol at 50 °C for 1 h under continuous stirring. Solution A was mixed drop wise with solution B under continuous stirring for 10 min. The mixture solution was then incubated at 60 °C in preheated water bath for 2 h. The zinc oxide nanoparticles in ethanol were transferred to de-ionized water in phase change by mixing equal amount of water to the solution and kept at control temperature of 84 °C to facilitate the evaporation of ethanol. The resultant transparent colloidal stock solution of zinc oxide nanoparticles (1.33 mM) was used for further antimicrobial assays.

2.2. Characterization of zinc oxide nanoparticles

The zinc oxide nanoparticles were characterized by following Akbar and Anal (2014), with the help of UV–Vis (UNICAM UV/Vis Spectrophotometer, UK) and Fourier transform infrared (FTIR) spectrometer (Perkin Elmer, USA). The morphological characteristics of nanoparticles were examined under transmission electron microscope (TEM), (FEI, TECNAI T20, Japan).

The bacterial strains of *S. aureus* and *S. typhimurium* were acquired from Bioprocess Technology laboratory of the Asian Institute of Technology, Thailand and the bacterial strains were reconfirmed by biochemical and immunological testing (Akbar and Anal, 2013).

2.3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of zinc oxide nanoparticles

MIC of ZnO nanoparticles was determined by following the method of Sadiq et al. (2015), with slight modification. The colloidal stock solution of zinc oxide nanoparticles (1.33 mM) was used in the preparation of nutritional media by mixing dehydrated nutrient broth (HiMedia, India) powder as per manufacturer instruction and heated for 5 min at 60 °C.

The ZnO nanoparticles containing nutrient were diluted to different concentrations by mixing with nanoparticles free broth to make final volume of 5 mL in clean and dried test tubes and sterilized under pressure at 121 °C for 15 min in autoclave. Target bacteria (*S. aureus* and *S. typhimurium*) approximately 10^7–10^8 CFU/mL were inoculated separately in different concentrations of ZnO (NP) stock solution (0–100%) and incubated at 37 °C for 24 h. The nanoparticles free nutrient broth with target bacteria inoculum was used as growth control, whereas ZnO (NP) containing nutrient broth without target bacteria was used as negative control. The lowest concentration of ZnO (NP) which showed no visible growth after incubation period (24 h) was marked as the MIC. MBC of ZnO (NP) was evaluated by standard plate count method for bacterial enumeration by sub-culturing 1 mL of test broth (containing 0–100% stock solution of ZnO nanoparticles) over the surface of nutrient agar plates with the help of sterile spreader, and incubated at 37 °C for 24 h. After subsequent incubation period (24 h) the lowest concentration that showed no visible growth on agar plates was measured as the MBC.

2.4. Kill time assay

Kill-time analysis was used to estimate the bactericidal effects of ZnO nanoparticles, following the method of Joray et al. (2011) with slight modifications. The stock solution of ZnO nanoparticles (1.3 mM) was used to prepare nutrient broth. The kill-time curves were established by inoculating *S. aureus* and *S. typhimurium* (10^7 – 10^8 CFU/mL) in broth containing ZnO nanoparticles. Nutrient broth containing test bacteria without nanoparticles was used as control. The samples were taken from tested bacterial culture at selected time intervals (0, 2, 4, 8, 12, 16, 20 and 24 h), serially diluted in sterile water and incubated in plate count agar. The bacteria were counted (CFU/mL) after 24 h of incubation at 37 °C. Moreover, the effect of nanoparticles was observed on test bacteria at selected time intervals by measuring the optical density (OD_{600}) with the help of UV–Vis spectrophotometer (UNICAM UV/Vis Spectrophotometer, UK) and standard plate count techniques.

2.5. Determination of bacterial morphological changes by TEM

The interactions and effects of ZnO nanoparticles on morphological characteristics of target bacteria were studied under TEM (FEI, TECNAI T20 Japan) (Akbar and Anal, 2014).

Fresh test bacterial culture (10^7–10^8 CFU/mL) was inoculated in nutrient broth supplemented with ZnO nanoparticles (1.3 mM stock solution) and incubated at 37 °C for 10 h, whereas, ZnO nanoparticles free nutrient broth was used as a control. Following the incubation period, bacterial cells were harvested by centrifugation (1500 g for 10 min), washed three times and resuspended in glutaraldehyde (2.5% w/v) in 0.1 M sodium cacodylate buffer pH (7.2–7.4) at 4 °C for overnight. The treated bacterial cells were fixed with osmium tetroxide (1.0%) in sodium cacodylate buffer (0.1 M) at 4 °C for 1 h. Finally, the bacterial cells were dehydrated with different concentration of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) at 4 °C, twice for 15 min each, followed by staining and observed under TEM (FEI, TECNAI T20, Japan) at acceleration voltage 120 kV.

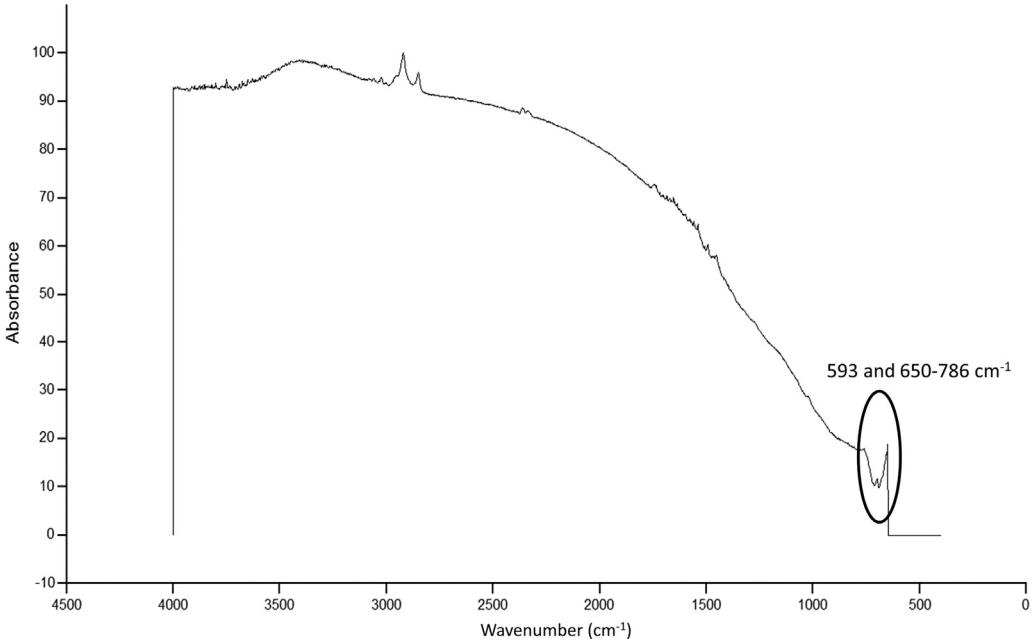

Fig. 1. Fourier transform infrared spectrum of zinc oxide nanoparticles.

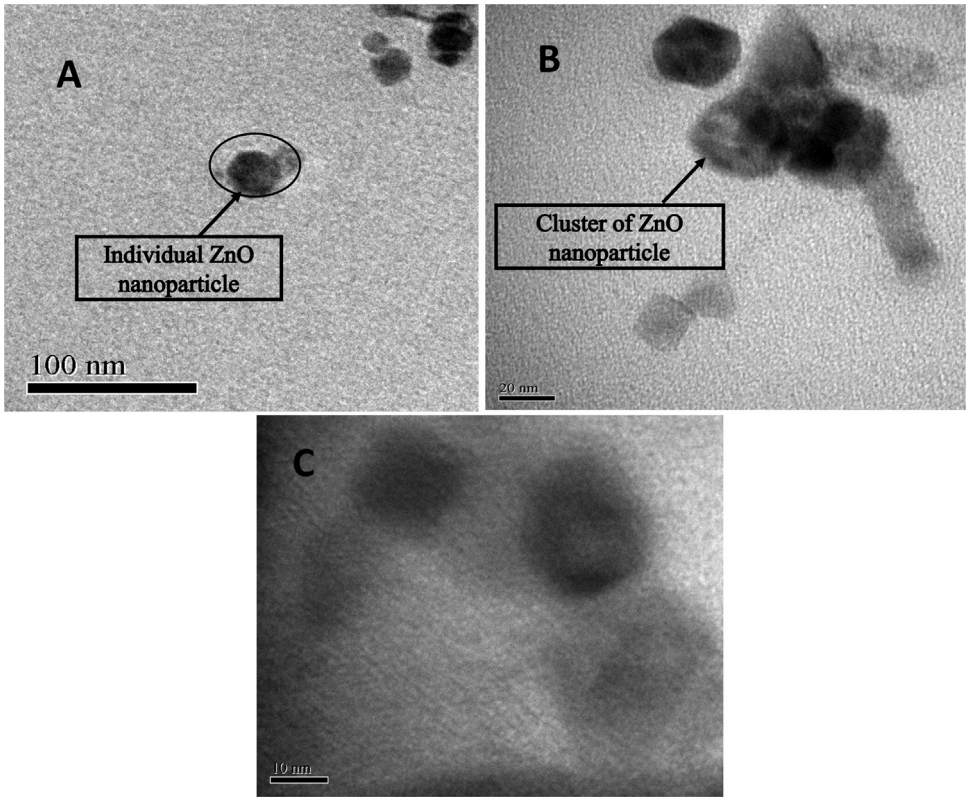

Fig. 2. Transmission electron micrograph of ZnO nanoparticles, showing average size equal to 20 nm in all A, B and C images.

2.6. Statistical analysis

All experiments were conducted in triplicates and one-way analysis of variance (ANOVA) was performed to estimate the significant differences ($p < 0.05$) between means by using SPSS statistical software package (SPSS, version 17.0, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Zinc oxide nanoparticles synthesis and characterization

The shift in UV–Vis spectrum from 370 to 385 nm and FTIR peaks (Fig. 1) at 593 cm^{-1} and shifting from 650 to 786 cm^{-1} confirmed the formation of ZnO nanoparticles (Akbar and Anal, 2014; Raja et al., 2018). Based on previously published studies, the absorption peaks in the range of 400–700 cm^{-1} could be attributed to the Zn-O stretching

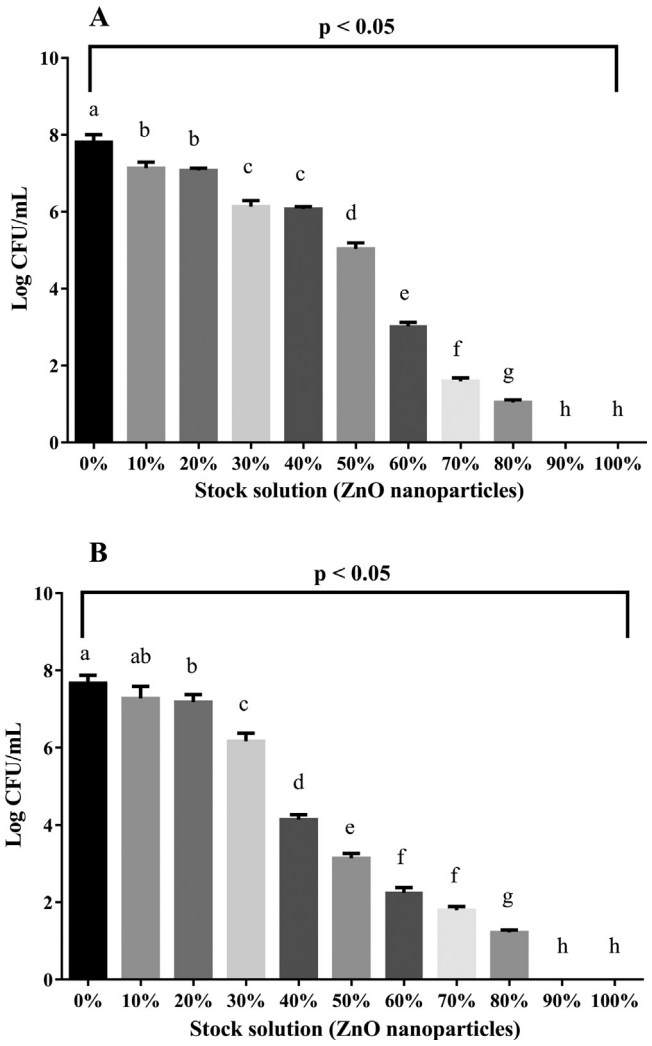

Fig. 3. Determination of minimum bactericidal concentration (MBC) of ZnO nanoparticles against *S. typhimurium* (A) and *S. aureus* (B) by plate count method. Different letters (a-h) above the bars indicated significant ($p < 0.05$) difference among mean observations and one-way analysis of variance (ANOVA) was performed to estimate the significant differences ($p < 0.05$) between means.

modes (Djaja et al., 2013). The Fig. 2 shows the particle size distribution and morphology, studied under TEM. The average particles size was in the range of 20 nm with spherical in shapes, predominantly.

3.2. Determination of MIC and MBC of zinc oxide nanoparticles

MIC and MBC of ZnO nanoparticles were found to be 80% and 90% of the stock solution (1.33 mM) respectively, for both *S. typhimurium* and *S. aureus*. MIC (80% in ZnO nanoparticles stock solution) was determined by evaluating the lowest concentration at which there was no visible turbidity in the test tubes with lowest CFU/mL in SPC for the test pathogens. MBC was determined by plate count method and there was significant ($p < 0.05$) reduction of bacterial count with increase in the concentration of nanoparticles (Fig. 3). After treatment with 80% nanoparticles stock solution the bacterial counts were 1.21 CFU/mL and 1.043 CFU/mL for *S. aureus* and *S. typhimurium*, respectively. After treatment with 90% of nanoparticles stock solution there was no viable growth observed for both the test bacteria, hence 90% of the nanoparticles stock solution was considered MBC. The results were in accordance with Duffy et al. (2018), who reported that the antibacterial effect of ZnO nanoparticles was increased against foodborne pathogens with increase in concentration.

ZnO nanoparticles showed strong antimicrobial effect against both gram positive and gram-negative bacteria (*S. aureus* and *S. typhimurium* respectively), which was in accordance with the previous research reports (Premanathan et al., 2011). Gunalan et al. (2012), reported 0.8 mM concentration of ZnO nanopartilces as MIC and MBC value against *S. aureus* which was in accordance with the current investigation. Padmavathy and Vijayaraghavan (2008), reported bacteriostatic effect of ZnO nanoparticles at concentration of 1 mM solution against gram negative bacteria (*E. coli*), however they reported the bactericidal effect at a concentration of 5 mM. The bacteriostatic and bactericidal concentrations in the current study were lower than reported by them, the reported difference might be due to different strain of gram negative bacteria and test conditions (Sadiq et al., 2017).

3.3. Kill time analysis

The test pathogens showed significant ($p < 0.05$) decrease in viability with passage of time when compared with control treatment (bacterial incubations without nanoparticles). The effects nanoparticles on viability of test pathogens are shown in Fig. 4. Antimicrobial agent was considered bacteriostatic at the lowest concentration that reduced the original inoculum size 0–3 log CFU/mL and bactericidal if, inoculum size was reduced by > 3 log CFU/mL (National Committee for Clinical Laboratory Standards, 1992).

S. typhimurium when treated with ZnO nanoparticles showed significant decrease in viability from 7.97 log CFU/mL to 1.825 log CFU/mL after 4 h of incubation. After 2 h of incubation there was decrease in *S. typhimurium* viability from 7.97 log CFU/mL to 4.84 log CFU/mL. The observed decrease in bacterial count was more than 3 log CFU/mL indicating bactericidal action of nanoparticles within 2 h of incubation. Whereas, in case of *S. aureus* the bactericidal effects were observed after 4 h of incubation period as the observed decrease in bacterial count was more than 3 log CFU/mL. The complete bacterial elimination was observed after 8 h of incubation of *S. typhimurium* with nanoparticles, whereas, for *S. aureus* the complete elimination was found after 12 h.

Moreover, the kill time analysis was studied by measuring the optical density (OD_{600}) of ZnO nanoparticles treated bacteria (Fig. 4 C and D). A similar time kill pattern, like plate count was observed for both the test bacteria. During kill time analysis, *S. typhimurium* and *S. aureus* treated with ZnO nanoparticles for 24 h showed the optical densities of 0.15 and 0.35 respectively, whereas, the control treatments for *S. typhimurium* and *S. aureus* optical densities were 0.699 and 0.657 respectively after 24 h of incubation.

When *S. typhimurium* was treated with nanoparticles, initially there was slight increase in optical density after 2 h of incubation followed by no significant change in optical density throughout the incubation period (24 h). The kill time analysis by optical density method was in accordance with the plate count method as bactericidal effects were observed after 2 h of incubation for *S. typhimurium*. *S. aureus* treated with nanoparticles showed significant increase in the optical density till 8 h of incubation followed by approximately stable optical density till 24 h, compared to control treatment. This was in accordance with plate count method indicating the delayed elimination of *S. aureus* compared to *S. typhimurium*.

The complete elimination of test pathogens was obtained at much lower concentration of ZnO nanoparticles (1.33 mM) compared to previously reported by Gunalan et al. (2012), who reported inhibition of bacterial growth at concentration of 8 and 16 mM of ZnO nanoparticles by measuring optical density (OD_{595}). Jones et al. (2007) studied the effect of various metallic nanoparticles on bacterial viability and found that only ZnO nanoparticles showed significant reduction in bacterial viability compared other metallic nanoparticles. Ren et al. (2009) studied the antimicrobial potential of CuO and ZnO nanoparticles and found that both the metallic nanoparticles showed the bactericidal activity in the same range of concentration. ZnO is bio-safe,

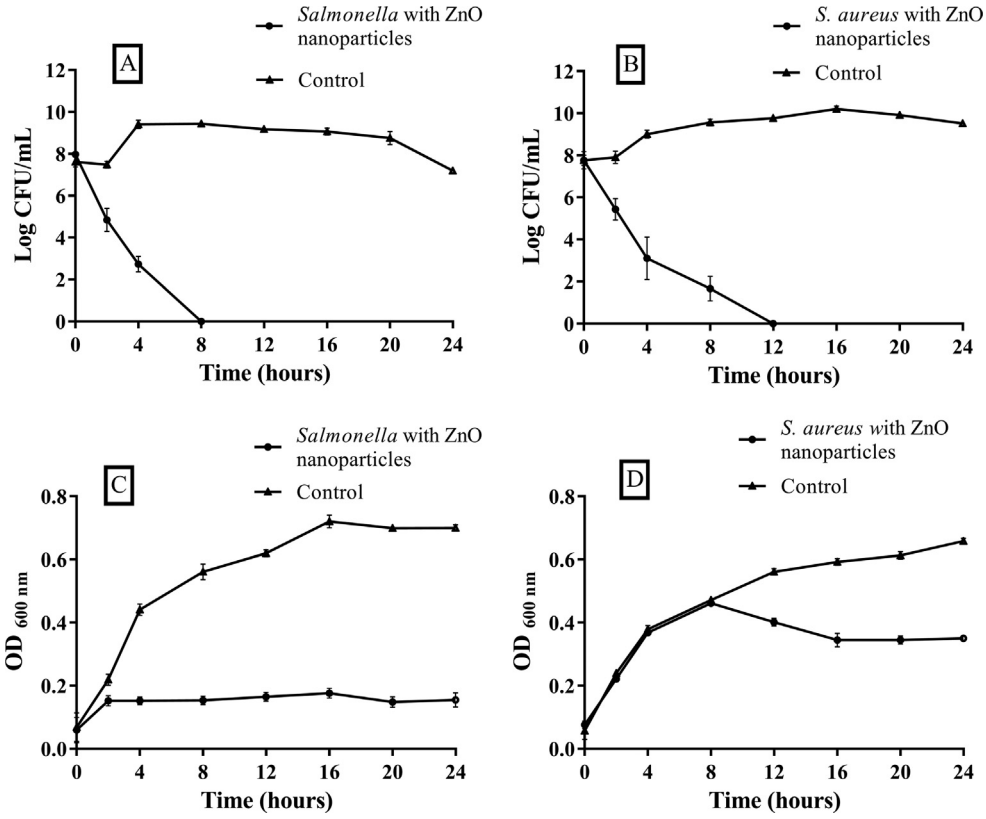

Fig. 4. Kill time analysis of *S. typhimurium* and *S. aureus* by plate count method (A and B) and by measuring the optical density (OD_{600}) (C and D). one-way analysis of variance (ANOVA) was performed to estimate the significant differences ($p < 0.05$) between means.

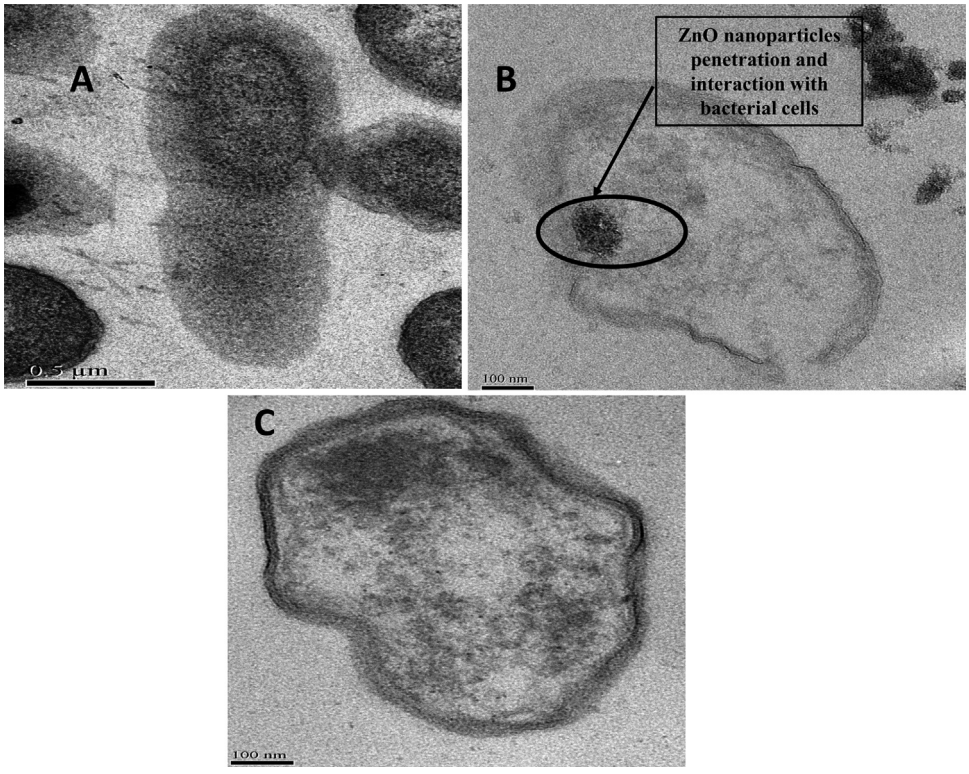

Fig. 5. TEM images (A = control, B and C = Bacteria treated with ZnO nanoparticles) for morphological changes in *Salmonella typhimurium* after treatment with ZnO nanoparticles.

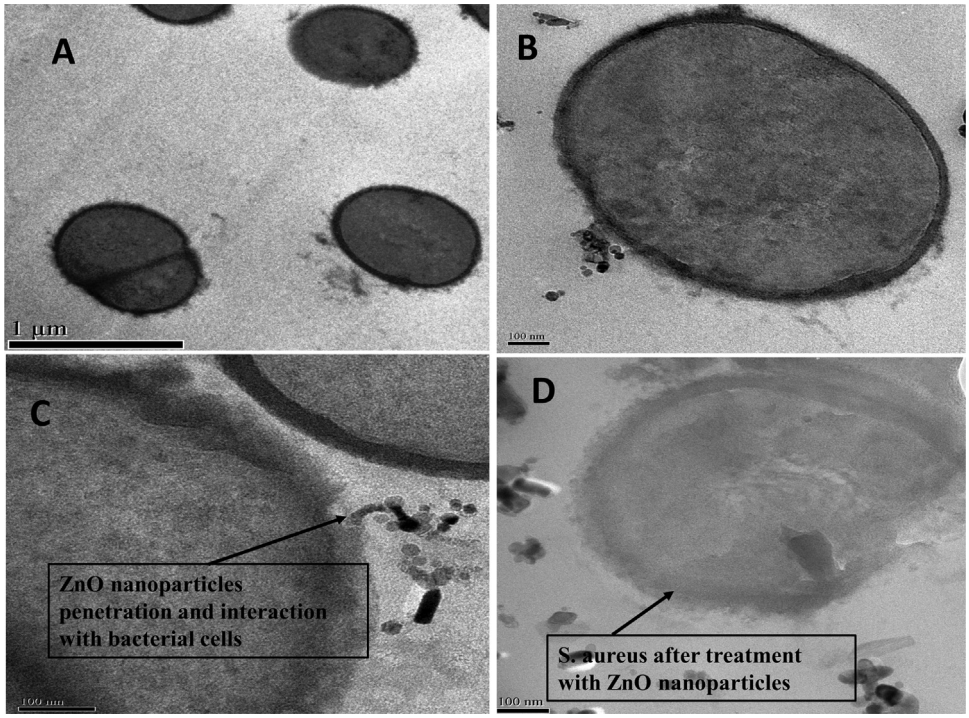

Fig. 6. TEM images (A = control; B, C and D = Bacteria treated with ZnO nanoparticles) for morphological changes in *S. aureus* after treatment with ZnO nanoparticles.

biocompatible and the main application of ZnO nanoparticles is the utilization of them as an effective drug delivery system (Mirzaei and Darroudi, 2017). Kim et al. (2016) reported ZnO nanoparticles as safe by evaluating acute dermal toxicity, dermal irritation and corrosion, and skin sensitization. ZnO nanoparticles have an additional application in food science as they are the source of Zn which is one of the most essential trace elements for humans (Wang et al., 2013). ZnO nanoparticles are reported by several studies as non-toxic to human cells (Colon et al., 2006). Khatami et al. (2018), reported that the bandages impregnated with ZnO nanoparticles showed antibacterial effect against Acinetobacter baumannii and Pseudomonas aeruginosa.

3.4. Bacterial morphological changes by TEM

The treated *S. typhimurium* became pitted and deformed as compared to control bacterial cells (Fig. 5). A similar pattern was observed for *S. aureus* after treatment with ZnO nanoparticles (Fig. 6). The electron micrographs clearly showed that the ZnO nanoparticles exhibit direct effect on the cell wall of bacteria resulting in the rupturing and death of the cells. The effects of the nanoparticles on cells surface affecting the cell wall is clear in the electron micrographs. The physiological and morphological changes in treated bacterial cells supported the results of kill time analysis, indicating the bacterial cell death by possible interactions of ZnO nanoparticles with cell walls and plasma membranes.

4. Conclusion

In this study ZnO nanoparticles were synthesized and evaluated for antimicrobial potential against *S. typhimurium* and *S. aureus*. The average size of synthesized nanoparticles was in the range of 20 nm. The nanoparticles showed potent antimicrobial effect against tested bacteria and significant ($p < 0.05$) decrease in viability in concentration dependent manner. The trend of using natural antimicrobials is becoming an attractive approach in the field of food preservation and safety because synthetic antimicrobials are associated with various health hazards. ZnO nanoparticles might be an alternate approach as antimicrobial agents or preservatives in food and pharmaceutical industries because of their safety, relatively low cost and effectiveness against various pathogens. However, further purification and stability of nanoparticles should be estimated in the final food and pharmaceutical formulations.

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Conflict of interest

Authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.005.

References

Akbar, A., Anal, A.K., 2014. Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in ready-to-eat poultry meat. Food Control 38, 88–95.
Akbar, A., Anal, A.K., 2013. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. Asian Pac. J. Trop. Biomed. 3 (2), 163–168.
Aydin, A., Sudagidan, M., Muratoglu, K., 2011. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *Staphylococcus aureus* strains isolated in the Marmara Region of Turkey. Int. J. Food Microbiol. 148 (2),

99–106.
Colon, G., Ward, B.C., Webster, T.J., 2006. Increased osteoblast and decreased *Staphylococcus epidermidis* functions on nanophase ZnO and TiO2. J. Biomed. Mat. Res. Part A 78 (3), 595–604.
Costa, L.F., Paixão, T.A., Tsolis, R.M., Bäumler, A.J., Santos, R.L., 2012. Salmonellosis in cattle: advantages of being an experimental model. Res. Vet. Sci. 93 (1), 1–6.
Djaja, N.F., Montja, D.A., Saleh, R., 2013. The effect of Co incorporation into ZnO nanoparticles. Adv. Mater. Phys. Chem. 3 (01), 33.
Duffy, L.L., Osmond-McLeod, M.J., Judy, J., King, T., 2018. Investigation into the antibacterial activity of silver, zinc oxide and copper oxide nanoparticles against poultry-relevant isolates of *Salmonella* and *Campylobacter*. Food Control 92, 293–300.
Espitia, P.J.P., Soares, N.D.F.F., dos Reis Coimbra, J.S., de Andrade, N.J., Cruz, R.S., Medeiros, E.A.A., 2012. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food Bioprocess Tech. 5 (5), 1447–1464.
FDA, 2011. Part 182—Substances Generally Recognized as Safe.Food and Drug Administration, Washington DC, USA.
Gunalan, S., Sivaraj, R., Rajendran, V., 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Prog. Nat. Sci. Mater. Int. 22 (6), 693–700.
Jaisai, M., Baruah, S., Dutta, J., 2012. Paper modified with ZnO nanorods–antimicrobial studies. Beilstein J. Nanotechnol. 3, 684–691.
Jevšnik, M., Ovca, A., Bauer, M., Fink, R., Oder, M., Sevšek, F., 2013. Food safety knowledge and practices among elderly in Slovenia. Food Control 31 (2), 284–290.
Jones, N., Ray, B., Ranjit, K.T., Manna, A.C., 2007. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol. Lett. 279 (1), 71–76.
Joray, M.B., del Rollán, M.R., Ruiz, G.M., Palacios, S.M., Carpinella, M.C., 2011. Antibacterial activity of extracts from plants of central Argentina–isolation of an active principle from *Achyrocline satureioides*. Planta Med. 77 (1), 95–100.
Khatami, M., Varma, R.S., Zafarnia, N., Yaghoobi, H., Sarani, M., Kumar, V.G., 2018. Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages. Sustain. Chem. Pharm. 10, 9–15.
Kim, S.H., Heo, Y., Choi, S.J., Kim, Y.J., Kim, M.S., Kim, H., Lee, K., 2016. Safety evaluation of zinc oxide nanoparticles in terms of acute dermal toxicity, dermal irritation and corrosion, and skin sensitization. Mol. Cell. Toxicol. 12 (1), 93–99.
Kuo, T.R., Wu, C.L., Hsu, C.T., Lo, W., Chiang, S.J., Lin, S.J., Chen, C.C., 2009. Chemical enhancer induced changes in the mechanisms of transdermal delivery of zinc oxide nanoparticles. Biomaterials 30 (16), 3002–3008.
Long, T.C., Saleh, N., Tilton, R.D., Lowry, G.V., Veronesi, B., 2006. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ. Sci. Technol. 40 (14), 4346–4352.
López-Gómez, A., Fernández, P.S., Palop, A., Periago, P.M., Martinez-López, A., Marin-Iniesta, F., Barbosa-Cánovas, G.V., 2009. Food safety engineering: an emergent perspective. Food Eng. Rev. 1 (1), 84–104.
Magrez, A., Kasas, S., Salicio, V., Pasquier, N., Seo, J.W., Celio, M., Forró, L., 2006. Cellular toxicity of carbon-based nanomaterials. Nano Lett. 6 (6), 1121–1125.
Mirzaei, H., Darroudi, M., 2017. Zinc oxide nanoparticles: biological synthesis and biomedical applications. Ceram. Int. 43 (1), 907–914.
National Committee for Clinical Laboratory Standards, 1992. Methods for determining bactericidal activity of antimicrobial agents. Tentative guidelines. In: Proceedings of the National Committee for Clinical Laboratory Standards document M26-T. Vol 12, No 19, NCCLS, Villanova, PA.
Padmavathy, N., Vijayaraghavan, R., 2008. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci. Technol. Adv. Mater. 9 (3), 035004 (7 pp).
Premanathan, M., Karthikeyan, K., Jeyasubramanian, K., Manivannan, G., 2011. Selective toxicity of ZnO nanoparticles toward gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomed. Nanotechnol. Biol. Med. 7 (2), 184–192.
Raja, A., Ashokkumar, S., Marthandam, R.P., Jayachandiran, J., Khatiwada, C.P., Kaviyarasu, K., Swaminathan, M., 2018. Eco-friendly preparation of zinc oxide nanoparticles using *Tabernaemontana divaricata* and its photocatalytic and antimicrobial activity. J. Photochem. Photobiol. B Biol. 181, 53–58.
Ren, G., Hu, D., Cheng, E.W., Vargas-Reus, M.A., Reip, P., Allaker, R.P., 2009. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int. J. Antimicrob. Agents 33 (6), 587–590.
Sadiq, M.B., Hanpithakpong, W., Tarning, J., Anal, A.K., 2015. Screening of phytochemicals and in vitro evaluation of antibacterial and antioxidant activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. Ind. Crops Prod. 77, 873–882.
Sadiq, M.B., Tarning, J., Aye Cho, T.Z., Anal, A.K., 2017. Antibacterial activities and possible modes of action of *Acacia nilotica* (L.) Del. against multidrug-resistant *Escherichia coli* and *Salmonella*. Molecules 22 (1), 47.
Sahoo, S.K., Parveen, S., Panda, J.J., 2007. The present and future of nanotechnology in human health care. Nanomed. Nanotechnol. Biol. Med. 3 (1), 20–31.
Sawai, J., Yoshikawa, T., 2004. Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. J. Appl. Microbiol. 96 (4), 803–809.
Sawai, J., 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J. Microbiol. Methods 54 (2), 177–182.
Seo, W.S., Lee, J.H., Sun, X., Suzuki, Y., Mann, D., Liu, Z., Dai, H., 2006. FeCo/graphitic-shell nanocrystals as advanced magnetic-resonance-imaging and near-infrared agents. Nat. Mater. 5 (12), 971–976.
Silvestre, C., Duraccio, D., Cimmino, S., 2011. Food packaging based on polymer nanomaterials. Prog. Polym. Sci. 36 (12), 1766–1782.
Singla, M.L., Kumar, M., 2009. Optical characterization of ZnO nanoparticles capped with various surfactants. J. Lumin. 129 (5), 434–438.
Sungkaworn, T., Triampo, W., Nalakarn, P., Triampo, D., Tang, I.M., Lenbury, Y., 2007. The effects of TiO2 nanoparticles on tumor cell colonies: fractal dimension and morphological properties. Int. J. Biomed. Sci. 2, 67–74.
Wang, H., Du, L.J., Song, Z.M., Chen, X.X., 2013. Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food. Nanomedicine 8 (12), 2007–2025.
Wang, Y., Gao, S., Ye, W.H., Yoon, H.S., Yang, Y.Y., 2006. Co-delivery of drugs and DNA from cationic core–shell nanoparticles self-assembled from a biodegradable copolymer. Nat. Mater. 5 (10), 791–796.