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# Research Article
# Green Synthesis of Zinc Oxide Nanoparticles and Their Antibiotic-potentiation Activities of Mucin Against Pathogenic Bacteria

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## Abstract

**Background and Objective:** Antimicrobial resistance is one of the biggest threats to world health, food security and development. Generally, infections-such as pneumonia, tuberculosis, gonorrhoea and salmonellosis-are becoming harder to treat as the drugs used to treat them become less efficacious. Present research was designed to investigate the antibacterial activities of green synthesized zinc oxide nanoparticles and their antibiotic-potentiating effects in the presence of mucin against some Gram-negative pathogenic bacteria. **Materials and Methods:** The broth micro-dilution method was used for determination of minimum inhibitory concentrations (MICs) of zinc oxide nanoparticles alone or in association with mucin using ciprofloxacin as a positive control. **Results:** Minimum inhibitory concentration indicated that zinc oxide nanoparticles and their combinations with mucin were able to inhibit the growth of all the studied bacteria within a concentration range of 0.140-6.420 and 0.006-1.024 μg $mL^{-1}$, respectively while the MIC range of 0.100-1.080 μg $mL^{-1}$ was recorded for ciprofloxacin positive standard antibiotic agent used. Significant synergistic effect was noted between zinc oxide nanoparticles and mucin combination on all tested bacteria except *S. enteritidis*. Present research has shown that zinc oxide nanoparticle is a potential antibacterial agent and the antibiotic effects were obviously potentiated in the presence of mucin. **Conclusion:** A novel topical formulation containing zinc oxide nanoparticle and mucin will be beneficial in wound management especially in accident and emergency units.

Key words: Zinc oxide nanoparticles, antimicrobial, gram-negative, antibiotic-potentiating, pathogenic bacteria, ciprofloxacin, mucin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Gram-negative bacterial resistance to convectional anti-microbial agents proceeds via multiple mechanisms including reduced membrane permeability[1] and activation of efflux mechanisms by biological efflux pumps. Efflux pumps are known to reversibly excrete entered antimicrobial agents to the extracellular compartments[2]. Other mechanism of resistance may be intrinsic and extrinsic in nature. The intrinsic resistance mechanism can include outer membrane permeability, down regulation of genes[3] and chromosomal resistance[4].

Clinically isolated Gram-negative bacteria are known to possess a structured envelope in a manner that prevents the penetration of metal and metal oxide nanoparticles[5]. The outer cell envelope is composed of a bi-layer formed by lipopolysaccharide (LPS) proteins and phospholipids. The overall effect of expression of the above mentioned pumps in pathogenic bacteria gave rise to observed emergence of pathogenic strains that are clinically resistant to many antimicrobial agents including metal and metal oxide nanoparticles[6].

It has been reported that both metal and metal oxide nanoparticles can inhibit efflux pumps of bacteria and can be classified under efflux pump inhibitors (EPIs)[7]. Natural products with EPI effect has also been reported including plant extracts such as *Berberis vulgaris*, also mucin from African Giant Snail contain compounds that can enhance antibacterial actions of some antimicrobial agents and also have many beneficial effects such as anti-bacterial and anti-microbial efficacy[8]. Present research aimed to investigate the antibacterial activities of zinc oxide nanoparticles and their synergistic effects with mucin from African giant snail against some Gram-negative pathogenic bacteria.

## MATERIALS AND METHODS

**Chemicals:** De-ionized Milli Q water, analytical grade zinc nitrate and sodium hydroxide, respectively were obtained from Merck, Germany and Oxoid, Hampshire, UK. Ciprofloxacin (Cipro) were product of Sigma-Aldrich.

**Study area:** The synthesis of nanoparticles and antibacterial studies were carried out at the Department of Pharmaceutical and Medicinal Chemistry and Department of Pharmaceutical Microbiology and Biotechnology, University of Port Harcourt respectively, while the characterization of zinc oxide nanoparticles were carefully performed at the prestigious nanotechnology laboratory of the Chemistry Department, Rhodes University, South Africa in September, 2018.

**Plant materials:** A previously identified, fresh leaves of *Solanium torvum*[9] were collected from the Medicinal plant garden of the Pharmacognosy Department, Faculty of Pharmacy, University of Port Harcourt, Nigeria. (UP/PHCOG00056). The dried leaves were manually ground into powder and stored in an air tight container for further use.

**Leave extract preparation:** Ground leaves were boiled in water for 45 min at 100° C, near ultra-filtration was ensured using 0.45 µm sintered glass funnel. The resultant filtrate was stored at 4 °C until use, this acted as the reducing and capping agent in the synthesis of the zinc oxide nanoparticles.

**Green synthesis of zinc oxide nanoparticles:** The zinc oxide nanoparticles used in this research were synthesized and duly characterized using the method of Ezealisiji *et al.*[10]. Briefly, 200 mL of aqueous zinc nitrate solution (1.5 mM) was mixed with 20 mL of the aqueous leaf extract of *Solanum torvum* L. and subsequently treated with 1.0 M sodium hydroxide (10 mL). The ions which initiated the reaction were afforded by the zinc nitrate in de-ionized water. The reaction mixture was incubated with constant stirring in the dark at 60 °C to avoid photo-catalysis. An observed off-white colour marked the formation of ZnO NPs at the end of 24 h. The resultant product was further purified by centrifugation and washed in double-distilled water and ethanol, respectively, dried and kept in an amber-coloured sample bottle until use.

**Extraction of snail mucin:** Mucin were extracted from the African giant Snail (*Archachatina marginata*) using the method described by Adikwu *et al.*[11]. The Snail shell was cracked and their fleshly bodies removed. Other unwanted materials were also removed. The slime was carefully washed and squeezed off the flesh to give a pool of 1.0 L. Mucin was precipitated out of the pooled washings using 2.0 L of acetone. Precipitate was filtered and dried for use.

**Bacterial cultures and medium:** The micro-organisms under study included typed strains of *Pseudomonas aeruginosa* ATCC 8037, *Escherichia coli* ATCC 325022, *Salmonella enteritidis* ATCC 2845, *Klebsiella pneumonia* ATCC 30605, *Proteus mirabilis* ATCC 160821, respectively were kept in Luria Bertani broth at 4 °C and sub-cultured on appropriate agar plates for 24 h prior to antimicrobial assay.

**Anti-microbial activity of synthesized zinc oxide nanoparticles and mucin:** All anti-microbial experiments herein were carried out under high aseptic condition. The method of disk-diffusion assay was employed to investigate the anti-microbial efficacy of zinc oxide nanoparticles and mucin respectively as well as their combination product at different concentration level[12]. The micro-organisms under study were seeded on the nutrient agar plates and then, 4 mm diameter paper disk were saturated at a known concentrations of 5.0 μg $mL^{-1}$, for zinc oxide nanoparticles and mucin respectively. This process was repeated for zinc oxide nanoparticles and mucin combination, ciprofloxacin (0.2 μg $mL^{-1}$) and distilled water, respectively. The latter were used as positive and negative control, respectively. The paper disk was placed on the solidified agar plates and was allowed to incubate at 37°C for 24 h. The inhibition zone diameter was accessed.

**Determination of minimum inhibitory concentration (MIC):** Determination of minimum inhibitory concentration values was carried out employing a twofold standard broth micro-dilution on examined test drugs against 5 standard bacteria strains, the minimum inhibitory concentration for the combination products were also determined following the official guidelines of the Clinical and Laboratory Standard Institute[13]. In summary, the bacteria cultures were incubated aerobically at 37°C for 18-24 h. The turbidity of the cultures adjusted to 0.5 McFarland ($1.5\times10^8$ CFU $mL^{-1}$) and then diluted in saline solution so as to obtain an inoculums size of $5.0\times10^5$ CFU/well. The first well of each 96 well micro plate row inoculated with 4 MIC of test drugs followed by double dilution in successive wells to detect any possible antagonistic or synergistic combinations. Two last wells serve as positive and negative controls. With through shaking, the inoculated micro-plates were aerobically incubated for 18 h at 37°C. The lowest concentration that inhibited visible growth after incubation was defined as MIC[14-18]. Activity of zinc oxide nanoparticles and mucin were compared with ciprofloxacin for potentiating.

## RESULTS

**Characterization of zinc oxide nanoparticles:** The average DLS particle size of ZnO NPs was observed to be 24 nm (Fig. 1). A TEM image shows ZnO NPs average size of 28.0±02.1 nm (Fig. 2a).The UV-VIS Spectroscopic analysis of synthesized zinc oxide NPs, showed a single peak corresponding to zinc oxide nanoparticles at 359 nm (Fig. 2b).

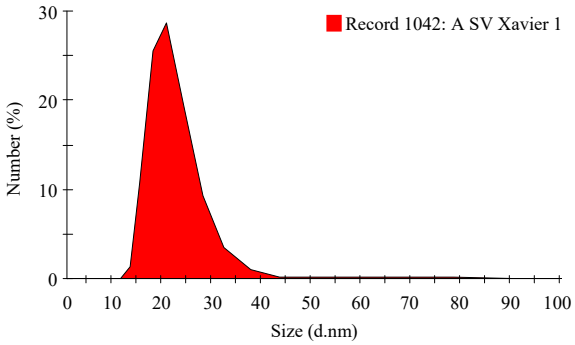


Fig. 1: DLS particle size distribution of synthesized ZnO NPs

Table 1: MICs (μg $mL^{-1}$) for ZnO NPs, mucin, ZnO NPs+mucin and ciprofloxacin against some Gram-positive bacteria

| Bacteria | ZnO NPs | Mucin | ZnO NPs+Mucin | Ciprofloxacin |
|---|---|---|---|---|
| *P. aeruginosa* | 0.260 | 0.160 | 0.048* | 0.290 |
| *E. coli* | 2.010 | 1.850 | 0.409* | 0.262 |
| *S. enteritidis* | 6.420 | 8.020 | 1.024 | 1.080 |
| *K. pneumonia* | 0.180 | 0.140 | 0.006* | 0.140 |
| *P. mirabilis* | 0.140 | 0.120 | 0.018* | 0.100 |

ZnO-NPs: Zinc oxide nanoparticles, Potentiation, appeared as steric numbers

**Result of antimicrobial efficacy:** The results of antimicrobial efficacy are presented below, with the histogram showing the inhibition zone diameter for the various test agents under study (Fig. 3). Also presented are the minimum inhibitory concentrations of the test agents and standard against some pathogenic organisms (Table 1).

## DISCUSSION

Synthesized zinc oxide nanoparticles were examined to detect synergy with mucin. Ciprofloxacin positive control was also assayed on the examined strains as shown in Table 1. It was observed that Gram-negative pathogenic test organisms were highly sensitive to the antimicrobial activities of ZnO NPs, mucin and their combinations. The ZnO NPs (5 μg $mL^{-1}$) gave a remarkable mean inhibition zone diameter (IZD) of 18±1.02, 16±0.08, 08±0.82, 20±1.04 and 22±1.08 mm for *P. aeruginosa*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *P. mirabilis*, respectively. Mucin (5 μg $mL^{-1}$) also showed an outstanding antibacterial affect with mean zone of inhibition of 20±0.06, 18±0.08, 10±1.04, 22±0.9 and 23±0.14 mm for *P. aeruginosa*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *P. mirabilis*, respectively. Combination of ZnO NPs and mucin (5 μg $mL^{-1}$) gave highest inhibition zone diameter (IZD) of 30±0.09, 24±0.18, 12±0.02, 32±0.84 and 34±0.18 mm for *P. aeruginosa*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *P. mirabilis*, respectively and this is in agreement with previous

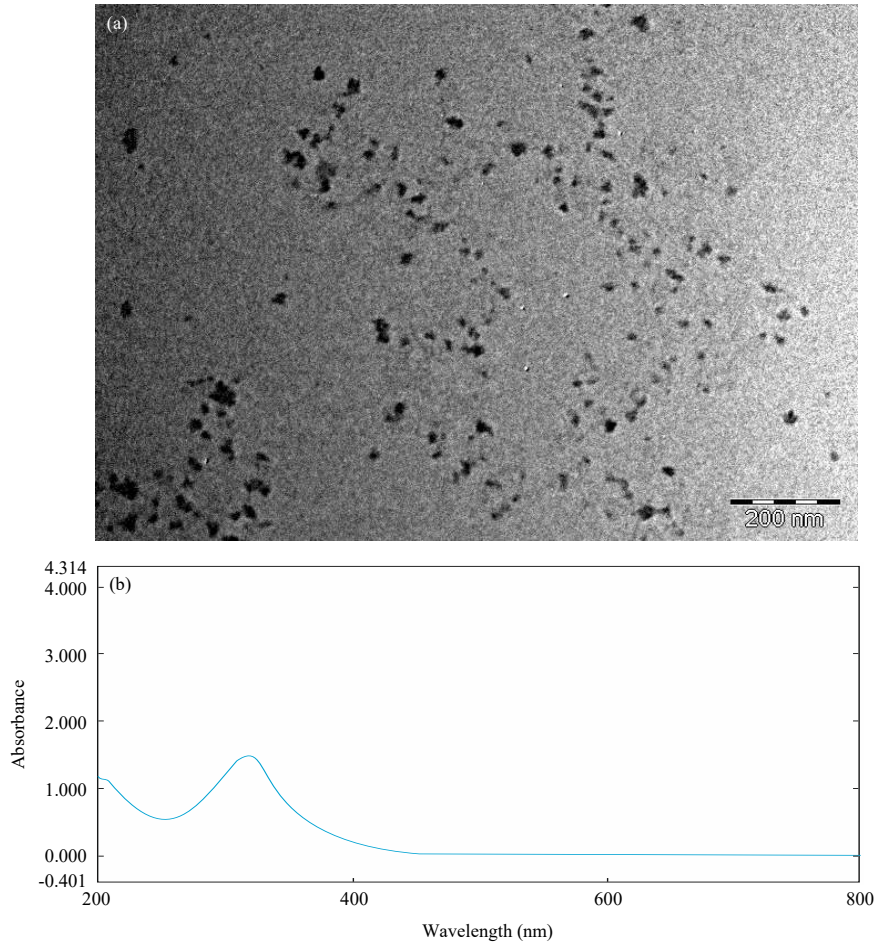


Fig. 2(a-b): (a) TEM image of biosynthesized ZnO NPs and (b) UV-vis spectroscopic analysis of synthesized zinc oxide NPs, a single peak corresponding to zinc oxide nano particles (359 nm)

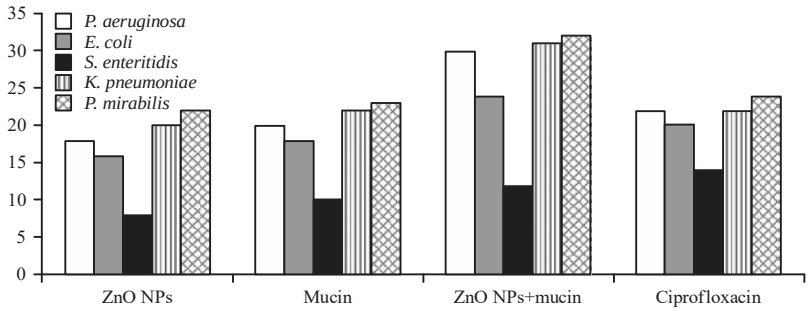


Fig. 3: Inhibition zone diameter for ZnO NPs, mucin, ZnO NPs+mucin and ciprofloxacin

work of Makarovsky *et al.*[8] which showed that mucin-silver nanoparticle composite possess strong antibacterial activity against several pathogenic bacteria including both Gram-negative and Gram-positive specie. Already established work of Farzana *et al.*[19] revealed that at the maximum concentration of 1 mg $mL^{-1}$, inhibitory zone formed by the combined effect of Imipenem and zinc oxide nanoparticles was so remarkable (29 mm). A quite related study by Iram *et al.*[20] tends to support our result where ZnO NPs composite showed greater antimicrobial activity. The standard drug ciprofloxacin yielded a mean inhibition zone diameter of 22±0.02, 20±0.08, 14±1.02, 22±1.03 and 24±1.06 mm

for *P. aeruginosa*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *P. mirabilis*, respectively. Bacterial growth inhibition was minimal for ZnO NPs against *S. enteritidis* while highest inhibition was recorded for ZnO NPs against *P. mirabilis*. Mucin at 5.0 μg $mL^{-1}$ concentration showed maximum bacterial inhibition against *P. mirabilis* while minimum inhibition was observed for *S. enteritidis*. The ZnO NPs and Mucin combination gave a remarkable bacterial inhibition against *K. pneumoniae* while least zone of inhibition was recorded for *S. enteritidis*. The standard drug, ciprofloxacin followed same trend when compared with mucin.

A very low but well acceptable minimum inhibitory concentration range of between 0.140-0.180 μg $mL^{-1}$ was recorded for zinc oxide nanoparticles against *P. mirabilis* and *K. pneumoniae*, respectively. *Salmonella enteritidis* showed a higher minimum inhibitory concentration value for ZnO NPs (6.420 μg $mL^{-1}$). Mucin gave an outstanding MIC value of 0.120 and 0.140 μg $mL^{-1}$ against pathogenic micro-organisms, *P. mirabilis* and *K. pneumoniae*, respectively compared to the much more reduced value of 0.006 and 0.008 μg $mL^{-1}$ for the same set of organisms against the combination of mucin and ZnO NPs. This implies that the combination of mucin and ZnO NPs, gave a better clinically acceptable MIC when compared with ZnO NPs and Mucin, respectively. The above result when projected was also found to be better than those of the standard drug ciprofloxacin. Mechanism of nanoparticle bacterial cell penetration is believed to be due to the modification and distortion of the cell wall architectural integrity followed by pincytocis[21,22], while the mucin are known to interfere with the bacteria cell DNA synthesis and hence protein synthesis. These combined effects gave an outstanding synergistic activity as observed for the combination hence, the observed excellent results.

## CONCLUSION

The synergism observed in present study highlighted the advantage of metal oxide nanoparticle combination with natural product such as Mucin in combating drug resistant pathogenic bacteria.

## SIGNIFICANT STATEMENT

This study discovers the use of natural product in combination with Biosynthesized nanoparticles in Biomedical application, this approach can be used as a promising solution for fighting bacterial infection in hospitals especially, with regards to intensive wound management in the accident and emergency unit.

## REFERENCES


1. Van Nguyen, J.C. and L. Gutmann, 1994. [Resistance to antibiotics caused by decrease of the permeability in gram-negative bacteria]. Presse Medicale, 23: 522-527, (In French).
2. Szumowski, J.D., K.N. Adams, P.H. Edelstein and L. Ramakrishnan, 2012. Antimicrobial efflux pumps and *Mycobacterium tuberculosis* drug tolerance: Evolutionary considerations. Curr. Top. Microbial. Immunol., 374: 81-108.
3. Hall, C.W. and T.F. Mah, 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol. Rev., 41: 276-301.
4. Beceiro, A., M. Tomas and G. Bou, 2013. Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial world? Clin. Microbiol. Rev., 26: 185-230.
5. Slavin, Y.N., J. Asnis, U.O. Hafeli and H. Bach, 2017. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. J. Nanobiotechnol., Vol. 15. 10.1186/s12951-017-0308-z.
6. Kadiyala, U., N.A. Kotov and J.S. VanEpps, 2018. Antibacterial metal oxide nanoparticles: Challenges in interpreting the literature. Curr. Pharmaceut. Des., 24: 896-903.
7. Kvist, M., V. Hancock and P. Klemm, 2008. Inactivation of efflux pumps abolishes bacterial biofilm formation. Applied Environ. Microbiol., 74: 7376-7382.
8. Makarovsky, D., L. Fadeev, B.B. Salam, E. Zelinger and O. Matan *et al*., 2018. Silver nanoparticles complexed with bovine submaxillary mucin possess strong antibacterial activity and protect against seedling infection. Applied Environ. Microbiol., Vol. 84, No. 4. 10.1128/AEM.02212-17.
9. Ndebia, E.J., R. Kamgang and B.N. Nkeh-ChungagAnye, 2007. Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum* (*Solanaceae*). Afr. J. Tradit. Complement. Altern. Med., 4: 240-244.
10. Ezealisiji, K.M., X. Siwe-Noundou, B. Maduelosi, N. Nwachukwu and R.W.M. Krause, 2019. Green synthesis of zinc oxide nanoparticles using *Solanum torvum* (L.) leaf extract and evaluation of the toxicological profile of the ZnO nanoparticles-hydrogel composite in Wistar albino rats. Int. Nano Lett., 9: 99-107.
11. Adikwu, M.U., C.O. Okolie and A.A. Agboke, 2009. The effect of snail mucin on the ulcer healing rate of clarithromycin. J. Pharmaceut. Res., 8: 6-8.
12. Koeth, L.M., J.M. DiFranco-Fisher, N.E. Scangarella-Oman and L.A. Miller, 2017. Analysis of MIC and disk diffusion testing variables for gepotidacin and comparator agents against select bacterial pathogens. J. Clin. Microbiol., 55: 1767-1777.
13. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal., 6: 71-79.

14. Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother., 48: 5-16.
15. Kavanagh, A., S. Ramu, Y. Gong, M.A. Cooper and M.A. Blaskovich, 2019. Effects of microplate type and broth additives on microdilution MIC susceptibility assays. Antimicrob. Agents Chemother., Vol. 63, No. 1. 10.1128/AAC.01760-18.
16. Karuppusamy, S. and K.M. Rajasekaran, 2009. High throughput antibacterial screening of plant extracts by Resazurin redox with special reference to medicinal plants of Western Ghats. Global J. Pharmacol., 3: 63-68.
17. Kerridge, A., H. Lappin-Scott and J.R. Stevens, 2005. Antibacterial properties of larval secretions of the blowfly, *Lucilia sericata*. Med. Vet. Entomol., 19: 333-337.
18. Ebrahimi, A., Z. Arvaneh, M. Mahzounieh and S. Lotfalian, 2017. Antibiotic resistance induction by benzalkonium chloride exposure in nosocomial pathogens. Int. J. Infect., Vol. 4, No. 2. 10.5812/iji.40296.
19. Farzana, R., P. Iqra, F. Shafaq, S. Sunaira, K Zakia, T. Hunaiza and M. Hushna, 2017. Antimicrobial behavior of zinc oxide nanoparticles and β-lactam antibiotics against pathogenic bacteria. Arch. Clin. Microbiol., Vol. 8, No. 4. 10.4172/1989-8436.100057.
20. Iram, S., J.A. Khan, N. Aman, A. Nadhman, Z. Zulfiqar and M.A. Yameen, 2016. Enhancing the anti-enterococci activity of different antibiotics by combining with metal oxide nanoparticles. Jundishapur J. Microbiol., Vol. 9, No. 3. 10.5812/jjm.31302.
21. Lopez-Romero, J.C., H. Gonzalez-Rios, A. Borges and M. Simoes, 2015. Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. Evidence-Based Complement. Altern. Med., Vol. 2015. 10.1155/2015/795435.
22. Wang, L.L., C. Hu and L.Q. Shao, 2017. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Int. J. Nanomed., 12: 1227-1249.