DOI : 10.35124/bca.2019.19.2.3489

*Biochem. Cell. Arch.* Vol. 19, No. 2, pp. 3489-3494, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

# ANTIBACTERIAL ACTIVITY OF ZnO AND $CO_3O_4$ NANOPARTICLES SYNTHESIZED BY CO-PRECIPITATION METHOD

**Ali Mohammed Al-Tamimi[1], Esam H. Hummadi[2,*] and Mustafa Hammadi[1]**

[1]Department of Chemistry, College of Education for Pure Science, University of Diyala, Iraq.
[2]Department of Biotechnology, College of Science, University of Diyala, Iraq.
*e-mail esam_hummadi@sciences.uodiyala.edu.iq

**(Received 25 April 2019, Revised 21 July 2019, Accepted 8 August 2019)**

**ABSTRACT : Nanoparticles (NPs) are widely and successfully used in medicine as an alternative to antibiotics. Metal oxide NPs such as ZnONPs and $Co_3O_4$NPswere found to be effective against antibiotic resistant bacteria. *Staphylococcus* and *Escherichia* and other food borne pathogens are important causes of enteric illness and hospital-acquired infections. In this work, ZnONPs and $CO_3O_4$NPs were synthesized by co-precipitation technique. These NPs were characterized by several techniques including Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscope (SEM). XRD pattern showed the crystalline nature of ZnONPs and $CO_3O_4$NPs. SEM demonstrated the morphology of ZnONPs and $Co_3O_4$NPs. The average diameter of ZnONPs and $Co_3O_4$NPs calculated by XRD was around 27.41nm and 13.61nm, respectively. Energy dispersive XRD pattern suggested that prepared ZnO and $Co_3O_4$NPs were highly pure. The antibacterial activity of these NPs were investigated individually and in conjugation with amoxicillin against *Staphylococcusaureus* and *Escherichia coli*. ZnONPs and $Co_3O_4$ NPs alone showed high growth inhibition against tested bacteria compared with amoxicillin. The results showed that the combination of amoxicillin with ZnONPs and $Co_3O_4$NPs provides enhanced antibacterial activity. This synergistic effect could be used against resistant microorganisms in the future. The synergistic mechanisms of antibacterial activity of ZnONPs and $Co_3O_4$NPs needs to be studied further.**

***Key words :*** Nanoparticles, ZnONPs, $Co_3O_4$NPs, Co-precipitation method, antibacterial activity.

## INTRODUCTION

Nanoparticles (NPs) are particles whose size ranges from 1-100 µm, which possess nanoscopic, sized dependent properties. High proportion of surface area to volume of NPs confer the particles significant effect on their properties in biological applications (Gupta and Xie, 2018). For instance, NPs activity against microorganisms is known to be a characteristics of the surface area in contact with the microbes (Sirelkhatim *et al*, 2015). Tiny size and largeratio of surface to volume of the NPs enhances their interaction with the microorganism to carry out a lot of potential antimicrobial activities (Wang *et al*, 2017). Recently, NPs research is an attracting field in scientific studies, because of its wide variety of practicaluses in the area of medicine, agriculture, industry and food packaging (Khan *et al*, 2017; Gupta and Xie, 2018). In medicine, NPs are used as drug delivery system, detection of pathogens, probing of DNA structure and antimicrobial agents against resistant pathogens (Khan *et al*, 2017; Gold *et al*, 2018). For example, copper and zinc NPs have gained more attention as safe and effective antimicrobial compounds (Ghasemi and Jalal, 2016; Gold *et al*, 2018). NPs can be categorized into distinct groupsby size, morphology, physico-chemical characteristics. These are including Carbon-based NPs, Metal NPs (e.g. Cu, Ag and Au, Co, Zn), Semiconductor NPs, Ceramics, NPs, Lipid-based NPs and Polymeric NPs (Khan *et al*, 2017). Among these groups, metallic NPs (e.g. ZnO, $Co_2O_4$, $Fe_2O_3$ and $Ag_2O$) extensively investigated as potential antimicrobials (Masri *et al*, 2018).

Synthesize of NPs can be categorized into physical methods (e.g. laser ablation, mechanical milling), chemical methods (e.g. hydrothermal, precipitation, vapor deposition and sol-gel processing) and biological methods (e.g. bacteria and plants) (Dhand *et al*, 2015). Co-precipitation technique is the most widely used to produce nanoparticles because it is simple, rapid, eco-friendly,can be used for inorganic or organic substancesand may avoid some disadvantages of the other extraction techniques (Rajaeiyan and Bagheri-Mohagheghi, 2013; Bader *et al*, 2014).

Many researchers have proved the antimicrobial efficacy of NPs on bacteria, fungi and other organisms (Otari *et al*, 2016; Ró¿añska *et al*, 2017; Dobrucka and Dlugaszewska, 2018). Zinc oxide has proved to be a

powerful antimicrobial agent for therapeutic applications. ZnONPs have bactericidal effects on *S. aureus* and *E. coli* (Banoee *et al*, 2010). They can also being effective on microbial spores which are resistant to high temperature and high pressure (Ghasemi and Jalal, 2016).

NPs showed high antimicrobial activity when combine with standard antibiotics (Banoee *et al*, 2010). For example, conjugation of ZnONPs with ceftazidime and ciprofloxacin enhanced antibacterial activity toward multidrug-resistant Acinetobacter baumannii (Ghasemi and Jalal, 2016). Silver nanoparticle combination increased antibacterial efficacy of cephradine,vildagliptin and ceftriaxone against different clinical bacteria (*e.g. E. coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, methicillin-resistant *S. aureus* (MRSA) and *Bacillus cereus*) (Shah *et al*, 2014; Gold *et al*, 2018; Masri *et al*, 2018).

In the current study, ZnONPs and $Co_3O_4$NPs were synthesized using co-precipitation technique and examine their antibacterial effect on *E. coli* and *S. aureus*. Structural property of ZnONPs and $Co_3O_4$NPs was assessed by XRD and SEM fitted with energy dispersive X-ray diffraction spectroscopy. Antibacterial activity of ZnONPs and $Co_3O_4$NPs was examined by a broth microdilution method against human pathogenic bacterial strains individually and in combination with amoxicillin.

## MATERIALS AND METHODS

### Materials

The chemicals used for preparing the solutions were of analytical grade. Cobalt(II) Chloride Hexahydrate ($CoCl_2.6H_2O$), Zinc acetate ($Zn(CH_3COO)_2.2H_2O$, Sodium hydroxide (NaOH), 96% ethanol were purchased from Alfa Aesar (Germany). Polystyrene 96-well plates were purchased from Greiner Bio-One (Germany). Amoxicillin was purchased from Ajanta Pharma (India). All the culture media used in the study were products of HiMedia Laboratories Pvt. Ltd, Mumbai (India). Cultures of two clinical strains of *E. coli* and *S. aureus* were obtained from Laboratory of Microbiology Research at Al-Kindi Teaching Hospital, Al-Rusafa (Baghdad, Iraq). Cultures were maintained in Nutrient agar in refrigerator at 4°C.

### Synthesis and characterization

### Preparation of cobalt oxides nanoparticles

The cobalt oxide ($Co_3O_4$) nanoparticles were synthesized using co-precipitation method. A 0.9 g of Cobalt(II) Chloride Hexahydrate ($CoCl_2.6H_2O$)was suspendedin 50 ml deionized water with magnetic stirring until completely melt the substance. After complete dissolution of $Co_3O_4$, 150 ml of the alkaline solution (0.8 g NaOH in deionized water) was gradually added under constant stirringat 80°C for 2h. The pH was adjusted to 9 in the reaction mixture. Then it was permitted to hold on for 12h at 25°C. The resulting slurry was repeatedly washed with deionized water and EtOH(to eliminate the intermediate by-products). Finally, the precipitate was desiccated at 80°C for 30 min and burned at 400°C for 2 h and driedin drying chamberat 25°C for 24 h.

### Preparation of zinc oxides nanoparticles

A 9.2 g of Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) was suspended in 100 ml deionized water on a magnetic stirrer until completely melt the substance. After complete dissolution of Zinc acetate, drops of alkaline solution (1 M) was dropped gradually under constant stirring at 70°C for 2 h. The pH of the mixture was adjusted at 9 throughout the reaction. Then it was permitted to hold on at room temperature for 12 h. The resulting slurry was repeatedly washed with deionized water and EtOH (to eliminate the intermediate by-products). Finally, the precipitate was dried at 80°C for 30 min and burned at 500°C for 5 h and desiccated indrying chamberat 25°C for 24 h.

### Characterization techniques

The metal oxides nanoparticles were characterized by several techniques including X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy and Scanning electron microscope (SEM). Crystallinity, structure and crystallite size of nanoparticles were determined by XRD using a Shimadzu (Kyoto, Japan) Miniflex X-ray diffractometer with Cu-Kα radiations ($\gamma$ = 0.15406 nm) in the 2θ range from 20° to 80°C. FTIR spectra of the samples were obtained using Shimadzu (Tokyo, Japan) FTIR spectrophotometer using KBr pellet. SEM examination was conducted using a 200 kV ZeissSEM (Germany).

### Antibacterial activity

### Broth microdilution method

For preparation of stock NPs solutions, 1 mg ZnONPs or $Co_3O_4$NPs was suspended in 1 ml sterilized deionized water with shaking. The suspensions were sonicated in water bath sonicator for 30 min of repeating the cycle after every 5 min. The suspension was kept at 4°C and shake vigorously on a vortex mixer prior to bioassays. The antibacterial activity of metal oxides nanoparticles and antibiotic were determined by the broth microdilution technique. Serial dilutions of NPs or amoxicillin (100-500 µg/ml) were made in sterile 96-well flat-bottomed microtiter plate. The optical density of *E. coli* and *S. aureus* was adjusted using Mueller-Hinton Broth corresponding to 0.5 McFarland standard ($10^8$ CFU/ml).

For antibacterial testing, Aliquots of 100 µl of the bacterial inoculums were added to each well containing 100 µl/ well of NPs or amoxicillin at the tested concentration. Three wells containing bacterial inoculum without tested agents used as growth control and three wells containing only medium as a blank control were involved in the plate. The microtitreplates were then incubated at 37°C for 24 h. The inhibition of bacterial growth was determined by measuring the absorbance at 630 nm using a Biotek Synergy HT Multimode Microplate Reader. At each concentration, growth inhibition (GI%) was calculated as:

$$GI\% = (OD_{630 \text{ of antibacterial agent}} - OD_{630 \text{ of positive control}})/ OD_{630 \text{ of positive contro}} \times 100.$$

**Determination of MIC**

The MIC was determined in accordance with CLSI (Clinical and Laboratory Standards Institute) guidelines. Aliquots of 100 µl of each NPs with different concentrations were added in 96-well plate and inoculated with 100 µl of the microbial inoculum. Plates were incubated without shaking for 24 h at 37°C. The MIC readings were performed spectrophotometrically with a microplate reader at 630 nm. The MIC was defined as the lowest concentration of the tested NPs resulting in growth inhibition of microbial growth of up to 50% (Giannousi *et al*, 2017).

**Combining activity of NPs with amoxicillin**

The combined activity between NPs and amoxicillin was determined. Bacterial suspensions were loaded to each well of 96-well microtiter plate and a NPs in combination with the required concentration of amoxicillin was added. The ratio of amoxicillin: NPs were selected in the range of 1:3, 1:1 and 3:1 (v/v) to give final concentrations of (75:25, 50:50 and 25:75 µg/ml). Plates were incubated for 24 h at 37°C and the optical density was measured at 630 nm and the GI% was recorded.

**Statistical analysis**

Statistical analyses were conducted using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). One-way ANOVA and Tukey's multiple comparisons test were used to compare between the tested groups. Results are presented as mean ± standard deviation (SD) and $p< 0.05$ was considered significant.

## RESULTS AND DISCUSSION

**Morphological analysis**

**XRD study**

ZnONPs and $Co_3O_4$NPs were prepared by co-precipitation technique. The patterns of typical XRD examination of the NPs spectra are presented in Fig. 1. The XRD spectra of cobalt oxide were agreed with the standard spectrum of cobalt oxide according to the database (JCPDS number 42-1467). The composition of $Co_3O_4$crystalline cobalt oxide was determined by diffraction peaks (19.00, 31.27, 36.85, 38.54, 44.81, 56.66, 59.36, 65.24 and 74.12). No impurity diffraction peaks were detected in the spectrum of XRD. The average particle size of the crystalline $Co_3O_4$was increased by 13.61 nm using Debye-Scherrer equation: $D=K\lambda/\beta\cos\theta$ (Otari *et al*, 2016). The XRD spectra of zinc oxide were agreed with the standard spectrum of zinc oxide according to the database (JCPDS number 36-1451). The composition of ZnO crystalline zinc oxide was determined by diffraction peaks (31.77, 34.43, 36.21, 47.71, 56.71, 63.00, 66.49, 68.09 and 69.16). No impurity diffraction peaks were detected in the spectrum of XRD. The average size of the crystalline $Co_3O_4$was increased by 27.41 nm. The data of XRD spectrum of $Co_3O_4$ and ZnO are clearly indicating that the products is high purity.

**FTIR study**

The FTIR spectra of the ZnO and $Co_3O_4$ samples calcined at 500 and 400°C, respectively. NPs were embedded in KBr matrix and the FTIR spectra were recorded by scanning the samples over a range of wavelengths (400-4000 $cm^{-1}$), correspond to vibration manners of various chemical groups (*e.g.* hydroxyl, carboxylate and alkane) in the particles. FTIR spectrum of the ZnO showed a broadband at 451 $cm^{-1}$ is the distinctive absorption of Zn–O bond (Xiong *et al*, 2007). FTIR spectrum of $Co_3O_4$ sample exhibited significant absorption peaks at 671 $cm^{-1}$ is equivalent to O-Co-O bond and 578 $cm^{-1}$ is assigned to Co-O (Manigandan *et al*, 213) (Fig. 2).

**SEM analysis**

Fig. 3 shows the SEM image of ZnONPs and $Co_3O_4$NPs, respectively. The average SEM size of ZnONPs and $Co_3O_4$ NPs were around 62.22 nm and 34.45 nm, respectively, supporting the XRD results. The morphology of the NPs were investigated using SEM imaging shown in Fig. 3. The images show that the ZnONPs and $Co_3O_4$NPs are approximately uniform and sphere-shaped with an average diameter around 62.22 nm and 34.45 nm, respectively, supporting the XRD results. NPs synthesized were higher agglomeration, which can be due to weak forces exist between nanoparticles, for example electrostatic interactions, van der Waals for cesor surface tension (Gaikwad *et al*, 2014).

**Antibacterial activity**

Antibacterial effect of ZnONPs and $Co_3O_4$NPs was examined toward human pathogenic bacteria *E. coli* and

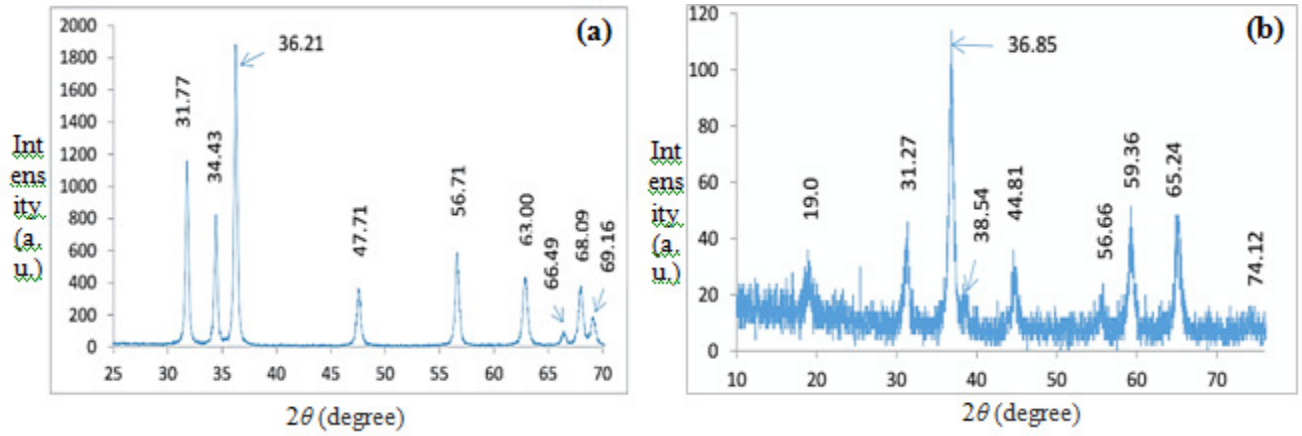


**Fig. 1 :** X-ray diffraction pattern: (a) ZnONPs, (b) $Co_3O_4$NPs.

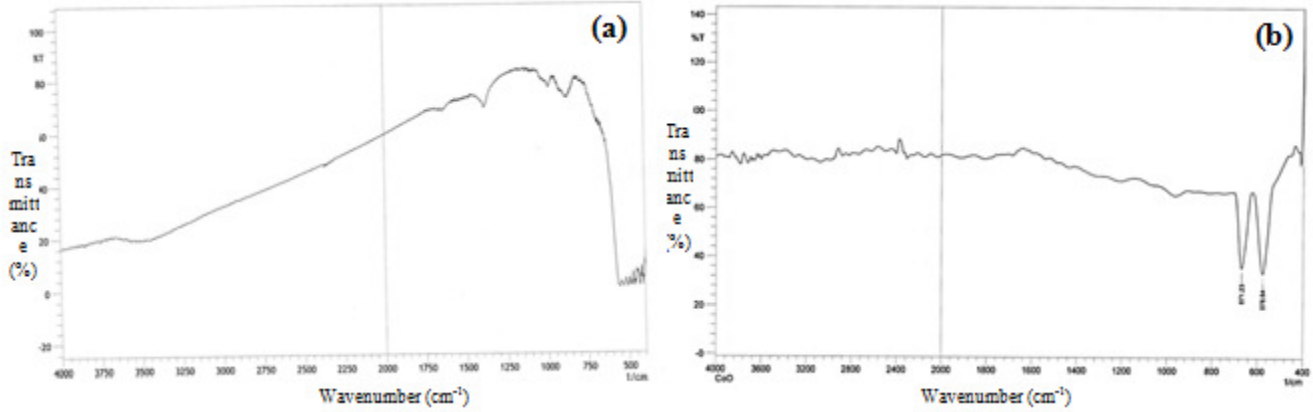


**Fig. 2 :** Fourier-transform infrared spectra: (a) ZnONPs, (b) $Co_3O_4$NPs.

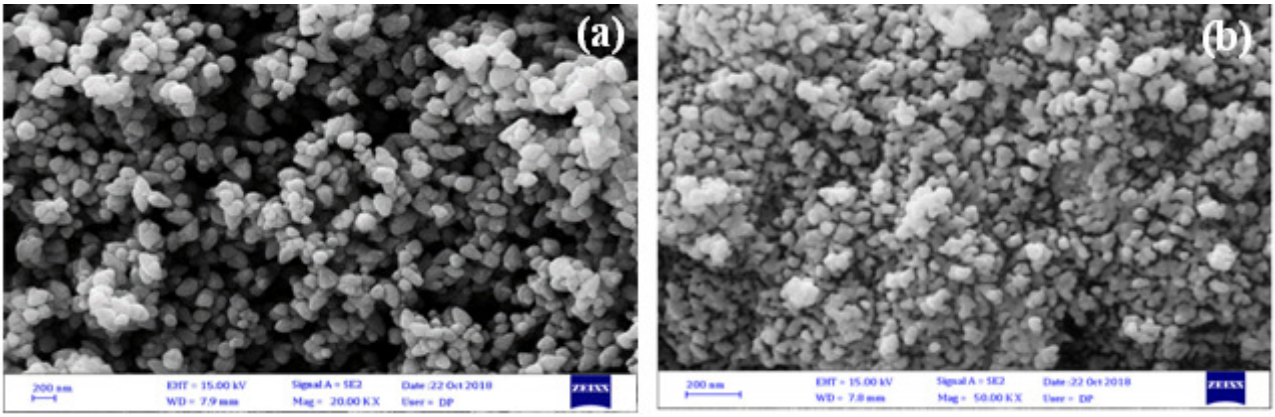


**Fig. 3 :** SEM images: (a) ZnONPs, (b) $Co_3O_4$NPs.

*S. aureus*. These bacteria were applied as models for examination of antibacterial activity because of its major roles in various humans and animals infections. The GI% reflects magnitude of susceptibility of bacteria in dose-dependent manner. Results showed that NPs has exhibited strong antibacterial activity against the bacteria used in this study. The antibacterial assay revealed that the NPs were significantly ($p< 0.05$) higher than amoxicillin (Fig. 4). In addition, NPs were significantly ($p< 0.05$) more effective toward *S. aureus* than *E. coli* at concentrations ranged between 100-300 µg/ml and 100-400 µg/ml, respectively. Table 1 shows MIC index of the tested NPs. The MIC values of $Co_3O_4$NPs (*E. coli* 200 µg/ml and *S. aureus* 100 µg/ml) were lower than those of ZnONPs for the two bacteria. It is not precisely known how NPs act on microorganisms. However The antibacterial effectiveness of NPs can be due to (1) the electrostatic interaction between NPs and bacterial surfaces which interrupt of the cell wall; (2) interaction with the cell surfaces change the permeability of membranes by which NPs can enter the cell; (3) inducing synthesis of Reactive Oxygen Species (ROS)

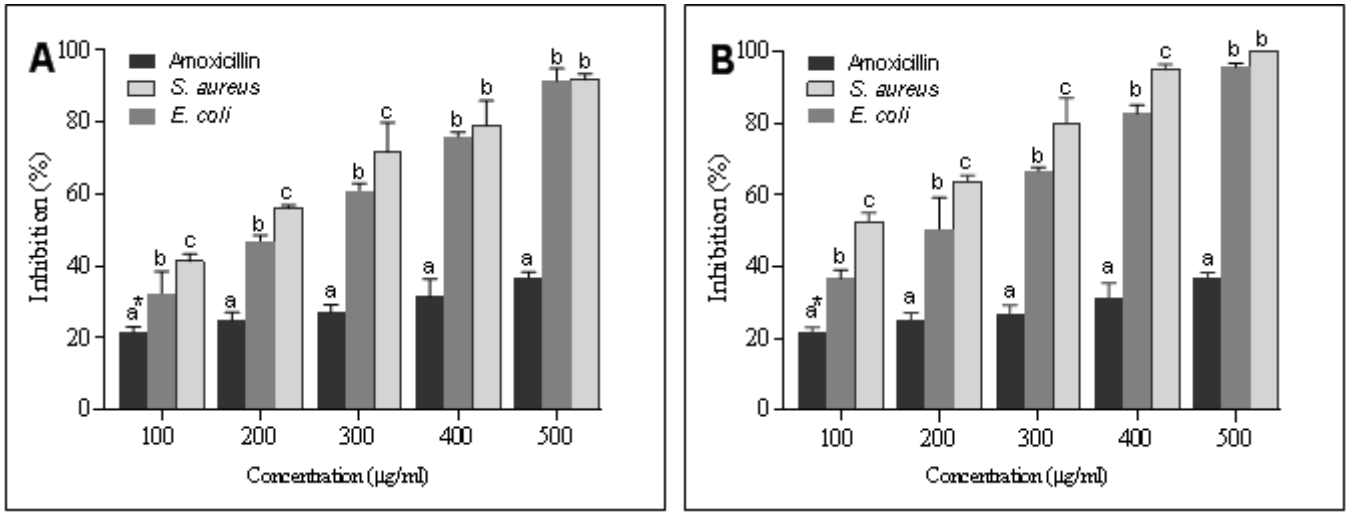


**Fig. 4 :** The percent inhibition of bacterial growth of (A) ZnONPs and (B) $Co_3O_4$NPs against *E. coli* and *S. aureus* when compared to amoxicillin. Data presented as mean inhibition±SD. *Mean with a letter in common are not significantly different according to Tukey's test ($p< 0.05$).

**Table 1 :** The minimum inhibitory concentration (MIC) of metal oxide nanoparticles against *E. coli* and *S. aureus*.

| Bacteria | MIC (µg/ml) | |
|---|---|---|
| | ZnONPs | $Co_3O_4$NPs |
| *E. coli* | 300 | 200 |
| *S. aureus* | 200 | 100 |

**Table 2 :** Combined activity of ZnONPs and $Co_3O_4$NPs with amoxicillin against *E. coli* and *S. aureus*.

| Antibacterial agent | Final concentration (µg/ml) | Growth inhibition (%)mean±SD | |
|---|---|---|---|
| | | *E. coli* | *S. aureus* |
| ZnONPs | 100 | 32±2.91 | 41±1.79 |
| $Co_3O_4$NPs | 100 | 37±2.13 | 52±2.42 |
| Amoxicillin | 100 | 34±1.75 | 22±1.24 |
| Amoxicillin:NPs | 75:25 | 100±0.00 | 100±0.00 |
| | 50:50 | 100±0.00 | 100±0.00 |
| | 25:75 | 100±0.00 | 100±0.00 |

and cellular oxidative stress of the constituents (Xie *et al*, 2011; AbdalDayem *et al*, 2017; Dogra *et al*, 2019).

In another experiment, we determined the interaction between nanoparticles and amoxicillin on inhibitory activity. Means of growth inhibition percentage of amoxicillin in the lack and presence of NPs are shown in Table 2. Results of combined activity clearly show that the synergistic effect is observed for both bacterial strains with resistance to amoxicillin (Table 2). The result emphasize the probable of the NPs combination to combat antibiotic-resistant isolates of *E. coli* and *S. aureus*. It has been reported earlier that ZnONPs have potential antibacterial activity toward *E. coli* (Liu *et al*, 2009) as well as pathogenic microbes (Zvekiæ *et al*, 2011; Jin *et al*, 2019; Nazoori and Kariminik, 2018). It was suggested that the interactive impact between NPs and antibiotics was triggered by antimicrobial export inhibition by blocking efflux pumps or by enhancing the entry of these agents into the bacterial cell by damaging the membrane (Banoee *et al*, 2010). Accordingly, the NPs synthesized in the present study could lead to a more significant antimicrobial efficiency against a wide range of pathogenic microbes at a lower level of concentration. In that instance, these NPs could be non-toxic at the potential concentration at which they might be used for therapeutics. However, it is advised to consider the *in vivo* toxicity of NPs as they can lead to serious health problems in human beings or animals (Flores *et al*, 2013). These results suggest that NPs can be promising antibacterial agents for antibiotic resistant bacterial strains.

## CONCLUSION

In this work, we have described the synthesized zinc oxide and cobalt oxide via cheap and practical co-precipitation method. The characterization of the structural properties of ZnONPs and $Co_3O_4$NPs were carried out using FTIR, XRD and SEM. The FTIR showed a broad absorption bands related to Zn-O and Co-O vibration band. The XRD and SEM examination showed that the average size of ZnO and $Co_3O_4$ particles sized 13.61-62.22nm. In addition, the SEM results showed the production of sphere-shaped NPs. Antibacterial activity assessment was conducted using broth microdilution technique showed that the synthesized nanoparticles showed excellent activity against *E. coli*, *S. aureus* versus amoxicillin. NPs individually and in mixture with amoxicillin evidenced to be active in the control of *E. coli* and *S. aureus*.

## REFERENCES


AbdalDayem A, Hossain M, Lee S, Kim K, Saha S, Yang G M and Cho S G (2017) The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. *Int. J. Mol. Sci.* **18**(1), 120.

Bader N A B I L, Benkhayal A A and Zimmermann B (2014) Co-precipitation as a sample preparation technique for trace element analysis: an overview. *Int J Chem Sci*. **12**, 519-25.

Banoee M, Seif S, Nazari Z E, Jafari Fesharaki P, Shahverdi H R, Moballegh A and Shahverdi A R (2010) ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **93**(2), 557-561.

CLSI (2012) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-9th ed.. In: CLSI document M07-A9. Wayne: Clinical and Laboratory Standards Institute.

Dhand C, Dwivedi N, Loh X J, Ying A N J, Verma N K, Beuerman R W and Ramakrishna S (2015) Methods and strategies for the synthesis of diverse nanoparticles and their applications: a comprehensive overview. *Rsc Advances* **5**(127), 105003-105037.

Dobrucka R and Dlugaszewska J (2018) Antimicrobial activity of the biogenically synthesized core-shell Cu@ Pt nanoparticles. *Saudi Pharmaceutical Journal* **26**(5), 643-650.

Dogra V, Kaur G, Jindal S, Kumar R, Kumar S and Singhal N K (2019) Bactericidal effects of metallosurfactants based cobalt oxide/ hydroxide nanoparticles against *Staphylococcus aureus*. *Science of The Total Environment* **681**, 350-364.

Flores C Y, MinÞaìn A G, Grillo C A, Salvarezza R C, Vericat C and Schilardi P L (2013) Citrate-capped silver nanoparticles showing good bactericidal effect against both planktonic and sessile bacteria and a low cytotoxicity to osteoblastic cells. *ACS Applied Materials and Interfaces* **5**(8), 3149-3159.

Gaikwad S S, Gandhi A C, Pandit S D, Pant J, Chan T S, Cheng C L and Wu S Y (2014) Oxygen induced strained ZnO nanoparticles: An investigation of Raman scattering and visible photoluminescence. *Journal of Materials Chemistry C* **2**(35), 7264-7274.

Ghasemi F and Jalal R (2016) Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant *Acinetobacter baumannii*. *Journal of Global Antimicrobial Resistance* **6**, 118-122.

Giannousi K, Pantazaki A and Dendrinou-Samara C (2017) Copper-Based Nanoparticles as Antimicrobials. In : *Nanostructures for Antimicrobial Therapy* (pp. 515-529). Elsevier.

Gold K, Slay B, Knackstedt M and Gaharwar A K (2018) Antimicrobial Activity of Metal and Metal Oxide Based Nanoparticles. *Advanced Therapeutics* **1**(3), 1700033.

Gupta R and Xie H (2018) Nanoparticles in daily life: applications, toxicity and regulations. *J. Environ. Pathol., Toxicol. and Oncol.* **37**(3).

Jin S E, Jin J E, Hwang W and Hong S W (2019) Photocatalytic antibacterial application of zinc oxide nanoparticles and self-assembled networks under dual UV irradiation for enhanced disinfection. *International Journal of Nanomedicine* **14**, 1737.

Khan I, Saeed K and Khan I (2017) Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*.

Liu Y, He L, Mustapha A, Li H, Hu Z Q and Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157: H7. *J. Appl. Microbiol.* **107**(4), 1193-1201.

Manigandan R, Giribabu K, Suresh R, Vijayalakshmi L, Stephen A and Narayanan V (2013) Cobalt oxide nanoparticles: characterization and its electrocatalytic activity towards nitrobenzene. *Chemical Science Transactions* **2**(Supplement 1), S47-S50.

Masri A, Anwar A, Ahmed D, Siddiqui R, Raza Shah M and Khan N (2018) Silver Nanoparticle Conjugation-Enhanced Antibacterial Efficacy of Clinically Approved Drugs Cephradine and Vildagliptin. *Antibiotics* **7**(4), 100.

Nazoori E S and Kariminik A (2018) *In Vitro* Evaluation of Antibacterial Properties of Zinc Oxide Nanoparticles on Pathogenic Prokaryotes. *J Appl. Biotechnol Rep*. **5**(4), 162-165.

Otari S V, Yadav H M, Thorat N D, Patil R M, Lee J K and Pawar S H (2016) Facile one pot synthesis of core shell Ag@ SiO2 nanoparticles for catalytic and antimicrobial activity. *Materials Letters* **167**, 179-182.

Rajaeiyan A and Bagheri-Mohagheghi M M (2013) Comparison of sol-gel and co-precipitation methods on the structural properties and phase transformation of $\gamma$ and $\alpha$-$Al_2O_3$ nanoparticles. *Advances in Manufacturing* **1**(2), 176-182.

Rakshit S, Ghosh S, Chall S, Mati S S, Moulik S P and Bhattacharya S C (2013) Controlled synthesis of spin glass nickel oxide nanoparticles and evaluation of their potential antimicrobial activity: A cost effective and eco friendly approach. *RSC Advances* **3**(42), 19348-19356.

Ró¿ańska A, Chmielarczyk A, Romaniszyn D, Sroka-Oleksiak A, Bulanda M, Walkowicz M and Knych T (2017) Antimicrobial properties of selected copper alloys on *Staphylococcus aureus* and *Escherichia coli* in different simulations of environmental conditions: with vs. without organic contamination. *Int. J. Environ. Res. Public Health* **14**(7), 813.

Shah M R, Ali S, Ateeq M, Perveen S, Ahmed S, Bertino M F and Ali M (2014) Morphological analysis of the antimicrobial action of silver and gold nanoparticles stabilized with ceftriaxone on *Escherichia coli* using atomic force microscopy. *New Journal of Chemistry* **38**(11), 5633-5640.

Sirelkhatim A, Mahmud S, Seeni A, Kaus N H M, Ann L C, Bakhori S K M and Mohamad D (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Letters* **7**(3), 219-242.

Wang L, Hu C and Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int. J. Nanomed.* **12**, 1227.

Wu S H and Chen D H (2003) Synthesis and characterization of nickel nanoparticles by hydrazine reduction in ethylene glycol. *Journal of Colloid and Interface Science* **259**(2), 282-286.

Xie Y, He Y, Irwin P L, Jin T and Shi X (2011) Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **77**(7), 2325-2331.

Xiong G, Pal U and Serrano J G (2007) Correlations among size, defects, and photoluminescence in ZnO nanoparticles. *J. Appl. Phy. 101*(2), 024317.

Zvekiæ D, Srdiæ V V, Karaman M A and Matavulj M N (2011) Antimicrobial properties of ZnO nanoparticles incorporated in polyurethane varnish. *Processing and Application of Ceramics* **5**(1), 41-45.