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Antibacterial Activity of Silver-Conjugated Magnetic Iron Oxide Nanoparticles

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Abstract

Silver-conjugated magnetic iron oxide nanoparticles (Ag-MNPs) were successfully synthesized and characterized and its effect on certain bacteria was evaluated. The synthesized nanoparticles were tested against six isolates which include *Serratia marcescens, Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Salmonella enterica* and *Acinetobacter baumannii*. The result showed that antibacterial activity of the synthesized Ag-MNPs was found to be effective against *Serratia marcescens* and *Staphylococcus aureus*, while the compound was ineffective against some clinical bacterial isolates. The diameters of the zones of inhibition were found to be 20 mm and 27 mm for *Serratia marcescens* and *Staphylococcus aureus*, respectively. The Minimum Inhibitory Concentration (MIC) was 10 mg/ml for both organisms. Also, the Minimum Bactericidal Concentration MBC for the two organisms was found to be 20 mg/ml. Therefore, the synthesized compound has antibacterial activity and could be a reliable compound of choice for treating bacterial infections.

Keywords: Bacterial isolates, nanoparticles, drug discovery, antibacterial activity.

INTRODUCTION

The difficulty of multidrug resistance of pathogenic bacteria to antimicrobial drugs has hampered the successful detection and therapy of infectious diseases (Dakal *et al*., 2016). The resistant microorganisms (fungi, viruses, bacteria and parasites) can have the capability to resist the antimicrobial drug thereby causing repeated occurrence and spreading of infection (Tanwar *et al*., 2014). Most of these organisms have developed a resistance against the existing antibiotics, hence the need to develop alternative means of treating bacterial infections in order to solve multidrug resistance problem. This problem has made researchers to focus on new agents which are capable of inhibiting microbial growth. Recent development in the field of nanotechnology is of great benefit which may lead to the development of nanoparticles as a new antibacterial agent.

The use of nanoparticles in various fields is due to their unique physical, chemical, and effective biological properties (Azam *et al.,* 2012). These unique properties have led to their applications in different areas of biomedical sciences such as sensing application, gene delivery, biomolecules detection and clinical diagnostics (Pandey *et al.,* 2008). Some metal and metal oxide nanoparticles like silver, ZnO, TiO_2, have been used as antibacterial agents (Behera *et al*., 2013; Arakha *et al.,* 2015). Silver nanoparticles has found its applications over the years in various field which include catalysts, biotechnology and bioengineering, medicine, metal electrodes and water treatment owing to their unique optical, electronic and catalytic properties (Singh & Bahadur 2015; Wang *et al.,* 2012; Lee *et al.,* 2012; Kumar-Krishnan, S Chakaravarthy *et al.,* 2016). Also, they have equally attracted great attention due to their excellent antimicrobial properties (Apalangya *et al.,* 2014; Huang *et al.,* 2010).

It has been found that the exposure of silver nanoparticles to wastewater has led to reduction in the in the amount of nitrifying bacteria present in the sludge (Nagy *et al.,* 2011). The use of magnetic nanoparticles in various field has been a research of interest especially in the biomedical field which include molecular detection (Haun *et al.,* 2011), bio-separation

(Lee *et al*., 2018; Fatima & Kim 2017), drug delivery (El-Boubbou *et al*., 2017; Wahajuddin & Arora 2012; Xiong *et al*., 2018), hyperthermia (Guardia *et al*., 2012; Hilger 2013) and MRI (Rümenapp *et al.*, 2012; McGrath *et al.*, 2017). In present study, Ag-MNPs were successfully synthesized and characterized by EDXRF and XRD. In this study, Ag-MNP was tested against six selected bacterial species and their antimicrobial activity was evaluated.

MATERIALS AND METHODS

Chemicals and Reagents

Silver nitrate ($AgNO_3$ > 99.7%), Ferric chloride hexahydrate ($FeCl_3.H_2O$; technical grade) ferrous chloride tetrahydrate ($FeCl_2.4H_2O$ technical grade), trisodium citrate dehydrate ($Na_3C_6H_5O_7$, >99% technical grade) were purchased from Sigma Aldrich, Ammonia solution (NH_4OH; 28%) were purchased from Sigma Aldrich. All the reagents were used as received. High purity deionized water was used throughout the experiments.

Preparation of Magnetic Nanoparticles (MNPs)

Magnetic nanoparticles (MNPs) was prepared through conventional in situ co-precipitation method using aqueous solution of ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions at 2:1 mole ratio of Fe^{3+} : Fe^{2+}. Typically, $FeCl_3.6H_2O$ (64.8 mmol) and $FeCl_2.4H_2O$ (32.4 mmol) were dissolved in 300 mL of absolute ethanol and water (v:v; 1:2) the mixture was stirred at 80^oC for 30 miniutes. Then 15 mL ammonia ($NH_3.H_2O$) was added to the mixture drop wise and the reaction was stirred for another 30 minutes. 2 mM of trisodium citrate (0.05g in 20ml) was directly added into the solution and allowed to stir for another 30minutes. The black crude magnetic nanoparticles were recovered magnetically, and the slurry was washed three times with deionized water and ethanol. The magnetic nanoparticles were then dried in the oven for 24 hours. The magnetic nanoparticles are represented as MNP.

Preparation of Silver Coated Magnetic Nanoparticles (Ag-MNPs)

About 1% (w/v) of magnetic nanoparticles was sonicated for one hour, to form a homogenous solution The solution was further stirrer for few minutes and 0.25g of silver nitrate was directly added to the solution and it was allowed to stir for 30 minutes at room temperature (Solution A). To a separate beaker, a solution of trisodium citrate was prepared (1.8 g into 50 ml of water). The already prepared trisodium citrate solution was added to solution A. The solution was allowed to stir for 60 minutes. The nanoparticles were recovered magnetically, and washed three times with deionized water and ethanol. The nanoparticles were then dried in the oven for 24 hours. The nanoparticles are represented as Ag-MNP.

Characterization of Silver Coated Magnetic Nanoparticles (Ag-MNPs)

The synthesized Ag-MNPs were characterized using X-ray diffraction (XRD) and Energy-dispersive X-ray fluorescence (EDXRF).

Preparation of Media

Nutrient agar medium was prepared by weighing approximate amount of nutrient agar powder (28 g) in 1 liter of distilled water. The mixture was homogenized by boiling to fully dissolve all components. It was then autoclaved to dissolve mixture at 121^oC for 15 minutes. Once the nutrient agar has been autoclaved it was allowed to cool to about 45^oC and poured into Petri-dish. The plates were allowed to set/solidify before inoculation.

Test Organisms

Six different clinical isolates were collected from the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko. The morphological and biochemical characteristics of the isolates were confirmed using Bergey's manual of determinative bacteriology 9th edition. The isolates include *Serratia marcescens, Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Salmonella enterica* and *Acinetobacter baumannii*. All bacterial cultures were maintained on nutrient broth and stored at 4^oC throughout the study period.

Determination of Antibacterial Activity

The bacterial cultures were maintained on nutrient broth. Inoculum size containing 10^8 cfu/ml of each bacterium was used to seed already solidified Petri plates of Mueller-hinton agar. The antibacterial activity of Ag-MNPs was determined using agar well diffusion method. A sterile 7 mm cork borer was used to make 3 wells on already solidified agar and one of the well was filled with Ag-MNPs**,** the second well with positive control (ampiclox) and the third well was filled with distilled water (negative control). The plates were allowed to stand for about 2 hours to allow absorption of the samples into the media after which they were incubated at 37^oC for 24 hours. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using a method described by Adeoyo *et al.,* 2019 with MIC concentrations of 80 mg/ml, 60 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml and 1 mg/ml. Each suspension was adjusted to 0.5 McFarland standards. After which the standardized inoculums were seeded on the plates. The bacterial plates were incubated at 37^oC for 24 hours. The results were observed and recorded. MIC was determined as the lowest concentration of compound permitting no visible growth. For MBC determination, the test MIC dilutions were cultured on fresh agar medium and incubated at 37^oC for 24 hours. The lowest concentration of MIC tubes with no visible growth was taken as MBC.

RESULTS

Characterization silver coated magnetic nanoparticles

XRD patterns of silver coated magnetic nanoparticles

In Fig 1, the X-ray diffraction (XRD) pattern analysis of the synthesized silver coated magnetic nanoparticles indicated the presence of silver (Ag) with three 2θ peaks at 32.1°, 44.1°, and 77.6, Fe_3O_4 (Magnetite) with three peaks at 35.8°, 57.8°, and 63.4°. The presence of the peaks assigned to Ag-MNP was an indication that the compound is composed of magnetite and silver. The XRD spectrum revealed that the synthesized silver nanoparticles were crystalline and in nanocrystal form. The peaks can be assigned to the planes (122), (200), and (311) facet of silver crystal respectively (Jemal *et al.*, 2017). The XRD diffractograms clearly revealed the formation of the Fe_3O_4. The diffraction peaks show the characteristic of magnetite which were indexed for the following Miller Indices (311), (511) and (440) (Hui *et al.*, 2008; Yang *et al.*, 2005). The XRD spectrum revealed that the produced silver nanoparticles were crystalline and in nanocrystal form.

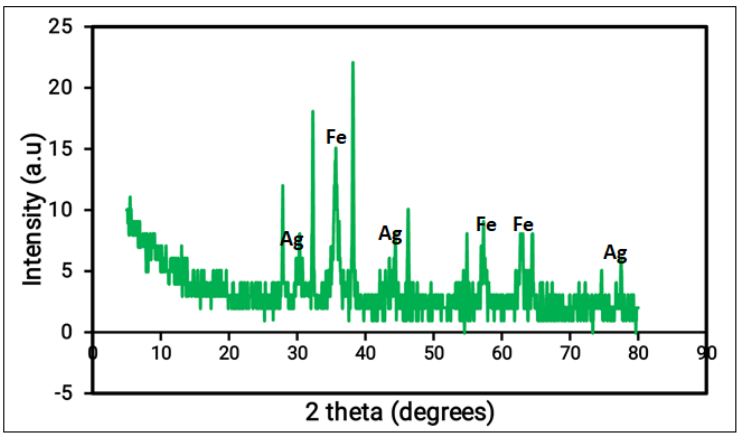

Figure 1: XRD diffractogram of Ag-MNPs

Energy-dispersive X-ray fluorescence (EDXRF) of silver coated magnetic nanoparticles

Energy-dispersive X-ray fluorescence spectrum is presented in Figure 2. Energy-dispersive X-ray fluorescence a non-destructive elemental analysis was used to characterize the synthesized Ag-MNPs to confirm the presence of the expected elements. The EDXRF analysis revealed the presence of iron peak and that of silver which confirms the successful synthesis of Ag-MNPs and the successful coating of magnetic nanoparticles with silver.

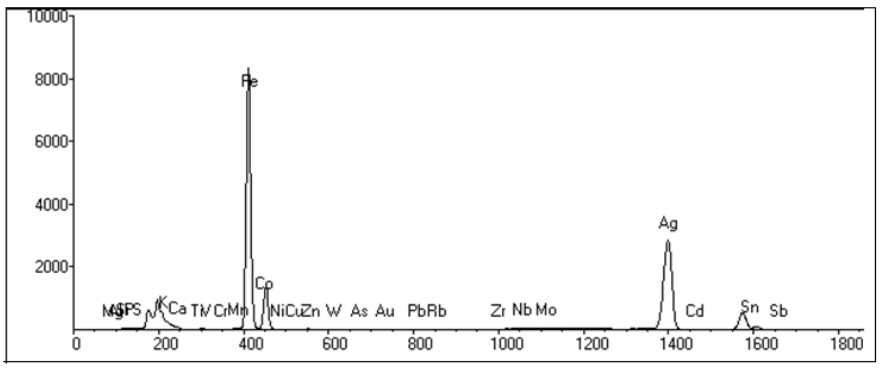

Figure 2: Energy-dispersive X-ray fluorescence (EDXRF) of silver coated magnetic nanoparticles

Antibacterial activity of silver coated magnetic nanoparticles

Antibacterial activity of silver coated magnetic nanoparticles (Ag-MNPs) against six different organisms (Table 1). Ag-MNPs exhibited an effective antibacterial activity against *S. marcescens* and *S. aureus*, while the compound was ineffective against other bacteria. The diameters of the zones of inhibition were found to be 20 mm and 27 mm for *S. marcescens* and *S. aureus*, respectively (Table 1). The MIC and the MBC were also investigated and the results were presented in Tables 2 and 3. The MIC for both organisms were found to be 10 mg/ml, this indicated that 10 mg/ml of Ag-MNPs inhibited the growth of *S. marcescens* and *S. aureus* while MBC result revealed that at a concentration of 20 mg/ml, none of these two organisms were able to grow, hence were both inhibited by Ag-MNPs (Table 3).

Table 1: Antibacterial Susceptibility test of silver coated magnetic nanoparticles against some bacterial isolates

Organism	Diameter of inhibition zone (mm)
Serratia marcescens	20±1.0
Staphylococcus aureus	28±2.5
Escherichia coli	9±0.5
Enterobacter cloacae	7±0
Salmonella enterica	10±0.5
Acinetobacter baumannii	8±0.5

Key: 1-14 = Resistant; 15-19 = Intermediate; 20 and above = Susceptible. Values presented with standard errors of the means (±SEM).

Table 2: Minimum inhibitory concentration of Ag-MNPs against selected bacterial isolates

Test organisms	80mg	60mg	40mg	20mg	10mg	1 mg
Serratia marcescens	+	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	+	-

Key: + = growth; - = no growth

Table 3: Minimum bactericidal concentration of Ag-MNPs against selected bacterial isolates

Test organisms	80mg	60mg	40mg	20mg	10mg
Serratia marcescens	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-

Key: + = growth; - = no growth

DISCUSSION

Silver coated magnetite nanoparticle was successfully synthesized and the elemental composition of the synthesized nanoparticles was characterized using EDXRF. The XRD result confirmed the successful synthesis of the nanoparticles, thus, revealing the peaks that are assigned to magnetite and silver respectively. In order to discover alternative agents for multidrug-resistant bacteria, silver and magnetic nanoparticles have exhibited exceptional antibacterial activity against a variety of microorganisms (Dakal *et al*., 2016; Li et al., 2018)

Ag-MNPs were investigated for antimicrobial activity against six bacterial species (*S. marcescens, S. aureus, E. coli, E. cloacae, S. enterica* and *A. baumannii)*. The compound exhibited an effective activity against two out of the six test organisms with *S. marcescens* and *S. aureus*, the obtained results showed that *S. aureus* was more susceptible Ag-MNPs than *Serratia marcescens*. Similar results were reported by Laskowska *et al*. (2018) who reported bactericidal effect and immunomodulatory properties of magnetic nanoparticles functionalized by 1,4-dihydropyridines. The results obtained from this study revealed that the findings of this research revealed that other organisms were resistant to Ag-MNPs (Table 1). Also, from the toxicity point of view, synthesized Ag-MNPs, under the same experimental conditions, selectively kills bacterial species (*S. marcescens* and *S. aureus*), and exhibited insignificant or lower level of toxicity towards other organisms (*E. coli, E. cloacae, S. enterica* and *A. baumannii*).

The susceptibility of the organisms (*S. marcescens* and *S. aureus*) towards Ag-MNPs could be attributed to the fact that the synthesized Ag-MNPs have the ability to penetrate the cell wall of organisms. The ability of silver to interact with bacteria causing severe disruption to the cell (Kim *et al*., 2007). Bacteria exposure to magnetic nanoparticles produce reactive oxygen species inside the cell, which damage a range of cellular constituents, disrupt cell function, and eventually leads to cell death (Li *et al*., 2018). The Ag-MNPs' antibacterial activity is influenced by the bacterial membrane's composition and structure (Kim *et al*., 2007). The Ag-MNPs' impact on bacteria results in membrane attachment, membrane penetration, and morphological alterations, all of which lead to cell death.

CONCLUSION

The synthesized silver coated magnetic nanoparticles demonstrated remarkable antibacterial activity against *S. marcescens*, and *S. aureus* whereas less activity was shown towards *A. baumannii, E. coli, S. enteric* and *E. cloacae.* Therefore, Ag-MNPs has antibacterial capacity and could be an effective compound of choice for treating bacterial infections, which serves as an alternative means for new drug discovery and overcoming the challenge of multidrug resistance.

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