ANTIBACTERIAL ACTIVITIES OF BIOENGINEERED SILVER CLAY NANOCOMPOSITE ON SPECIFIC CLINICAL ISOLATES

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ABSTRACT

Drug resistance is a global public health concern and the search for a better and efficient alternative cannot be overemphasized. In this study, antibacterial activity of bioengineered silver nanocomposite (BSN) on clinical isolates was examined using standard microbiological and analytical methods. Aqueous extract of *Moringa oleifera* leaves was used to synthesize the BSN, while characterization of BSN by transmission electron microscopy (TEM), scanning electron microscopy (SEM) x-ray diffraction (XRD), Braunuer Emmett Teller (BET) fourier-transform infrared spectroscopy (FTIR), and x-ray fluorescence (XRF) were employed for the structural and morphological elucidation. Result revealed evidence of significant agglomeration due to rapid oxidation and specks of tiny silver crystal on the surface of BSNs. The two clinical isolates were identified as *Staphylococcus aureus* and *Escherichia coli*. It was observed that the nanoparticles were generally more effective against *S. aureus* than *E. coli* as zones of inhibition ranged from 5 to 7mm for *E. coli* and 17 to 25 mm for *S. aureus.* Findings in this study suggest that BSN exhibited varied levels of antibacterial activity against *E. coli* and *S. aureus*. It could therefore be useful for the antimicrobial therapy of infections related to these organisms especially when resistance to conventional antibiotics becomes difficult to manage.

INTRODUCTION

Infectious diseases remain one of the leading causes of morbidity and mortality worldwide (Adegoke *et al*., 2020). The WHO and CDC have expressed serious concern regarding the continued increase in the development of multidrug resistance among bacteria. Therefore, the antibiotic resistance crisis is one of the most pressing issues in global public health (Adegoke *et al.*, 2020). Associated with the rise in antibiotic resistance is the lack of new antimicrobials. This has triggered initiatives worldwide to develop novel and more effective antimicrobial compounds as well as to develop novel delivery and targeting strategies. Because of the emergence and increase in the number of multiple antibiotic resistant microorganisms and the counting emphasis on health care costs, many scientists have researched methods to develop new effective antimicrobial agents that overcome the resistance of these microorganisms and are also cost effective. Recently developed nanomaterial system has been evaluated for their potentials in the area of nanobiotechnology and it is thought that the promising characteristics of these materials can be help in the fight against resistance to contemporary antimicrobials. In this context, metal oxide NPs have received great attention due to their unique physicochemical and biological properties and interestingly these properties may be altered by fine-tuning their structure and morphology at atomic level (Sondi and Salopek-Sondi, 2004). Nanomaterials based antibacterial are expected to revolutionize food technology and the fight against human pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Sondi and Salopek-Sondi, 2004). Thus, bactericidal properties investigation of the nanomaterial is currently at the forefront of scientific researches. Silver ions have long been known to exert strong inhibitory and bactericidal effect as well as to pose a broad spectrum of antimicrobial activities (Bankar *et al.*, 2010). It triggers the release of K^+ ions from bacteria; thus targeting the cytoplasmic membrane, which is associated with proper function in of many important enzymes and DNA (Fulrmann and Rothstein, 2006). It is reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganism at low concentrations without any side effects (Jeong *et al.,* 2005). The products derived from several herbs and plants, being a source of multifunctional curing agents and bioactive compounds, are relatively considered safe for consumption. According to the Food and Agriculture Organization's (FAO, 2014) report, about 70–80 % of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases (Ekor 2014). *Moringa oleifera* Lam. (syn. *M. pterygosperma* Gaertn., $2n = 28$) belongs to the family Moringaceae, commonly known as the 'drumstick' or 'horseradish' tree. It is an affordable and readily available source of major essential nutrients and nutraceuticals, and it has the potential to eradicate malnutrition (Kunyanga *et al.* 2013). The *Moringa oleifera* is often considered as important famine food because of its high resistance to drought and arid conditions owing to their tuberous roots (Padayachee and Baijnath 2012). Almost each and every part of *Moringa* tree is useful for medicinal, functional food preparations, nutraceuticals, water purification, and biodiesel production; including roots, leaves, flowers, green pods, and seeds (Saini 2015). Montmorillonite (MMT) is a class of clay that belongs to the smectite family with an octahedral sheet and tetrahedral sheet in the ratio 2:1 respectively. The volume of montmorillonite when in contact with water is reported to increase rapidly due to fragile bonds between the layers. More so, the negatively charged surface facilitates electrostatic attraction which synergistically increases its potency (Yuvakkumar *et al*., 2011). Importantly, it is pliant, abundant, stable and at the same time economical when compared to other conventional support materials (Li *et al*., 2019). Therefore, the development of the BSN would increase the stability of the silver ions on the surface of the montmorillonite coupled with the capping properties of the *Moringa oleifera* extract. The aforementioned informed the use of MMT as support for the silver nanoparticles in the preparation of BSN. Due to the established antibacterial activity of silver, it is projected that development of BSN would confer higher stability and efficiency than when occurring independently. Hence, this study sought to examine the potential of bioengineered silver nanocomposite as antibacterial on *Staphylococcus aureus* and *Escherichia coli.* These microorganisms are leading bacterial pathogens of healthcare associated infections and bacteremia in older-age populations.

Materials and Methods

Clinical isolates and *Moringa oleifera* plants were obtained from St. Luke's Hospital, Anua, Uyo, Akwa Ibom State and Uyo metropolis respectively. The clinical isolates were characterized based on their cultural and morphological attributes as well as their responses to standard biochemical tests as described by Cheesbrough (2006) and Brenner *et al*., (2005). Beckman Coulter DU 730 Uv-vis spectrophotometer was used to record the surface plasmon resonance (SPR) of BSN at a wavelength range of 300 to 650 nm while surface morphology was captured under a FEI™ scanning electron microscope (Nova Nano SEM 230, FEI, Hillsboro, OR, USA). Functional group analyses of the samples were recorded over the range 4000 – 400 cm^{-1} using Shimadzu FTIR-8400S. Similarly, X-ray diffraction (XRD) patterns of the nanomaterials were performed on a Rigaku (D/MAX-3B) diffractometer with Cu K_α radiation ($\lambda = 1.54056$ A˚) and a graphite monochromator at 40kV, 30 mA. The porosity and specific surface area were recorded on a BET surface area analyzer (Model Nova 2000e, USA). The agar well diffusion technique as described by (Cheesbrough, 2006). was employed to determine the effect of various concentrations (10 mg/ml, 25mg/ml, and 40mg/ml) of the nanoparticles on the test isolates.

RESULTS AND DISCUSSION

Transmission electron microscopy (TEM) image in Fig 1a shows the different sizes and shapes of BSN. Furthermore, as evident in Figure 1, there is a high distribution of majorly spherical silver and MMT support with sizes below 100 nm.

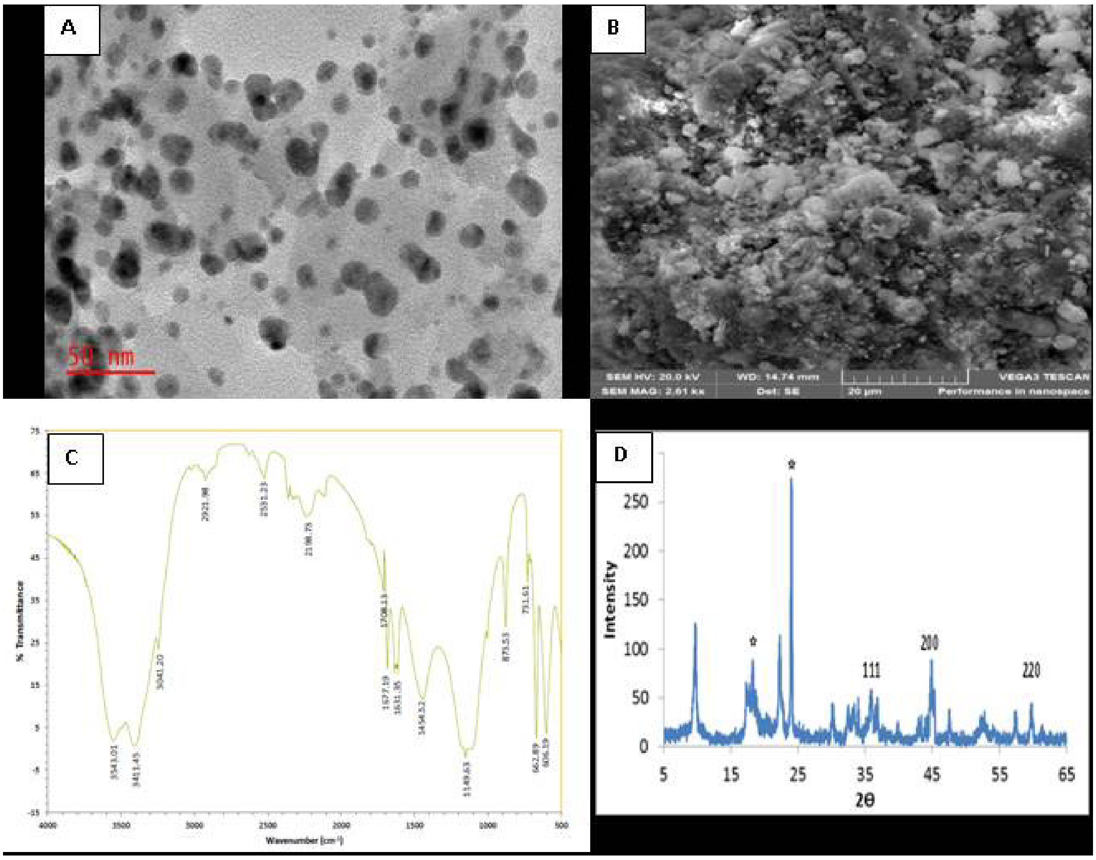

Figure 1: (a) TEM (b) SEM (c) FTIR and (d) XRD of BSN

X-ray Fluorescence (XRF) Analysis of BSN

The elemental distribution in the BSN is shown in Table 1. The concentration of Ag was higher in silver nanoparticle than the silver-clay nanocomposites due to the complexing effect of the clay mineral while other elements (K, Ca, Fe and Cu) increased significantly due to their natural presence in the clay.

Table 1: XRF of Ag nanoparticle and Ag-clay nanocomposites

Elements	Conc (%)
Ag	1.2814
K	0.5221
Ca	1.0860
Al	1.8284
Si	1.3031
Mn	0.0190
Fe	0.8350
Ni	0.0090
Cu	0.1096
Zn	0.0290
Sr	0.0138
Pb	0.0118

The morphological and biochemical characteristics of the clinical isolates are presented in table 2. The two isolates were identified as *Staphylococcus aureus* and *Escherichia coli*.

Table 2: Morphological and Biochemical Characteristics of clinical Isolates obtained from St. Luke's Hospital Anua.

Gram Staining	Shape	Catalase	Coagulase	Oxidase	Urease	Citrate	Indole	Motility	Methyl Red	Voges Proskauer	Lactose	Mannitol	Glucose	Organisms
+	S	+	+	N/A	NA	N/A	N/A	+	N/A	N/A	N/A	+	N/A	*Staphylococcus aureus*
-	R	+	N/A	N/A	-	-	+	+	+		-	+	N/A	+ *Escherichia coli*

Key: G.R = Gram Staining; MR. = Methyl Red; VP = Voges Proskauer; + = Positive; - = Negative; R = Rod; S = Spherical; A = Acid only; N/A = Not Applicable

The antibacterial activity of the BSN is presented in Table 3. BSN inhibited the growth of both clinical isolates tested although it was generally more effective against *S. aureus* compared to *E. coli*. Generally, zones of inhibition ranged from 5 to 7mm for *E. coli* and 17 to 25 mm for *S. aureus.*

Table 3: Antibacterial activities of different concentrations of BSN against *E. coli* and *S. aureus*.

Isolates	**Zone of Inhibition (mm)**			
	10 mg/ml	**25 mg/ml**	**40mg/ml**	**Control (75% Ethanol)**
E. coli	7	5	7	5
S. aureus	25	20	17	5

The average size of BSN particles in Figure 1a was 25 nm and as related to findings of Rajeswari *et al.* (2016), the shape, size and dispersion rate of the silver on the MMT are highly influenced by nature of *Moringa oleifera* extract and experimental conditions while further attributing the high level of mono-dispersion observed to capping properties of the plant extracts. This is similar and responsible for the observations in Fig. 1a. In contrast to deductions of Prasad *et al.* (2014), there were no pairing tendencies or assemblage of the BSN, primarily due to coating potentials of the phytochemicals, thereby enhancing their surface area. However, unlike Prasad *et al.* (2014), flavonoids and particularly phenols were established to inhibit clustering as a result of exceptional reducing and enveloping properties. According to the SEM result, the surface of the nanocomposites has significantly agglomerated due to rapid oxidation. However, tiny crystals of silver are found of the surface of the nanocomposite as shown in Figure 1b which is similar to reports of Damm and Munstedt, (2008). FTIR spectra of the BSN (Figure 1c), shows a strong broad O–H stretching absorption around 3411.45 cm^{-1} and a prominent C–H stretching absorption at about 2921.98 cm^{-1} while C-O stretch was observed at 1149.63 cm^{-1}. There was a general increase in the intensity and shift of the absorption bands and also the appearance of a peak at 2899.11 cm^{-1} and 1051.24 cm^{-1} of the Ag-clay nanocomposites that was not originally present in the spectrum of Ag nanoparticle (Coates, 2006).

The presence of active groups on the surface of BSN as a result of interaction with *Moringa oleifera* extract confers significant functionality on the BSN as corroborated by Tran *et al.*, 2015 and evident in Fig. 3. Absorption bands at 3700 cm^{-1}, 2820 cm^{-1} and 1618 $cm^{-1}$are ascribed to O-H stretching group of amide, CH_2 group bending vibration and C=O groups respectively while presence of Si-O bands at 1100 $cm^{-1}$to 900 cm^{-1}in all the spectra is attributed to the aluminum silicate nature of the MMT mineral. Sharp and less intense absorption bands around 3650 and 1620 cm^{-1} were duly ascribed to Al – Al – OH stretching band and bending modes of water molecules majorly due to the wet method of synthesis and the plant extract analogous to Liu *et al*., 2012. The XRD result revealed apparent peaks ( ) at 2θ of 23.58^o and 18.3^o that indicated the presence of quartz and MMT phase in the clay mineral. The apparent peaks ( ) at 2θ of 23.58^o and 18.3^o indicate the presence of quartz and MMT phase in the clay mineral. In Fig. 1d, the presence of silver in the clay matrix is noticed by the presence of peaks at 2Ɵ: 37.2^o (111), 44.8^o (200) and 53.3^o (220). The above observations corroborate the interaction of silver with the clay mineral as reported by Siamey and Farhadi, (2014). The presence of Ag was evident in BSN alongside other elements such as K, Si, Fe and Al due to their natural presence in the MMT mineral (Balati *et al*., 2015).

The two clinical isolates were identified as *Staphylococcus aureus* and *Escherichia coli*. It was observed that the nanoparticles were generally more effective against *S. aureus* than *E. coli*. There are many proposed mechanisms for Ag NPs action against bacteria. For example, nanoparticles attachment to bacterial cell wall via hydrophobic, electrostatic, and receptor ligand interactions and van der waals forces which leads to cellular damage and eventual death. Also, Ag NPs generate reactive oxygen species such as H_2O_2 adept at interaction with and subsequent disruption of microbial cells especially the cell membrane bilayer and influencing integrity, membrane fluidity, and lateral organization (Xie *et al*., 2011). These potential effects can be some of the reasons for NPs cytotoxicity. Important bacterial biomolecules can adsorb on Ag NPs. Also, protein structural changes and phospholipid molecular damage are more likely reasons for bacterial toxicity. Toxicity of NPs is probably due to the dissolved metal ions and from NPs tendency to interact with the cell walls.

CONCLUSION AND RECOMMENDATION

In this study, silver nanoparticles were successfully synthesized and examined for antibacterial activity against clinical isolates. It was observed that BSN was effective against *E. coli* and *S. aureus*. They could therefore be useful for the antimicrobial therapy of infections related to these organisms.

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