



Antibacterial efficacy of cotton nanofiber soaked in Ag, ZnO and TiO₂ nanoparticles

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ABSTRACT

In view of eco-benign nature of nanoparticles prepared via green routes, in the present investigation, silver, zinc oxide and titanium nanoparticles have been prepared using onion (*Allium cepa*) extract, which act a reducing and capping agent. The prepared nanoparticles were characterized using UV-visible, FT-IR, XRD, AFM, and SEM techniques. The nanoparticles efficacy was studied as antibacterial agent by applying on cotton fiber. Cotton nanofiber was coated by soaking in solutions of nanoparticles (Ag, ZnO and TiO₂) and their biological efficiency was evaluated and it was observed that the cotton fiber soaked with nanoparticles showed a strong biological efficiency against panel of selected strains. This coated nanofiber of cotton shows a mat with high surface area versus uncoated fiber. Results revealed that green synthesized nanoparticles enhanced the antibacterial activity of cotton fiber, which have potential applications in textile industry.

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Capsule Summary: This study was conducted to appraise the antibacterial efficacy of cotton nanofiber soaked in Ag, ZnO and TiO₂ nanoparticles and nanofiber soaked in nanoparticles showed promising antibacterial activity.

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INTRODUCTION

Modified cotton and textiles via coating with antibacterial agents have attracted much attention of the textile sector and interest by manufacturers for his distinctive features (Berendjchi et al., 2011; Rivero et al., 2015; Zhang et al., 2015). This modification applied in the field of fashion industry and manufacturing textiles for clothing (Zhang et al., 2015), domestic products (Rivero et al., 2015), furniture (Zhang et al., 2014) and engineering uses for consumer goods (Bick et al., 2018). Nanomaterials are often used with other chemical

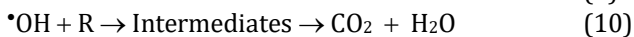
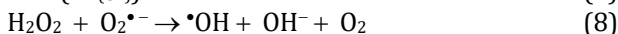
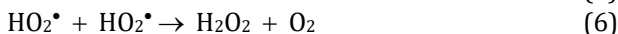
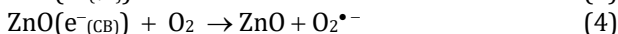
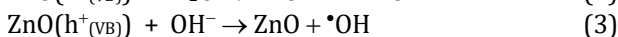
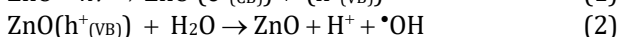
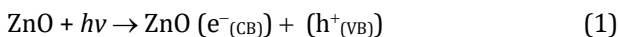
processes and innovations to incorporate or develop different clothing functionalities (Pensupa et al., 2017). Such chemicals may have a detrimental effect on humans and the environment (Unalan et al., 2019). The correct collection and cautious incorporation of nanomaterials into the fabric helps to reduce these detrimental effects (Bhattacharjee et al., 2019; Usenyuk-Kravchuk et al., 2020)].

Textiles with antimicrobial properties used in the manufacturing of knitwear and underwear benefit from undesirable odors owing to a prevalence of bacteria in body areas often exposed to suck (Handy et al., 2008). The efficacy of antimicrobial activity on textiles differs with the textile

antimicrobial with respect to concentration and mode of usage (Lin et al., 2016). The suspension of bacterial test is inoculated directly on the textile materials (Lin et al., 2010); bacteria are transferred to the material by means of an agar plate in which the test bacterial is placed (Jung and Bhushan, 2009); and a bacterial test filter in which the bacteria are placed is printed onto a textile material (Yang et al., 2018). Self-cleaning is achieved by ensuring a reliable and stable integration of nanomaterials in the fabric and nano-titanium dioxide is now used in textiles for UV protection and by combining nano-titanium dioxide and the nano-silver (Raafat and Sahl, 2009).

The researchers investigated the nanoparticles applicability for antibacterial purposes (Tan et al., 2013). These designed textiles must be capable of killing microorganisms, destroying organic compounds and neutralizing bad odors (Oberbek et al., 2019). According to research on the antibacterial properties of produced nanoparticles, silver nanoparticles made from garlic extract are very efficient against test isolates. The range of inhibition zone for all isolates was 17 mm, indicating that garlic extract promising efficiency for the synthesis of silver nanoparticles, which may be used as a treatment for human microbial illnesses (Monteiro-Riviere et al., 2012). It has been widely acknowledged that immobilizing TiO₂ nanoparticles on a suitable support would assist to reduce the need for pricey phase separation operations and increase the viability of using such catalysts in industrial processes. Recent research by Plesch et al. (2009) reported a promising photocatalytic activity of immobilized TiO₂ particles on microporous ceramic alumina foam.

The semiconductor oxides, such as titanium dioxide (TiO₂) and zinc oxide (ZnO), are utilized as photocatalysts in heterogeneous photocatalysis, which is employed in advanced oxidation processes (AOP) mechanisms (Guo et al., 2019). The photocatalytic mechanism of the ZFGO nanocomposite (which consists of ZnO nanoparticles, F₃O₄ nanoparticles, and GO nanosheets) as photocatalyst agents to degrade the Brilliant Green dye was studied, and a mechanism for this activity is presented in Eqs. 1-10 (Ayeleru et al., 2019).



Based on promising catalytic and antimicrobial activity of nanoparticles, in the present investigation, the garlic extract was used as a reducing and capping agent nanoparticles synthesis, which were then characterized using X-ray diffraction, scanning electron microscopy (SEM), energy

dispersion X-ray (EDX), and FTIR techniques. Then, cotton nanofiber was soaked in Ag, ZnO and TiO₂ nanoparticles and their antibacterial activity was evaluated.

MATERIAL AND METHODS

Nanoparticles preparation

All the supplies utilized in this study were from Sigma Aldrich. The nanoparticles (Ag NPs) were prepared using the approach already reported, i.e., silver (Sanches et al., 2020), zinc oxide (Nohynek and Dufour, 2012) and titanium dioxide (AL-Rubaye et al., 2020) using onion (*Allium cepa*) extract as the reducing and capping agent. Rietveld refinement of XRD patterns, carried out using FullProf software. The Debye-Scherrer technique was used to compute the average crystal size. Williamson-Hall method was used to investigate the effects of strain in X-ray line broadening of ZnO nanoparticles (NPs), and scanning electron microscopy (SEM) was used to conduct the morphological studies. The average particle size obtained by SEM matches that of the Debye-Scherrer and Williamson-Hall methods (Richa et al., 2018). By using the atomic force microscope AFM and X-ray diffraction (XRD), the produced nanostructures were analyzed. PVA that has been carboxymethylated has been created and described. (CPVA) composites were made with varying amounts of ZnO nanoparticles. Films are cast with the composites. An HP LCR meter was used to measure the films' dielectric constant characteristics (Latif et al., 2012).

Antimicrobial activity evaluation

On Mueller-Hinton agar, the antibacterial activity of nanofiber was assessed using the agar diffusion technique. Each petri dish was filled with sterilized Muller Hinton agar, which had been cooled to 50 ± 2 °C and allowed to gel firmly before being inoculated with bacteria. The challenge specimens were bacterial cultures of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Citrobacter* spp., and *Klebsiella* spp. Bacterial cultures were scraped off of nutrient agar plates and suspended in peptone water that had undergone sterilization. For evaluating bacterial turbidity, McFarland standard tube number 1, which is comparable to around 1×10⁸ CFU/mL, was used. To guarantee bacterial adhesion, Muller Hinton agar was smeared with cotton swabs that had been submerged in bacterial suspension. In the meantime, a borer applicator was flame sterilized, chilled, and then pushed upon Muller Hinton agar that had been seeded to create a well with a 6 mm radius. Because of the plate's aspect, wells were maintained apart from one another. Ag, ZnO, and TiO₂ nanoparticles were poured into each well of the plate, which was then incubated for 24 hours at 37 °C after being left in the lab for 10 minutes. Each plate was made in three copies, and the diameter of the inhibition zone was measured from the well's border. Following ASTM F2944-12 (Khan et al., 2019) with modification, 10 ml of bacterial culture of *S. aureus* and *S. epidermidis* counted 1×10⁸ CFU/ml were augmented with 7

mm discs of cotton coated with nano metal and incubated for 18 h at 37 ± 2 °C. Meanwhile, pouring technique was applied for bacterial enumeration; 1 ml of each treatment was mixed with 20 ml of pre-sterilized Muller Hinton agar (47 °C), poured in petri dish and incubated at 37°C for 18 h. Visible colonies were counted using colony counting equipment and number of viable bacterial cell was compared with the control trial that was augmented by conventional cotton disc.

RESULTS AND DISCUSSION

UV-visible, FT-IR, XRD, AFM, and SEM techniques were used to characterize the nanoparticles. The surface plasmon resonance peaks in the absorption spectra of silver colloidal solution revealed that the highest absorption range was around 380-440 nm. FT-IR was used to identify functional biomolecules in seaweed, such as carboxyl groups, that are responsible for the creation of silver nanoparticles. The XRD data revealed that the bio-organic phase crystallizes on the surface of the silver nanoparticles or vice versa. According to the findings, silver nanoparticles produced by onion extracts might be used as a therapeutic treatment for human microbial illnesses (Nahla et al., 2019).

FT-IR analysis

Plants have been used to synthesize silver nanoparticles, which is one of the best sources of preparation and a simple ecologically favorable benign approach. Because of its simplicity and lack of difficult conditions and criteria, this technology may be employed in commercial production of such nanoparticles at room temperature and in a single step. This study shows that plant extract stabilized nanoparticles are promising candidates for future research into their usage in biological and pharmaceutical applications. Because of the low synthesis temperatures and time required, this synthesis technique provides a less expensive and green alternative to standard procedures that can be easily scaled up for industry. Silver nanoparticles were used to prepared a chip from a new polymeric mixture nanocomposite consisting of (poly vinyl acetate, pectin and poly aniline) with silver nanoparticles. The electrical conductivity of this chip is very high compared to another chip prepared from the polymer mixture only. The chip was tested for electrical sensitivity by using a strip of polymeric mixture nanocomposites chip as one of the electrodes of the electrical cell and the other electrode with copper. After applied (6.5 V) the chip moves and has a bend at a certain angle (measured in a simple way using a ruler). When reversing the electrodes, the tape returns to its original shape. The motion range or bending (2.0 cm) response was more bending, and because of this response, the polymeric mixture nanocomposite which prepared is considered to be electrical sensitive smart materials and can be used in medical purposes (Vankar et al., 2012). Results shows FT-IR Characterization of the synthesized nanomaterials (Ag NPs, ZnO NPs and TiO₂ nanoparticle, where exhibited prominent

peaks at 501, 543 and 418 cm^{-1} due to silver nanoparticles and the peak at 3448 cm^{-1} of hydroxyl group, the peak at 1382 cm^{-1} due to NO₂ group, while analysis shows FT-IR spectra of Zn-O absorption band at 486.08 cm^{-1} and Results shows FT-IR analysis of TiO₂ nanoparticles and in line with previous reports (Theivasanthi and Alagar, 2012; Azam et al., 2010; Kasuga et al., 1999).

Structural analysis

Figure 1 depicts the XRD analyses of the generated nanomaterials at the range of 2 (10-80°); the X-ray diffraction patterns of the Ag-NPs manufactured using AgNO₃. All of the reflections correspond to pure silver metal with cubic symmetry at the face. Reflections were indexed as 111, 200, 220, and 221 with corresponding d values of 38.88, 45.15, 66.03, and 78.82 nm^{-1} (JCPDS 04-0783). (Xu et al., 2008). The strength of the peaks represented the silver nanoparticles' high degree of crystallinity. The diffraction peaks, on the other hand, were wide, indicating that the crystallite size is quite tiny. The Debye-Scherrer equation may be used to compute the average particle size of Ag-NPs (Langford and Wilson, 1978). $D = K \lambda / \beta \cos \theta$, D is the grain size, and K is the Scherrer constant, which ranges in value from 0.9 to 1 (shape factor). Other variables include the X-ray wave length, 1.5418, the breadth of the XRD peak at half-height, the Bragg angle, and. The Scherrer equation indicates that the typical Ag-NP crystallite size is (38.17nm). The hexagonal (wurtzite) structure of zinc oxide is depicted by the crystalline peaks at 2 at (31.78°, 34.46°, and 36.28°) connected with planes (100), (002), and (101) in Figure 1b (PDF 36-1451). (Peng et al., 2020).

The hexagonal structure of the polycrystalline ZnO nanostructures produced in a single step reaction at 80 °C was shown by the XRD pattern. Accurate values for the lattice parameters $a = 3.250353$ 0.00009, and $c = 5.207006$ 0.00020, where c/a ratio is 1.601981, were obtained using Rietveld refinement. The average crystalline size of ZnO nanoparticles (NPs) was around 30 nm, and the effects of strain on X-ray line widening showed that the peak broadening is caused by both a considerable strain distribution and a smaller coherently diffracting domain size. The internal lattice strain value was found to be and the extracted particle size is around 31.5 nm (6×10^{-4}). According to the conclusion of 29 = 40, the morphological and topographical analyses conducted by SEM revealed that the average particle size is comparable to results obtained by the Debye-Scherrer and Williamson-Hall techniques. The manufacture of ZnO nanostructures took place very quickly, and it was most astonishing that they could be made in a single step reaction at 80 °C without any additional treatments. Findings have shown that the material is a rutile phase and in a crystalline form as shown in Figure (1c) with a high purity of more than 99.5%, which revealed the efficiency of the method adopted for the synthesis of NPs (Tsuang et al., 2008).

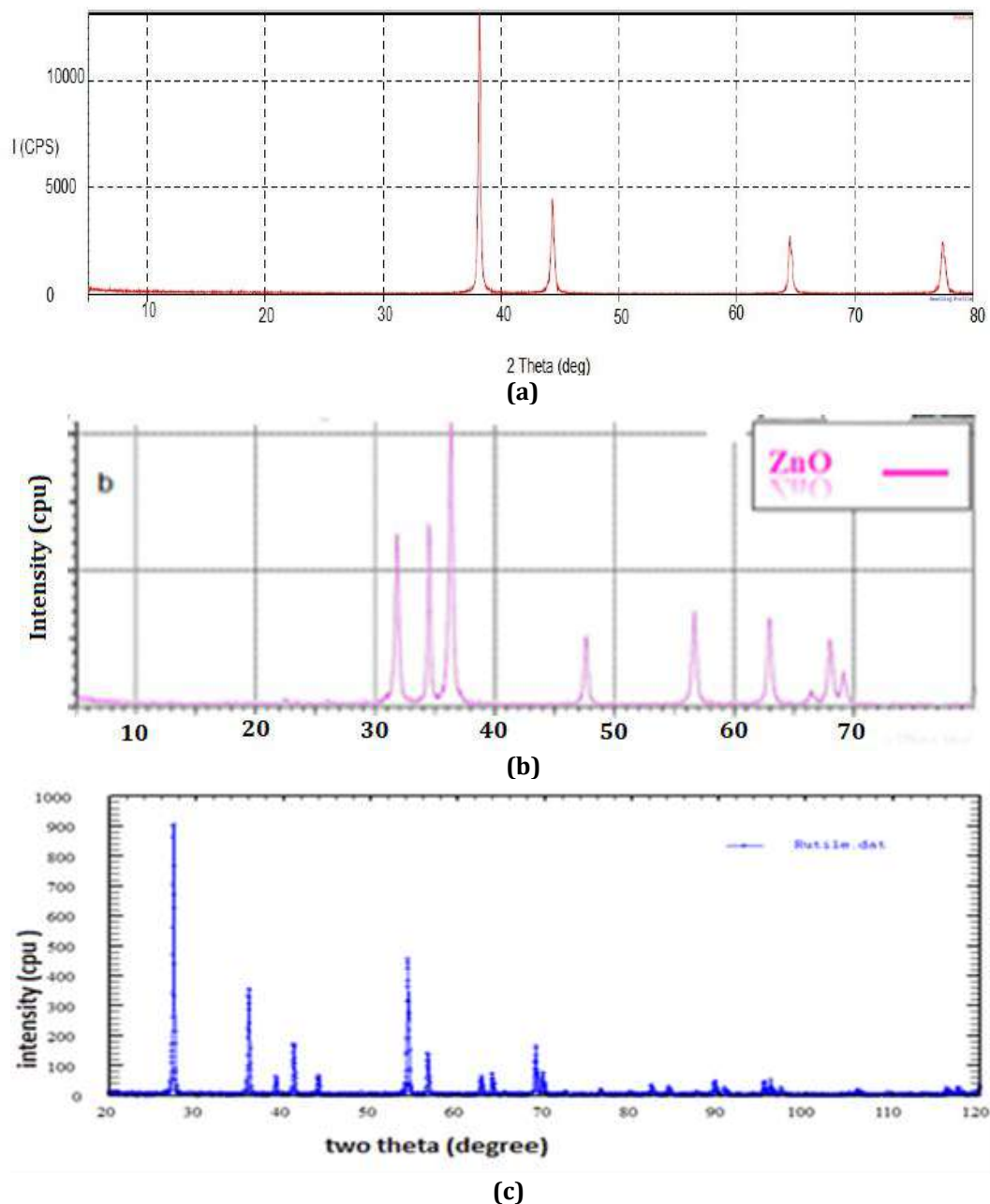


Fig. 1: The XRD patterns, (a) Ag (b) ZnO and (c) TiO₂

This information was obtained through the examination of X-ray diffraction of TiO₂ NPs. The Rutile phase is thermodynamically stable, according to the results of the XRD investigation, however Duchaniya et al. (2018) note that a minor quantity of rutile can convert to the Anatase phase at low temperatures. The semiconducting transition metal oxide substance titanium dioxide (TiO₂) has unique properties including low cost and ease of synthesis and resistance to photochemical and chemical erosion. Due to these benefits, TiO₂ is used in applications such as solar cells, chemical sensors, hydrogen gas evolution, pigments, self-

cleaning surfaces, and environmental purification, as previously mentioned by Hoffmann et al. (1995). Due to their superior electrical, optical, and magnetic qualities compared to their bulk counterparts, the oxide nanoparticles produced by various processes are proving to be more and more beneficial (Xu et al., 2008). Anatase, rutile, and brookite are the three primary crystalline polymorphs of titanium dioxide, which also occurs in amorphous and crystalline forms. While asbrookite has an orthorhombic structure, anatase and rutile both have tetragonal structures (Mahshid et al., 2007).

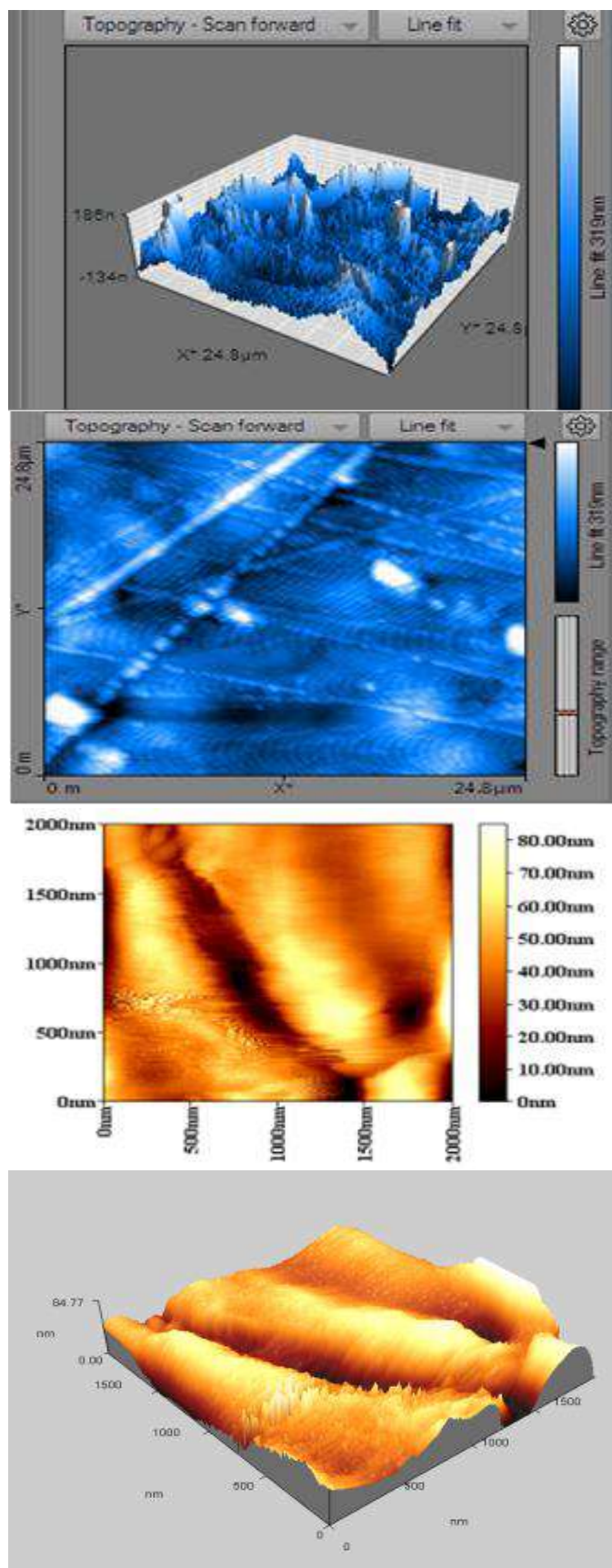


Fig. 2: AFM analysis of the prepared nanoparticles

AFM analysis

Figure 2 represents the Atomic Force Microscopy (AFM) pictures of topography of synthesized nanoparticles. Results shows the particles shapes of silver nanoparticles are balls or cubics (Andrade et al., 2013) with particles size histogram is about (31.92 nm). The analysis shows the particle size histogram of ZnO nanoparticles is about (77.92 nm) (Figure 2). The highest value for the Titanate nanotube lengths is (84.77 nm) (Figure 2), and when interpreting the results for the nanoparticle, their lengths were distributed differently, so the lengths of the nanoparticle ranged between (14-236 nanometers), as the average length of the tubes was about (32,5) and this rate included the ratios of lengths ranging in quantities (10%) with a length of 14 nm and (50%) with a length of 26 nm and the rest was of different lengths ranged from 26-236 nm.

SEM analysis

The silver nanoparticles under investigation were scanned by SEM as shown in Figure 3, which demonstrates that the shape of the particles seems to be spherical and that the particles cluster together. It is simple to see that the particles under examination are made up of several items that are only a few nanometers in size. However, due to challenges in obtaining higher magnification, we were unable to study the structure of the detected nanoparticles. A typical EDX spectrum obtained from the material under examination is displayed in Figure 3. A significant peak at 3 kV is found in the center of the given spectrum. The silver characteristic line L and this maximum have a direct relationship. The maximum, plainly seen at 0.2 kV in the left section of the spectrum Figure 3 ZnO nanoparticles are shown in SEM photos, and it was found that the particles had good form. The majority of the particles were hexagonal. Drawing a histogram for the SEM picture allowed us to determine the average crystallite size; the majority of the particles were nanoscale in size. Figure 3 shows a SEM picture of TiO₂ with approximately spherical, spongy form and aggregated nanoparticles.

Antimicrobial activity

Recent results indicated that cotton coated with nano metals shows biological activity against gram positive of bacterial species under study (Table 1). The bacterium *S. aureus* was susceptible to cotton coated with nano- silver followed by cotton coated with nano-titanium oxide. *S. epidermidis*, on the other hand, showed a high vulnerability to titanium nanotube-coated cotton. In contrast with gram negative bacterial species of *Citrobacter* spp., and *Klebsiella* spp., that showed very low susceptibility to cotton coated by nano- silver, nano-zinc and nano-titanium (Fig. 4). The prevention and treatment

of bacterial infections is become very difficult in medical care via developing of resistance to traditional synthetic and semi synthetic antimicrobial agents especially at hospitals. It has been proposed that differences in the cell wall structure, cell physiology and metabolism between gram positive and gram-negative bacterial species gave difference in antibiotic susceptibility (Prahald and Seenayya, 1988). Our efforts to overcome the bacterial resistance by formulated metal nano material to enhance antibacterial potency and such formulated could be used as an efficient material in modern medicine.

The mechanisms that might involve in the bacterial damage related with the physical mechanism through attaching onto the bacterial cell wall, biological mechanisms by interacting with lipopoly saccharide in membrane and chemically via formation of hydrogen peroxide as well as changing membrane permeability that led to disorganization of bacterial cell membrane following contact with metal nano material and such explanation highlight by (Patra et al., 2015). Despite that ZnO has potential and unique optical, electrical and chemical properties render their nano-products exhibit high antibacterial activity against both gram positive and gram negative bacterial species such as *S. aureus*, *Bacillus subtilis*, *Escherichia coli* O157:H7 and

Klebsiella pneumoniae; however, our results demonstrated that cotton coated with Zn Nps had no antibacterial activity against the four bacterial species under study (Padmavathy and Vijayaraghavan, 2008; Azam et al., 2012; Emami-Karvani and Chehrizi, 2017).

The activity of metal-based nanoparticles against bacterial population depended on numerous characters which include morphology, physicochemical, and electric properties. The most effector characters of nanoparticles were their size, shape, roughness, and surface energy (Wang et al., 2017). The differences in the chemical structure of bacterial cell wall between gram positive and gram-negative bacteria would interfere with the activity of cotton-coated with NPs. The net negative charge of cell surface; gram negative bacteria had higher negative charge in contrast with gram positive bacteria. Such criteria gave stronger interaction between gram negative and metal NPs in the early growth stage of bacterial culture which might cause a decline in the growth of bacteria. Since, the positive charge of nano particles metal attracts negative charge of bacterial cell wall via the electrostatic interaction. The strong bond formed between metal nano particles and the cell membrane resulting in disruption of cell wall and increases the permeability of membrane (Stensberg et al., 2011).

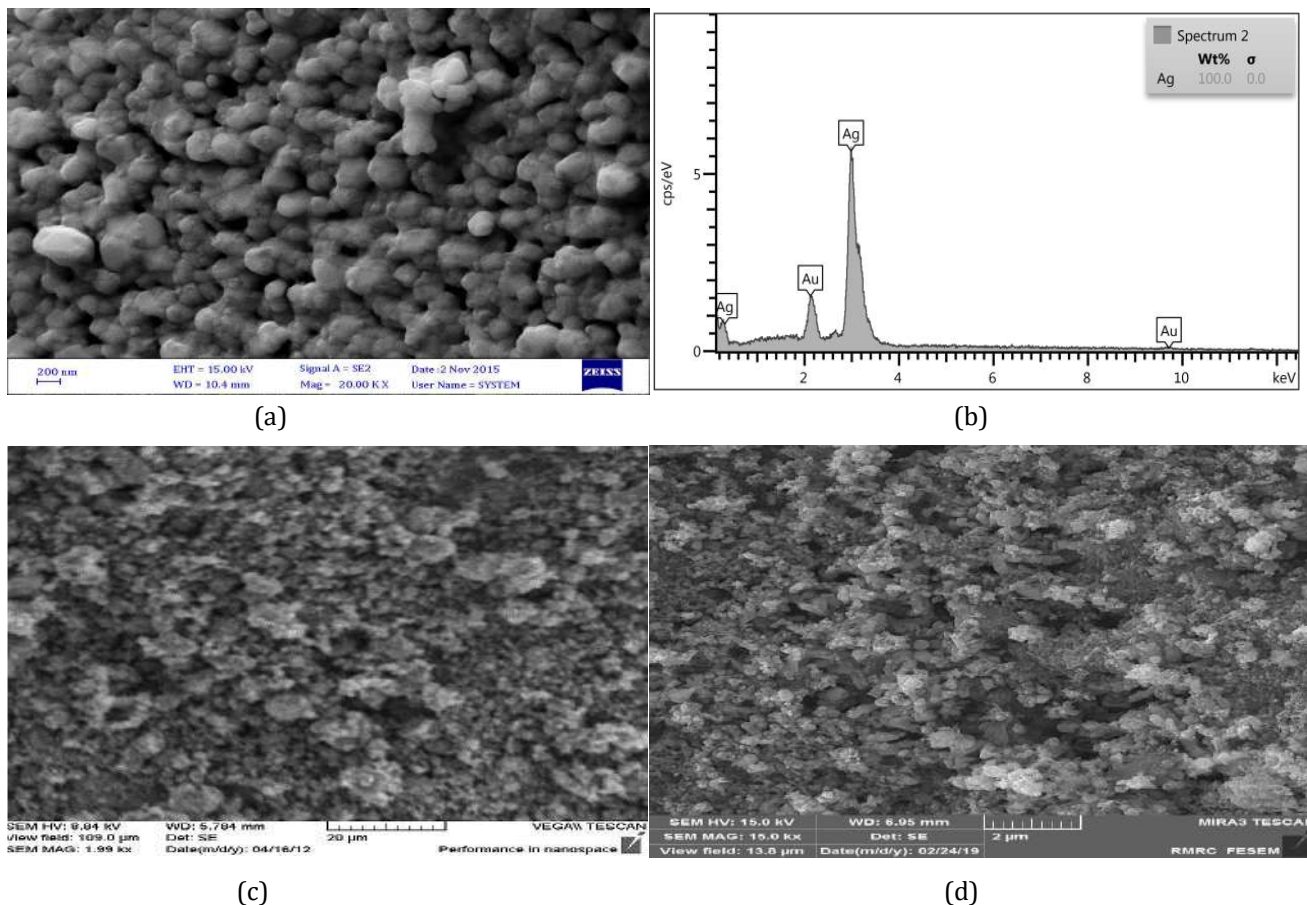


Fig. 3: (a) SEM analysis of the silver nanoparticles, (b) EDX analysis of silver nanoparticles, (c) SEM analysis of the synthesized ZnO nanoparticles, and (d) SEM analysis of the TiO₂

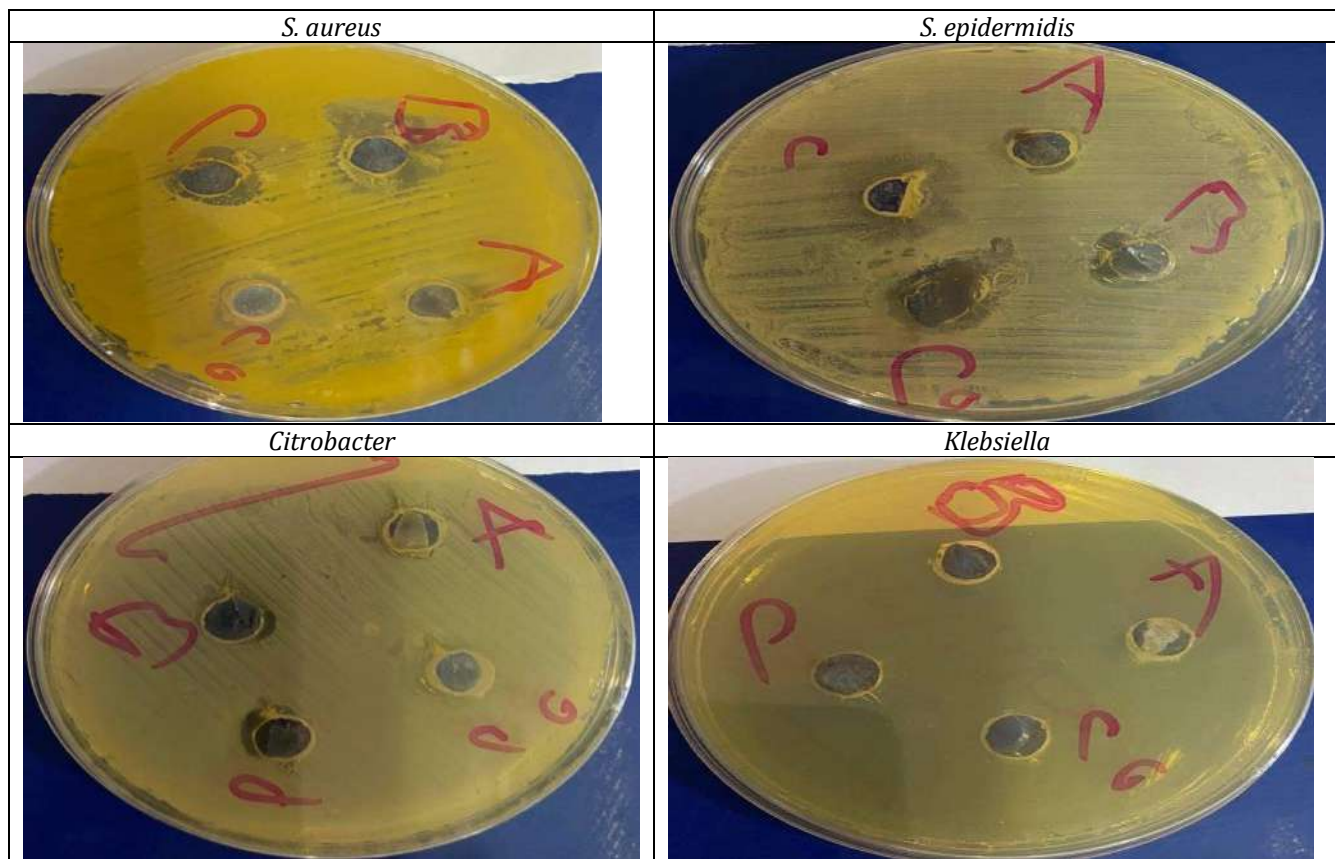


Fig. 4. Biological efficiency against gram positive and gram-negative bacteria, (a) ZnO nanoparticles, (b) Ag nanoparticles, (c) TiO₂ nanoparticles and control

Literatures cited that the first step adapted in the bacterial resistance to antibiotics is the membrane permeability. The OMPs constitute porines and play a major role in developing of antibiotic resistant in gram negative bacteria. OMPs directly interact with various environments surrounding the bacterial population and have essential roles in bacterial adaptation for hostile niches. Researchers found a significant difference in OMPs proteins in the presence of ZnO NPs (Lanka et al., 2014). Also, OMPs are charge macromolecules that undergo specific linkage with nano material disrupting the main function of OMPs. On the other hand, nano material might release ions that react with thiol group of protein imbedded in the cell membrane decreasing the membrane permeability to nutrients and causing cell death (Zhang and Chen, 2009). Nano material might involve with another cell membrane component, the LPS that responsible for bacterial adhesion to surfaces, attachment and virulence factor in many gram-negative bacteria. For this regard interactions affect the activity of LPS include metal or cations, polar group interaction, steric interference as well as specific reaction between functional group. Such assumption agrees with Yeh et al., 2006 who explained that ZnO Nps and Ag Nps could disrupt porins and LPS present in the bacterial cell membrane of *Klebsiella pneumoniae*.

Metal nanoparticles could induce the generation of reactive oxygen species (ROS) that leads to oxidation of glutathione, thus suppressing bacterial antioxidant defense mechanisms. As well as, metal nanoparticles can form strong coordination bonds with cellular macromolecules containing N, O and S such as proteins and DNA disrupting cell function (Yuan et al., 2018). Result presented here was in accordance with Shateri-Khalilabad (Shateri-Khalilabad, et al., 2013) who demonstrated a high antibacterial activity of cotton coated with Ag Nps against *S. aureus* bacterial challenge, as shown via formation of inhibition zone.

Result presented in Table 2 demonstrated that bacterial growth of both *S. aureus* and *S. epidermidis* were diminished after treatment with cotton coated with silver or titanium nanoparticles. This suggested that the technique used for coating cotton with nano metals was successfully prevalence with high activity against bacterial growth in the presence of moisture, humidity and even in socked situation. The most acceptable mechanism that responsible for the decline of bacterial viability was the formation of hydrogen peroxide or any other radical species which might contribute to the antibacterial activity of nano-metal hybrid polymer. Since UV light activated metals used in coating creating electron hole pairs and subsequently produced H₂O₂ that

Table 1: The biological efficiency against total count of bacteria for Ag, ZnO and TiO₂

Nanoparticles	<i>Klebsiella</i> spp	<i>Citrobacter</i> spp	<i>S. epidermidis</i>	<i>S. aureus</i>
ZnO	0.22±0.115	0.23±0.0152	0.76±0.030	0.39±0.012
Ag	0.39±0.023	0.42±0.0145	6.26±0.145	10.33±0.757
TiO ₂	0.31±0.020	0.33±0.020	12.5±0.32	8.06±0.305

Table 2: Cotton fiber: Total count of bacteria before and after treatment with nanoparticles

Treatment	Mean of total count before treatment (CFU/ml)	Mean of total count after treatment (CFU/ml)
1	T.M.C	20
2	T.M.C	40
3	T.M.C	40

1 = *Staphylococcus aureus* + cotton coated with Ag, 2 = *Staphylococcus aureus* + cotton coated ZnO, 3 = *Staphylococcus epidermidis* + cotton coated TiO₂, T.M.C= too many to count

penetrate cell membrane and kill bacteria such mechanism explained by (Blake et al., 1999).

For decades it has been recognized that nosocomial infections or hospital acquired infections (HAIs) were recurrent problems, identified chiefly in intensive care facilities, surgical, and medical wards. There are numerous reports regarding this problem in Europe and United State of America (USA); the species *S. aureus* considered as the most abundant and the more virulent in the hospital acquired infection. In French during 2007 there were a total of 12,188 *S. aureus* and many of them were encoding antibiotic resistance to many traditional drugs (Rogues et al., 2007). From reviewing serious nosocomial infections caused by *S. aureus* we found that methicillin resist *S. aureus* penetrate blood stream, serious damage to skin and soft tissue especially injured tissue or during burns and caused infections in respiratory tract (Elliott and Justiz-Vaillant, 2018).

Many reports reported that surgical or medical procedures may increase the probability of blood stream infections due the *S. aureus* that effect on the mortality rate, length to stay in a hospital beside the overall cost for medical care. Also, skin and soft tissue infections caused by *S. aureus* might encountered very important issue and consider as a risk factors in patients with diabetes mellitus, older age, immune compromise persons, alcohol consumers and prolonged hospitalization (Ki and Rotstein, 2008). Urgent of antibiotic resistant by *S. aureus* in nosocomial infections encourage scientists to continuous developing more effector strategies to overcome bacterial resistance. The coated cotton with Nano- metal had shown an effective antimicrobial activity against *S. aureus* that linked with dangerous nosocomial infections

CONCLUSIONS

The coated cotton nano fiber with nano- metal (silver, zinc oxide and titanium) was prepared for antibacterial applications. These nanoparticles have two shapes of (cubic, ball) that was show a strong biological efficiency against total

count of *S. aureus* and *S. epidermidis* bacterial species. Coated cotton nano fiber with Ag nanoparticles shows maximum biological efficiency against *S. aureus* then *S. epidermidis*, while for coated cotton with TiO₂ maximum biological efficiency against *S. epidermidis* then *S. aureus*. Nosocomial infections were responsible for delay healing in hospitalized patients as well as mortality. Recurrent of antibiotic treatment against diseases would take place of developing for bacterial antibiotic resistance beside multidrug resist microbes. Also, bacterial resistant would transfer vertically and horizontally among bacterial species due to the existence of different type of genetic transposable elements. All this encourage discovering well tolerated medical strategies in medicine formulation and production. One of the main advantages using metal- nano particles as antibacterial agents for their high output effectiveness against resistant strains of microbial pathogen, less toxicity and heat tolerance.

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