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Ag/ZnO Nano-composites as Novel Antibacterial Agent against Strain of *MRSA*

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With the appearance of microbial organisms resistant to multiple antibiotics, also, increase nosocomial infection, specially by Methicillin Resistant *Staphylococcus aureus* (MRSA), antibacterial effects of Nanocompositeshave attended by the many researchers in recent years. We report the synthesis of binary Nano-metal oxides such as Ag/ZnONanocompositesalso, Ag and ZnONanocompositesvia novel methods of thermal decomposition of oxalate precursor. XRD, FTIR, SEM and TEM were used to the thermal spectroscopic, structural, morphological characterization and surface area determination of the product, respectively. Strain of MRSA is prepared from ArdabilMedical University.Bacterial sensitivity to Nanocomposites was specially tested using by disc diffusion test and agar dilution test, and also with MIC, and MBC, against MRSA. The particle size was 12 nm, approximately. We reported that value of MIC Ag/ZnO bear on the MRSA was 8 µg/ml. Albeit, the value of MIC bear on the standard strain of *Staphylococcus aureus*(ATCC 1113) was 128 µg/ml. It is worthly noting that the value of MBC bear on the MRSA and the standard strain of *Staphylococcus aureus* were 128 µg/ml and 2048 µg/ml. The evidence was revealed that the antibacterial activity of Ag/ZnO were more vigorous to MRSA.

Key words*:* Antibacterial Activity, Ag/ZnONanocomposites, MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as an important Gram-positive bacterium involved in causing both hospital-acquired and community-acquired infections. In fact, MRSA is an important pathogen in the health care sector that has not been eliminated from the hospital nor community environment. The growing proportion of nosocomial and community associated isolates of MRSA, frequently displaying multidrug resistance against all semisynthetic penicillin and penems and to a variable extent macrolides, aminoglycosides, fluoroquinolones and glycopeptides[1,2], is an incentive to the development of novel antimicrobial agents active against such dangerous, hard-to-treat pathogens. In humans, *S. aureus* causes superficial lesions in the skin and localized abscesses, central nervous system infections, osteomyelitis, invasive endocarditis, septic arthritis, septicemia, pneumonia, and urinary tract infections. A bacteremia caused by *S. aureus* produces between 25% and 63% of mortality[3]. In 1960, the first strain of MRSA was isolated in the UK, just 1 year after methicillin started to be used as an alternative to penicillin. Nowadays, MRSA strains have a wide range of drug resistances, including to more than 16 types of antibiotics.

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Resistance to methicillin is related to the gen *mecA*, which codifies the protein PBP2a that has low affinity to methicillin and to all β-lactamics[3]. Antibiotic resistance is of growing concern in medical and veterinary circles and is identified by the World Health Organization as a major human health issue for the future. MRSA, a zoonotic bacterium resistant to standard antibiotic therapies, has become increasingly prevalent since the 1970s and is now endemic in many countries[4] and even epidemic in some. Methicillin-resistant *staphylococci* have recently been isolated from livestock that have been fed with growth-promoting antibiotics, thereby acting as a possible reservoir of human infections[5]. Consequently, the search for novel antimicrobial agents is critical. MRSA are inherently resistant to all β-lactam antibiotics, but some lineages (clones) have additionally evolved resistance to multiple antibiotic classes. Within the species, resistance to all known antibiotic classes has occurred due to mutation and horizontal gene transfer and this has led to anxiety regarding the future availability of effective chemotherapeutic options.

The prevalence of MRSA increased dramatically in many countries during the 1990s. In the UK, MRSA bacteraemia (as a percentage of *S. aureus* bacteriemia) rose from <2% in 1990 to 43% in 2001[6] and this trend was mirrored in other countries including the USA and Japan. These MRSA infections are often associated with systemic infections seeded from wounds surrounding indwelling devices such as venous catheters and stents. The burden of disease due to nosocomial MRSA infection is additional to that due to methicillin-sensitive isolates, however the former are associated with increased mortality, morbidity and length of stay. Investigations focused in the search of nanotechnology for the treatment of MRSA infections are continuously being held. Among the range of compounds whose bactericidal activity is being investigated, silver nanoparticles rise as a promising new antibacterial agent that could be helpful to confront this and other drug-resistant bacteria.

Different studies have established the bactericidal effect of nano-silver in Gram negative and Gram positive bacteria, but the bactericidal mechanism of this compound has not been clearly elucidated[7]. Morones *et al.* defined the antibacterial activity of silver nanoparticles in four types of Gram negative bacteria: *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa*, and *Salmonella typhi* and suggested that silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions[8].

Other groups determined a similar antibacterial activity in Gram positive bacteria, such as *Bacillus subtilis*[7], *S. aureus*[9], and *Enterococcus faecalis*[10], Silver nanoparticles have also been found to exert antibacterial activity against some drug-resistant bacteria[11,12]. However, limited information on the possible antimicrobial activity of Ag and ZnO nanoparticles are available[13] and the mechanism[14] of action of the Ag/ZnO monocrystals is not yet fully established but nowadays, we know that the bactericidal effect of metal nanoparticles can be attributed to their small size, photocatalystic of activity and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution[8]. In spite of the fact, they have believed that residual these metal ions may be adversely affect human health, but scientists experiments demonstrated selectivity in the toxic nature of ZnO monocrystals to different bacterial systems and human T lymphocytes. These results suggested that ZnO monocrystals may potentially prove useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimens[15]. Also Jayesh, believed that combination of metal oxide nanocrystals may give rise to more complete bactericidal effect against mixed bacterial population[13].

The Purpose of this study was synthesis of ZnO and Ag nanocrystals mono-metallic and Ag/ZnO nanocomposites via thermal decomposition of oxalate precursor method, for first time. In addition, the antibacterial activities to Ag, ZnO and Ag/ZnO nanocomposites, against strains of *Staphylococcus aureus* (PTCC 1113) and Methicillin-resistant *Staphylococcus aureus* (MRSA), were procured from the Persian Type Culture Collection (PTCC) and Ardabil Medicine University; were compared and antibacterial effects of them were explored. The antimicrobial effect was determined based on the inhibition zone measured in the disk diffusion tests and in the

agar dilution tests conducted in plates also by determining the minimum growth inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of nanocrystals in liquid batch cultures. In one comparative study we have also scrutinized antibacterial conducted of nanocrystals before & after to ultrasonic frequency by ultrasonic set.

MATERIALS AND METHODS

Synthesis of ZnO monometallic nanocrystals via oxalate decomposition method

Zinc acetate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M white solution. The temperature was elevated to 50 °C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn:OA was 1. The system was kept at 50 °C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80 °C overnight. The dried Zinc oxalate was ground and calcined at 550 °C for 2 h.

Synthesis of Ag monometallic nanocrystals via oxalate decomposition method

Silver nitrate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was elevated to 50 °C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Ag:OA was 1. The system was kept at 50 °C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80 °C overnight. The dried silver oxalate was ground and calcined at 550 °C for 2 h.

Synthesis of Ag/ZnO nano-composites via oxalate decomposition method

Zinc chloride (MERCK, Germany) and silver nitrate (MERCK, Germany) were added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was raised to 50 °C and after 30 min of continuous stirring, oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn/Ag:OA was 1. The system was kept at 50°C under reflux for 2 h and a gray precipitate was obtained; then the resulting viscous gel was dried at 80 °C overnight. The dried Ag/ZnO oxalate was ground and calcined at 550 °C for 2 h.

Characterization

Experiences of dependent on the crystallinity of the nanoparticles were carried out using a X-ray diffractometer set (XRD, Bruker D8-Advance Diffractometer using Cu Kα radiation). Also the nanoparticles were digested and analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, LIBERTY–RL, Varian Australia Co.) for determining the presence of residual chemical element in the nanoparticles. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker spectrophotometer in KBr pellets. Surface morphology of product were characterized by using a scanning electronic microscopy (SEM, Cam Scan MV2300) with an accelerating voltage of 30 KV and TEM.

Disk diffusion test

Bacterial sensitivity to antibiotics is commonly inspected by a disk diffusion test, employing antibiotic impregnated disk[16]. A comparable examination with nano-composites and mono-metallic nanocrystals loaded disks was utilized in this research. A 10 ml suspension of each nanoparticles (approximately, 16384 μgml^{-1}) was prepared into the Muller Hinton Broth medium (MERCK, Germany) and then suspension of each nanoparticles was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute, subsequently filtered through a membrane filter (0.2 µm, 15 mm diameter ShimieRasanTeb). The nanoparticles laden filter paper was dried in an oven for 1 h and small disks of uniform size (6 mm diameter) containing 16384 μgml^{-1} nanoparticles were punched out and stored in a desiccator at room temperature. For each type of the bacterial inoculums (1.5×10^8 $CFUml^{-1}$) were cultured completely on the surface of a Muller Hinton agar plate (MERCK, Germany) before placing the disks on the plate. The plates were incubated at 35 °C for 24 h, after which the average diameter of the inhibition zone enclosing the discs was measured with a ruler with up to 1 mm resolution. The examination were also replicated, without sonication and so the results were compared together. Subsequently, the tests were reported for each type of nanoparticles and with each microbial strain on three replicates.

Agar Dilution test

A 16384 μgml^{-1} suspension of each nanoparticles was prepared into the Muller Hinton Broth medium, approximately, and then of each nanoparticles was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute. For each type of the bacterial inoculums (1.5×10^8 CFUml^{-1}) were cultured completely on the surface of a Muller Hinton agar plate before excavating the cavity on the plate. Then 100 μgml^{-1} from suspension of each nanoparticles was filled into the cavities. The plates were incubated at 35 ^{o}C for 24 h, after which the average diameter of the inhibition zone enclosing the cavities was measured with a ruler with up to 1 mm resolution. The examination were also replicated, without sonication and so the results were compared together. Subsequently, the tests were reported for each type of nanoparticles and with each microbial strain on three replicates.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The lowest concentration of material that inhibits the growth of an organism was defined as the minimum inhibitory concentration[17] (MIC). From the serial dilution method, was employed for determine the MIC of the nanoparticles[18]. Each of the twelve test tubes was filled with 1ml of the liquid Muller Hinton broth medium. Into each of the test tubes number 1 and 2, one ml solution containing 16384 μgml^{-1} of nanoparticles that had been sonicated at room temperature and frequency of 28 KHz, during at the 10 minute, already, was added and mixed thoroughly with the culture medium. The concentration of nanoparticles in each test tube become 8192 μgml^{-1}. Then, 1 ml of the content of test tube number 2 was added to test tube number 3 and mixed completely. This process was performed serially to test tube number 16. Consequence, 1 ml content of test tube number 16 was discarded. In order to have equal amounts of material in all the test tubes, 0.9 ml of test tube number 1 was discarded. Finally, 0.1 ml of standard microbial suspensions (*Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA)) containing 1.5×10^8 CFUml^{-1} microorganism, were added to test tubes number 2 to 17, and the test tubes were incubated at 35 $^{\circ}$C for 24 h. Then, the microbial growth was studied by turbidimetric measurement, using a spectrophotometer[19] (Nano-volum spectrophotometer, Scandrop 250, Analytikjena Co.). The experiments also included a positive control (test tube containing nanoparticles and Muller Hinton broth medium, devoid of inoculum) and a negative control (test tube containing inoculum and Muller Hinton broth medium, devoid of nanoparticles). The negative controls indicated the microbial growth profile in the absence of nanoparticles[14,20]. All the experiments were carried out in triplicate. The minimum bactericidal concentration (MBC), i.e., the lowest concentration of nanoparticles that kills 99.9% of the bacteria was also determined from the batch culture studies. For growth inhibitory concentration (MIC) the presence of viable microorganisms was tested and the lowest concentration causing bactericidal effect was reported as MBC as suggested by Avadi[21]. To experiments for bactericidal effect, a loopful from each test tube (Specially, negative & positive test tubes) was inoculated on Muller Hinton agar and incubated at 35 $^{\circ}$C for 24 h. The nanoparticles concentration illustrating bactericidal effect was picked out based on absence of colonies on the agar plate. The release of Ag^+ and Zn^{2+} ions from the nanoparticles into DI water and Muller Hinton broth medium was deliberated by suspending 10 mg of nanoparticles in 100 ml DI water/medium and sonicating with ultrasonic set (PARSONIC 7500s, Pars Nahand ENGG. Co. IRAN) for 10 min. The suspension was kept in a rotary shaker (Gyrotwister 3-Dshaker, labnet Co. USA) under the same conditions as in the above studies and residual Ag^+ and Zn^{2+} concentration in the aqueous phase was defined by ICP-AES after 24 h.

RESULTS

The FT-IR spectra analysis

Fig. 1 shows FT-IR spectra of (a) Ag, (b) ZnO and (c) Ag/ZnO . The supplement of oxalic acid to the ethanol solution of Ag cation was cause to the precipitation of a gray solid of silver oxalate as shown by FT-IR spectrum in Fig. 1a. The broad band at 3427.33 cm^{-1} was allocated to both the ν_s(O-H) and ν_{as}(O-H) of hydration water. The extreme band at 1634.68 cm^{-1} was allocated to asymmetric and water tensional tremble δ(H-O-H). The shoulder at 1428.89 cm^{-1} is present in the spectrum

Table 1. Disc diffusion Test (μgml^{-1}), Agar dilution Test (μgml^{-1}), MIC (μgml^{-1}) and MBC (μgml^{-1}) of silver, zinc oxide and silver/zinc oxide nanoparticls for various microorganisms

	Nanoparticles	Disc diffusion Test (DIZ)	Agar dilution Test (DIZ)	MIC	MBC
Meticillin-resistant	Zn	12mm	13mm	512μg/ml	4096μg/ml
Staphylococcus aureus	Ag	15mm	14mm	32μg/ml	512μg/ml
(*MRSA*)	Ag/Zn	18mm	12mm	8μg/ml	128μg/ml
Staphylococcus aureus	Zn	12mm	15mm	256 μg/ml	2048 μg/ml
	Ag	8mm	10mm	1024 μg/ml	4096 μg/ml
	Ag/Zn	12mm	10mm	128 μg/ml	2048 μg/ml

evidence of (N-O) tremble and the closely spaced bands at 875.31 cm^{-1} and 577.35 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (M-O) tremble, respectively.

Fig. 1b depended to ZnO FT-IR spectrum. The broad band at 3445.05 cm^{-1} was allocated to both the ν_s(O-H) and ν_{as}(O-H) of hydration water. The extreme band at 1629.57 cm^{-1} was allocated to asymmetric and water tensional tremble δ (H-O-H). The shoulder at 1428.89 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 876.14 cm^{-1} and 551.12 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble respectively. Also, Fig. 1c conclude Ag/ZnO FT-IR spectrum.

The broad band at 3426.47 cm^{-1} was allocated to both the ν_s(O-H) and ν_{as}(O-H) of hydration water. The extreme band at 1628.14 cm^{-1} was allocated to asymmetric and water tensional tremble δ(H-O-H). The shoulder at 1458.22 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands 625.36 cm^{-1} are presents in the spectrum evidence of (Ag/ZnO) tensional tremble respectively.

The XRD spectra analysis

The XRD pattern of Ag, ZnO and Ag/ZnO nanoparticles (Fig. 2a, b and c) were compared and interpreted with standard data of International Centre of Diffraction Data (ICDD). The average

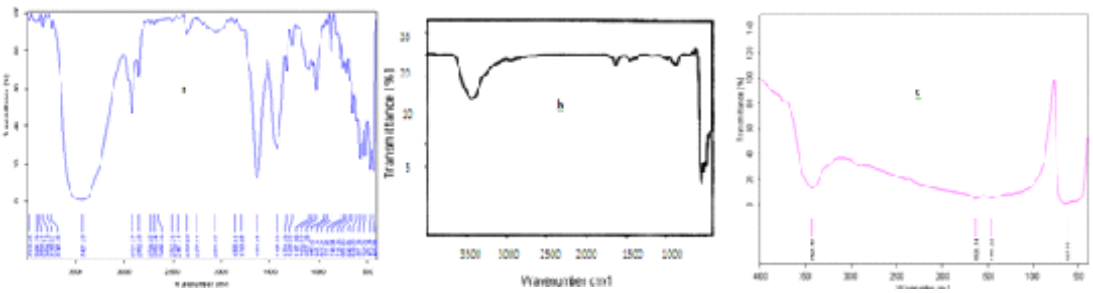

Fig. 1. FT-IR pattern of (a) Ag, (b) ZnO and (c) Ag/ZnOnanoparticls

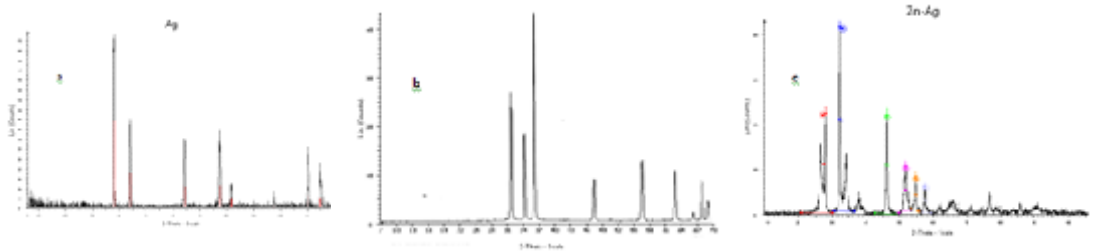

Fig. 2. XRD pattern of (a) Ag (b) ZnO and (c) Ag/ZnOnanoparticles

crystallite sizes (C.S) of the monocrystals were calculated using the Debye-Scherer Equation from the major diffraction peaks ($C.S = K.\lambda / \beta.\cos\theta$). Where K is a constant equal to 0.9 λ is the wavelength of Cu Kα radiation, β is the full width at half maximum (FWHM) of the diffraction peak in radiant and θ is the Bragg angles of the main planes. The average crystallite size of the Ag, ZnO and Ag/ZnO were 8.66 nm, 24.75 nm and 12.15 nm, respectively.

The ICP-AES spectra analysis

By ICP-AES analysis, we were succeed to estimation of residual ions, after digestion of nanoparticles by sonication. They indicated ions levels of 120 ppm to silver and ≤1 ppm to zinc oxide, in the silver/Zinc Oxide nanoparticles,

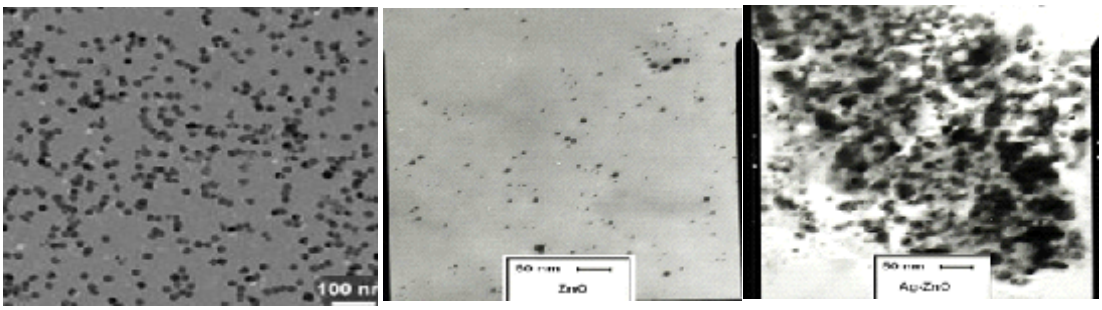

Fig. 3. TEM images of (a) Ag, (b) ZnO and (c) Ag/ZnOnanoparticles

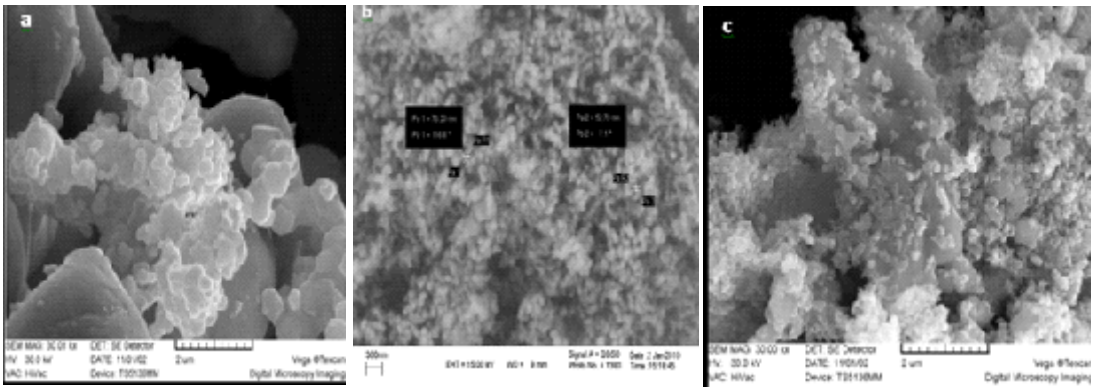

Fig. 4. SEM images (a) Ag, (b) ZnO and (c) Ag/ZnOnanoparticles

respectively.

The TEM and SEM images analysis

TEM image of silver nanoparticles were taken (Fig. 3) and approved that the metal particles were in the nano range, approximately. However, SEM images (Fig. 4) of nanoparticles were showed that silver; zinc oxide and silver/zinc oxide metal particles were exactly in the shape of spherical and clustered.

The antibacterial activity analysis

The antibacterial activity of silver, zinc Oxide and silver/zinc oxide nanoparticles was compared for *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA) using the diameter of inhibition zones in disk diffusion test and Agar dilution test. In fact, the diameter of inhibition zone (DIZ) reflects dimension of impressionability of the bacteria. We knew, the strains susceptible to disinfectants demonstrate larger DIZ, while resistant strains exhibit smaller DIZ. The disks with silver and Zinc oxide nanoparticles were compared to the silver/zinc oxide nanoparticles for all strains selected for this study. The DIZ for zinc oxide and silver/zinc oxide nanoparticles impregnated disks was almost greater than that studied with the silver nanoparticles impregnated disks for all the strains selected for this study.

Correspondingly, for Methicillin-resistant *Staphylococcus aureus* (MRSA) the silver and silver/zinc oxide nanoparticles impregnated disks were found to be more effective compared to zinc oxide nanoparticles impregnated disks, however the difference in the DIZ was merely 10–15% percent. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exist. Also, by contrast of size DIZ was measured on agar plates, before and after of sonication, were discovered that ultrasonic waves have not any efficacy on antibacterial feature of nanoparticles. The results in depended of the antibacterial effects of nanoparticles against different of bacterial via the disc diffusion test, the agar dilution test the MIC and the MBC are summarized in Table 1. A greater lag phase and lower maximum absorbance (at 600 nm) were observed as the concentration of nanoparticles increased. Similar observation was reported by Sondi and Salopek-Sondi[22]. We analysed effectivity of silver, Zinc oxide nanoparticles and silver/zinc oxide nanocomposite against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA). The bactericidal effect of nanoparticles is dependent on the concentration of nanoparticles and the initial bacterial concentration[23]. In this study, the initial bacterial concentration was constant at 1.5×10^8 CFU ml^{-1} regardless of nanoparticles concentration and microbial strain. The MIC observed for zinc oxide nanoparticles were 512 μgml^{-1} for Methicillin-resistant *Staphylococcus aureus* (MRSA) and 256 μgml^{-1} for *Staphylococcus aureus.* Surprisingly, antibacterial effect of the silver nanoparticles were weak for *Staphylococcus aureus*. The MIC observed for silver nanoparticles were 1024 $\mu g\ ml^{-1}$ for *Staphylococcus aureus* and 32 μgml^{-1} for Methicillin-resistant *Staphylococcus aureus* (MRSA). In contrast with the nanoparticles that picked out for this study, the most antibacterial effect was seen to silver/zinc oxide nanocomposite. Interestingly, the Methicillin-resistant *Staphylococcus aureus* (MRSA) was most sensitivity against of silver/zinc oxide nanocomposite. In fact, our research shows that silver/zinc oxide nanoparticles have got antibacterial effects against Methicillin-resistant *Staphylococcus aureus* (MRSA). The MBC observed in this study for silver/zinc oxide nanocomposite were 128 μgml^{-1} for Methicillin-resistant *Staphylococcus aureus* (MRSA) and 2048 μgml^{-1} for *Staphylococcus aureus.*

DISCUSSION

Gan strongly believed that colloidal and agglomerated nanoparticles may affect its ability in inhibiting or destroying bacteria and also influence the degree of MIC and MBC[24]. Regarding this theory, Guogang and Jayesh exposed the suspension of nanoparticles in liquid medium to the ultraviolet waves for 10 minutes to let them out of agglomeration and being dispersed and suspended[13,14]. Several studies performed by many authors on the antibacterial properties of Ag nanoparticles in colloidal phase[13,14,21,22,25]. However, no comparison reported on the antibacterial rate of metal nanoparticles, in both agglomerated and dispersed states. One of the aims of our study would be the examining and comparing of the rate of antibacterial effects of understudy nanoparticles - pre and post - exposed with ultrasonic waves, against standard strain of *Staphylococcus aureus*, also against strain of Methicillin-resistant *Staphylococcus aureus* (MRSA) using disc diffusion and agar dilution methods by sonicator machine. Data of the study indicated that in spite of the theories of authors like Gan, Guogang and Jayesh, the antibacterial effects of metal oxide nanoparticles against bacteria in colloidal or agglomerated phase showed no meaningful difference with unagglumerated phase and also the diameter of inhibition zone (DIZ) in plate was not significant[13,14,24]. Studies of Jayesh indicated that in aqua medium, no systematic change in the size of nanoparticles observed after 24 h. In the current study, after synthesis of nanoparticles of metal oxides Ag, ZnO and combined nanoparticles of Ag/ZnO, their antibacterial effects compared. Though, studies of several authors in recent years, confirmed the antibacterial effects of Ag nanoparticles[22,26,27]. In the current study, disc diffusion and agar dilution methods used for determining the antibacterial effects of nanoparticles. Jayesh performed extensive experiments in determination of microbial sensitivity of various bacteria to silver and copper nanoparticles, using disc diffusion method[14]. Regarding that the diameter of inhibition zone

(DIZ), reflects the sensitivity of the organism, strains of sensitive, show larger DIZ and the resistant strains show smaller DIZ. Results of the disc diffusion with Ag nanoparticles, by Thirumurugan against strains of pathogens *Escherichia coli, salmonella typhi, Bacillus subtilis, Staphylococcus aureus*, indicated higher sensitivity to silver nanoparticles which is in contrast with the results of our study[28]. Cho studies the MIC of Ag nanoparticles against *Pseudomonas aeruginosa* bacteria in which its growth in concentration of 7.5 μgml^{-1} completely inhibited[29]. Following their research project, cho reported the MIC rate of silver nanoparticles for *S. aureus* as 12.6 μgml^{-1}, but the MIC for this bacteria affected by silver nanoparticles reported[29] 1024 μgml^{-1}.

The *Staphylococcus aureus*, used in the current study, showed the least and the most sensitivity to silver and Ag/ZnO nanoparticles, respectively. Actually, the least degree of MIC in *Staphylococcus aureus* was related to combined nanoparticles of silver and zinc with concentration of 128 μgml^{-1}. i.e, not withstanding this nanoparticles had the most growth inhibitory effect in Methicillin-resistant *Staphylococcus aureus* (MRSA). Lock concluded that the most the dimension of silver nanoparticles, the better would be the MIC and their antibacterial effect would decrease[26]. The aim was to indicate that the sensitivity of different strains of one bacteria to silver nanoparticles shows meaningful difference. Kim studied the gram negative bacteria *E. coli* and gram positive *S. aureus,* also reported that the antibacterial silver nanoparticles mostly affects the *Escherichia coli,* which is due to the difference between cell wall of gram negative & positive microorganisms[25].

Rate of MIC obtained by Ping Li[30] for silver nanoparticles and against *S. aureus* were 0.625 μgml^{-1}. Limited studies performed on the antibacterial properties of ZnO. Reddy were amongst few authors worked on the toxicity of the ZnO nanoparticles in gram negative & positive bacteria[15]. They found that this nanoparticles are able to completely inhibit the growth of *E. coli.* Another idea presented in the study was processing of combined metal oxides of nanoparticles with antibacterial effects and the examining and comparing their antimicrobial effects. As already mentioned, for the first time, processed the Ag/ZnO combined metal oxide nanoparticles and following them, Guogang presented the theory of using ZnO combined metal nanoparticle for obtaining a more resistant antibiotic against methicillin resistant *S. aureus* (MRSA)[13]. According to their studies, strains of gram negative bacteria showed higher resistance to copper oxide nanoparticles combined with silver. However, Jayesh, suggested that combining of copper and silver nanoparticles may lead to the increased bactericidal effects[13]. Framework of the idea, formed performing the scientific study in the format of a project. Up to now, no complete and comprehensive study reported in the field of combining antibacterial nanoparticles and the comparison of their antibacterial properties between on *S. aureus* and Methicillin-resistant *S. aureus* (MRSA). Amongst few studies, Ling Yang, combined silver nanoparticles with Zn to improve antibacterial activity of Zn nanoparticles and investigate the antibacterial effect of Zinc oxide and silver nanoparticles and also comparing them with Ag/ZnO nanoparticles[31]. They obtained interesting results.

According to the findings of Kawashita and Pak-soo silver significantly increased antimicrobial activity[32,33]. Actually, Ling Yang believed that photocatalytic ability of ZnO nanoparticles plus silver nanoparticle improves and also increases its oxidation and reduction abilities, while suppressing bacteria growth[31]. However, silver ions, eventually release during sterilization and kill bacteria due to their high antibacterial activation. They theorized that silver ions release following bacteria death and colloid with other bacteria and repeat their sterilization behavior. It was also mentioned that silver covered in the surface of Zn nanoparticles has the ability to involve the electrons produced through photocatalytic reactions of Zn nanoparticles which increases electron isolation and makes gaps in cell membrane, so increase its antimicrobial activity. Regarding studies of these authors, antibacterial property of silver and zinc oxide nanoparticles improves with their combination. In fact, our study confirmed that the gram negative strains of bacteria had most sensitive to silver/zinc oxide nano-composite. Further, out study approved that combination of zinc oxide and silver nanoparticles, increased their bactericidal effect.

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