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Antimicrobial Activity of SnO_2 Nanoparticles against Escherichia Coli and Staphylococcus Aureus and Conventional Antibiotics

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Abstract

The prepared SnO_2nanoparticles solution sample was employed for the inactivation of Gram-negative Escherichia coli (ATCC 25922) and Gram-positive Staphylococcus Aureus (ATCC 29213). The antibacterial activity of the synthesized SnO_2 nanoparticles was evaluated using zone inhibition method. Antibacterial sensitivity of SnO_2 nanoparticles in different dilution and conventional antibiotics were tested. 5 and 10 times of SnO_2 nanoparticles solution shows strong inhibitory zone against E-coli and S. Aureus than conventional antibiotics Gentamycin and Nalidixic acid. Nalidixic acid gives no inhibitory zone against E-coli. The results showed that diameters of inhibited zones of different concentration SnO_2 nanoparticles against Staphylococcus Aureus presented good antibacterial performance than Escherichia coli. As the amount of the nanoparticles in the solution decreases, antibacterial activity decreases.SnO_2 nanoparticles show almost equivalent sensitivity like the conventional Gentamycin antibiotics and Nalidixic Acid antibiotic shows no resistance in E. Coli. SnO_2 nanoparticles show more sensitivity than the conventional Gentamycin and Nalidixic Acid antibiotics in S. Aureus.

Keywords: Antimicrobial activity; SnO_2 Nanoparticles; Escherichia Coli; Staphylococcus Aureus; Conventional Antibiotics.

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1. Introduction

With the outbreaks of infectious diseases caused by pathogenic bacteria and the rise of antibiotic resistance of bacteria [1], much attention in pharmaceutical and medical fields has been focused on creating new antibacterial agents [2]. In recent years, nano-scaled antibacterial materials as novel antimicrobial species have been seen as promising candidates for application owing to their high surface-to-volume ratio and their novel physical and chemical properties on the nano-scale level [3, 4].

Many kinds of nanometre-sized antibacterial materials such as TiO2, ZnO, MgO, chitosan, calamine, copper and silver have been reported on in this area [5–11]. Among them, nanocrystalline silver has been proved to be the most effective antimicrobial agent [12] since silver and its compounds have powerful antimicrobial capability [13] and broad inhibitory biocidal spectra for microbes including bacteria, viruses and eukaryotic microorganisms [14–16].

However, the removal of bacteria from water is an extremely important process for drinking and sanitation systems especially against concerns on growing outbreaks of water borne diseases [15]. In the United States, only between 2003 and 2005 there were four reported waterborne disease outbreaks attributed to pathogens in drinking water affecting 282 people [16]. Conventional methods for disinfection of water are dependent on chemical agents, that are ineffective against cyst-forming protozoa such as Giardia and Cryptosporidium and also these methods often produce harmful by-products. Nanotechnology is considered as a new generation of technology that can have a great impact on economies through new consumer products, manufacturing methods and materials usage [17]. This technology can lead to cost effective and high performance water treatment systems [18]. By the use of nanotechnology, implementation of oligodynamic nanoparticles for water disinfection is being explored. Oligodynamic nanoparticles based disinfection includes the use of metals such as silver, gold, zinc, tin and copper due to their antimicrobial properties. Large band gap semiconductors, such as TiO_2, SnO_2, SiO_2 and ZnO are suitable photocatalytic materials [19-21]. Among these Tin oxide (SnO_2) is an important n-type metallic oxide semiconductor with wide band gap (3.6 eV). Because of its unique electronic, optical, electrochemical and catalytic properties, SnO_2 were extensively used in solar cells, transparent conducting electrodes, solid-state sensors, rechargeable Li batteries and optical electronic devises [22, 23]. The conductivity and optical properties of SnO_2 are largely dependent on the particle size and shape of the nanocrystallites [24-26].

The antibacterial properties of copper, silver, have also been widely utilized in advanced coating technologies, such as the design of materials for biomedical devices, hospital equipment, food processing and storage equipment, household materials, and antifouling paints. There have also been several reports on the antimicrobial activities of metal nanoparticles. However, there are still challenges such as the instability of the nanoparticles, control of their size and shape, uniform dispersity in a matrix, and control of the release rate.

Finally, the biological activity of SnO_2 nanoparticles has been investigated against a gram negative bacterium, E. Coli (ATCC 25922) and gram positive bacterium, Staphylococcus Aureus (ATCC 29213) using zone inhibition method and the antibacterial activity of the nanoparticles have been compared with conventional

antibiotics Gentamycin and Nalidixic acid.

2. Materials and Methods

2.1. Materials

SnO_2 nanoparticles solution, E. Coli strain (ATCC 25922), Staphylococcus Aureus strain (ATCC 29213), sterile Nalidixic acid antibiotic, Gentamycin antibiotic, Mueller Hinton Agar were used as received without further purifications.

2.2. Equipments

Sterile Petri plates, sterile Cotton Swab, Incubator, Autoclave, Millimeter ruler, Conical flask, Microoven, Micropipettes, Marker, 6 mm blank filter paper.

2.3. Preparation of sterile nutrient Agar plate

Suspend 3.8 g Mueller Hinton Agar in 100.0 mL distilled water. Heat in a Microoven to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. Cool to 45-50^0C. Mix well and pour into sterile Petri plates.

2.4. Methods

Antibacterial activity of the SnO_2 nanoparticles was evaluated using Zone Inhibition Method.

2.4.1. Antimicrobial Activity measurement

Chemical substances that either kill bacteria or inhibit bacterial/viral growth are called *antimicrobial agents.* The effectiveness of each type of antimicrobial agent is influenced by many factors. Some of these factors include the environmental conditions in which the agent is applied, the chemical properties of the agent, how long the agent has been stored, and the rate of deterioration of the agent.

In this investigation, we have tested the effectiveness of one disinfectant (SnO_2 nanoparticles in solution in two dilution 5 and 10 times) on the growth of bacteria (E. Coli and Staphylococcus Aureus).

Part A: Inoculating a Sterile Nutrient Agar Plates

The following steps are involved in the inoculation of a sterile nutrient agar plates.

1. One sterile nutrient agar plate was obtained. It was noted that the agar was in the bottom of the Petri dishes**.** The Petri dish agar side was turned up on the worktable.

2. With a marker pen, the bottom of Petri dish was marked as shown in Figure 1.

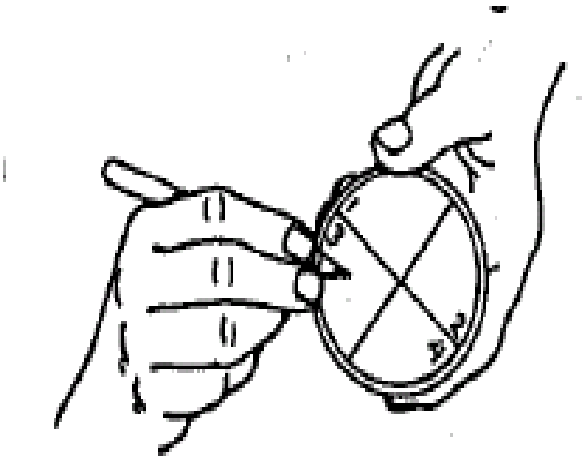

Figure 1: Marking up the bottom of perti dish.

3. Using sterile techniques, one sterile nutrient agar plate was slightly opened. Using a cotton swab the plate was streaked with bacteria and nutrient agar Petri dish became inoculated with as shown in Figure 2.

Figure2: Inoculating a sterile nutrient agar plate.

Part B. Controlling the Spread of the Bacterium with Disinfectants

The steps involved in controlling the spread of the bacterium with disinfectants are:

1. Sterile 6 mm filter paper blank disc and Nalidixic acid and Gentamycin disc were placed in different zones of Petri dish. 100 µL of SnO_2 nanoparticles diluted solutions (5 and 10 times dilution) were dropped in 6 mm filter paper blank disc of different zones of Petri dish.

2. The Petri dish was taped close with transparent tape. The Petri dish was turned upside down (agar side faces up) and was placed in a 37 ^{o}C in a incubator.

3. After 48 hours incubation, the zones of inhibition around each drop were measured in millimeters and recorded.

3. Results and Discussion

3.1. Antibacterial sensitivity of SnO_2 nanoparticles

Antibacterial sensitivity of SnO_2 nanoparticles in different dilution and conventional antibiotics were tested using zone inhibition method. The diameter of zone inhibition, expressed in millimeter, has been summarized in Table 1. Figure 3 shows photographically antibacterial test of SnO_2 nanoparticles of different concentration and conventional antibiotics in zone inhibition method. Figures 4 and Figure 5 show diameters of inhibited zones against *E. Coli* and Staphylococcus Aureus of different concentration SnO_2 nanoparticles respectively.

Table 1: Diameter of zone inhibition of SnO_2 nanoparticles at different dilutions and conventional antibiotics

Disinfectants		Concentration	Strains	Diameter of Zone inhibition (mm)
SnO_2 nanoparticles		5x 10x	E. coli S. Aureus E. coli S. Aureus	9 15 6.5 13
Antibiotics	Gentamycin		E. coli S. Aureus	6 No zone
	Nalidixic Acid			6
	Gentamycin			4.5
	Nalidixic Acid			

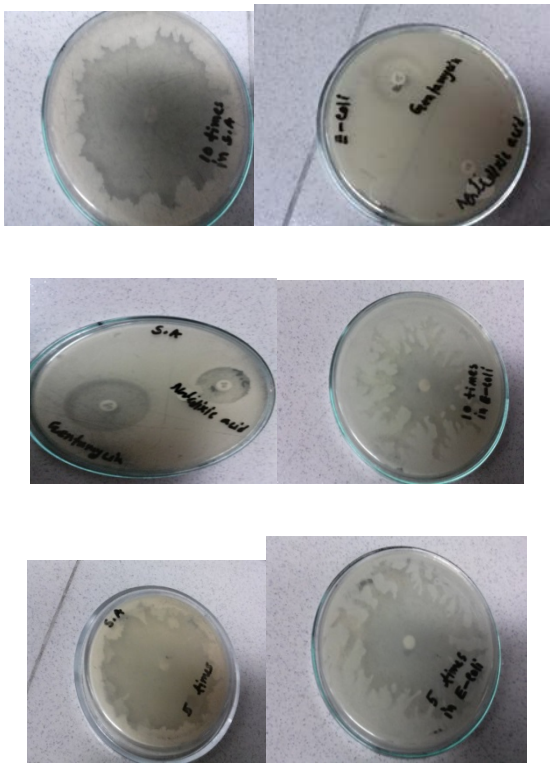

Figure3: Photographs of antibacterial test of SnO_2 nanoparticles of different concentration and conventional antibiotics in zone inhibition method

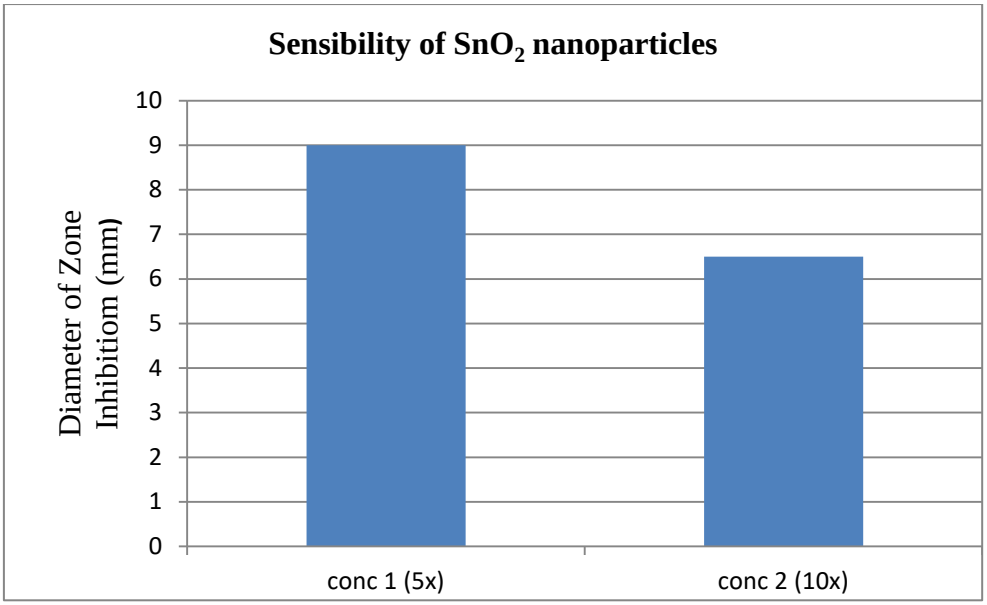

Figure 4: Diameter of inhibited zone against E. Coli of different concentration of SnO_2 nanoparticles.

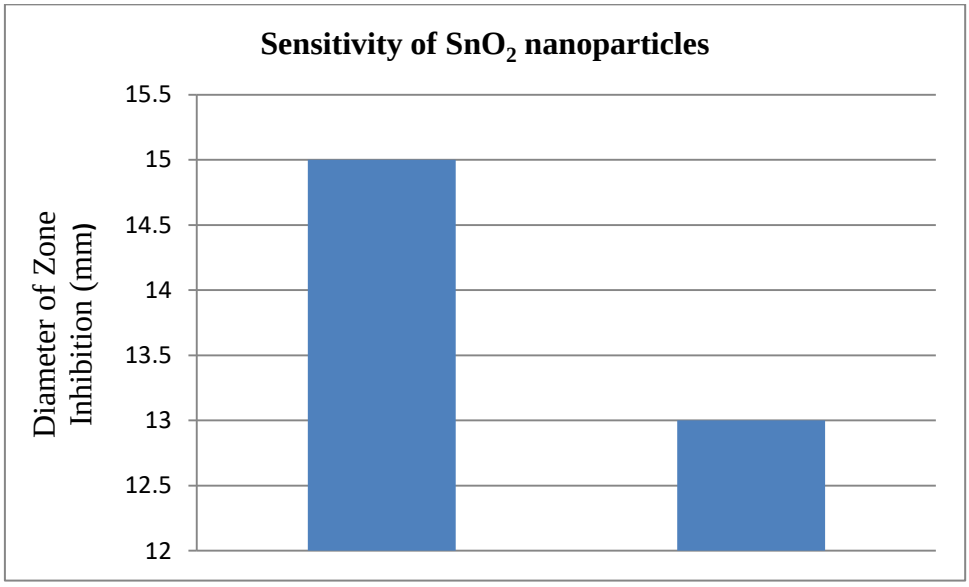

Figure 5: Diameter of inhibited zone against S. Aureus of different concentration of SnO_2 nanoparticles.

3.2. Antibacterial Activity of conventional antibiotics

Antibiotic discs are often used to determine if a particular bacterium is susceptible to a type of antibiotic. The bacteria are grown on a dish and discs saturated with different antibiotics are placed on top of the growing bacteria. If the antibiotic works successfully, a clear ring will appear around the disc in 24/48 hours. The ring is called the zone of inhibition. Zone of inhibition is the area around a paper disk or colony of bacteria or mold where no other organisms are growing It is measured in mm to see how wide it is. The larger this zone of inhibition, the more effective that antibiotic is against that particular type of bacteria. Figure 6 and Figure 7 show antimicrobial sensitivity of Gentamycin and Nalidixic Acid antibiotics against E. Coli and Staphylococcus

Aureus respectively.

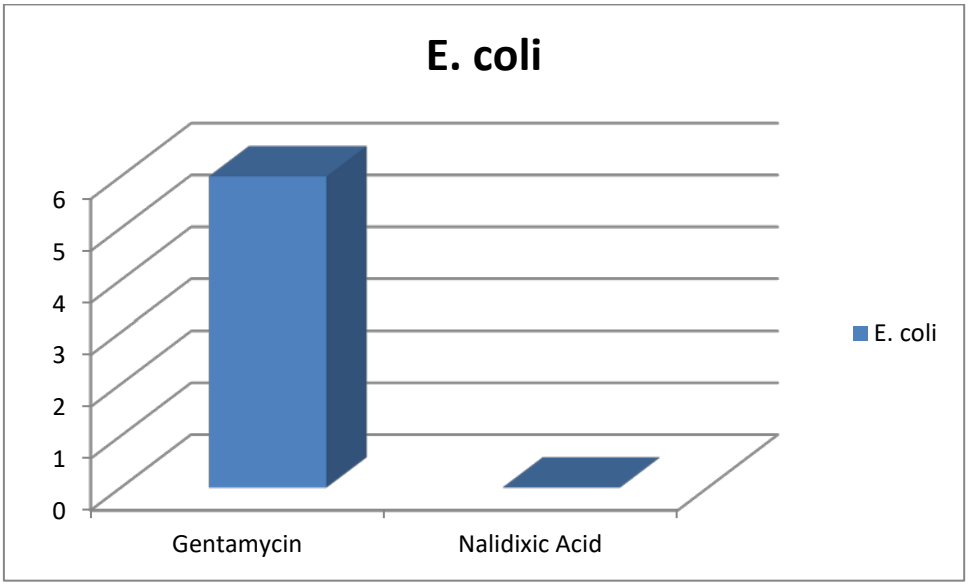

Figure 6: Antimicrobial sensitivity of Gentamycin and Nalidixic Acid antibiotics against E. Coli .

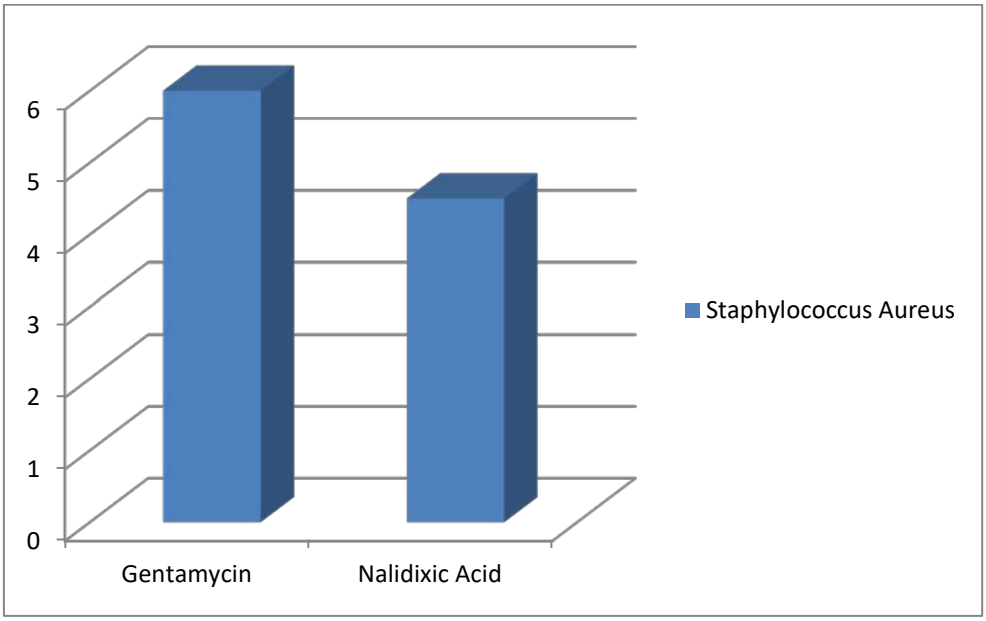

Figure 7: Antimicrobial sensitivity of Gentamycin and Nalidixic Acid antibiotics against Staphylococcus Aureus.

We have tested the antibiotic sensitivity of SnO_2 against Escherichia coli (E. Coli), a gram negative bacterium and Staphylococcus Aureus, a gram positive bacterium.SnO_2 shows a strong antibiotic activity against Staphylococcus Aureus where SnO_2 shows comparatively less antibacterial activity against E. Coli. Figure 8 compares the antibacterial sensitivity of SnO_2 nanoparticles in different dilution between E.coli and Staphylococcus Aureus. As the concentration decreases, antibacterial activity has been found to decrease gradually to infer that antibacterial activity has concentration dependence.

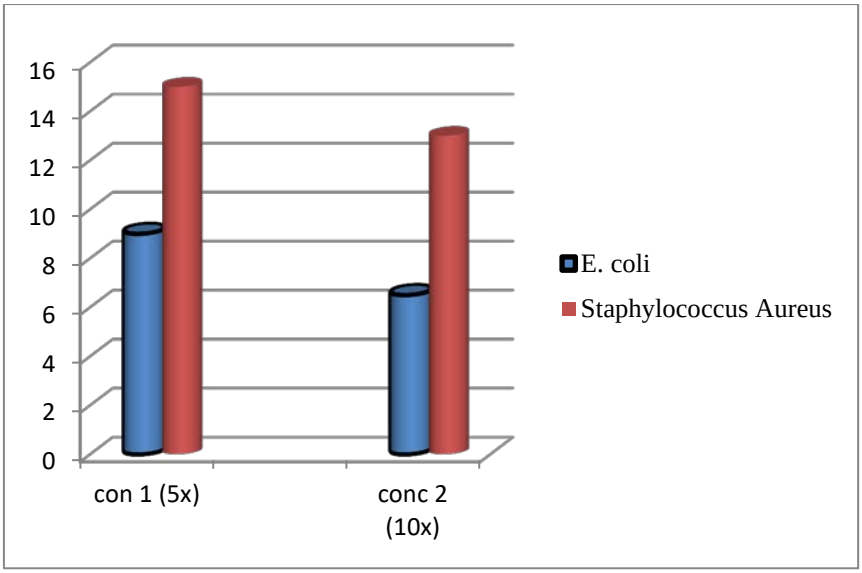

Figure 8: Comparison of antibacterial sensitivity of SnO_2 nanoparticles in different dilution between E.coli and Staphylococcus.

A comparison of sensitivity of SnO_2 nanoparticles with conventional antibiotics in E. Coli and S. Aureus respectively are also shown in Figure 9 and Figure 10. From Figure 9, it is clear that SnO_2 nanoparticles show almost equivalent sensitivity like the conventional Gentamycin antibiotics and Nalidixic Acid antibiotic shows no resistance in E. Coli. And from Figure 10, it is clear that SnO_2 nanoparticles show more sensitivity than the conventional Gentamycin and Nalidixic Acid antibiotics in S. Aureus.

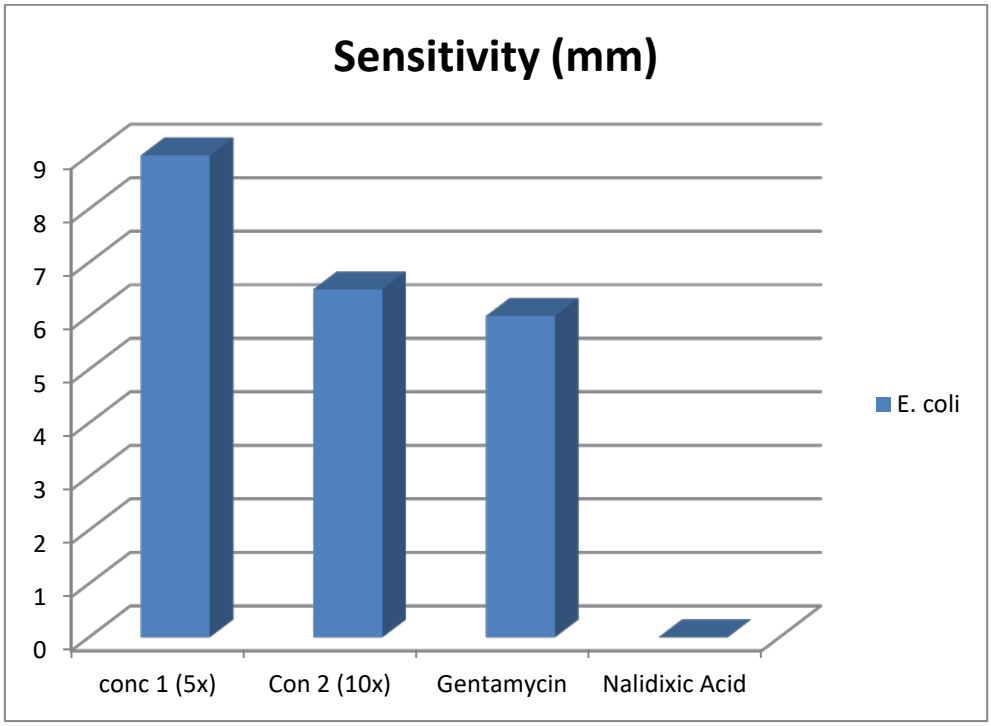

Figure 9: Comparison between SnO_2 nanoparticles and conventional antibiotics in E. Coli.

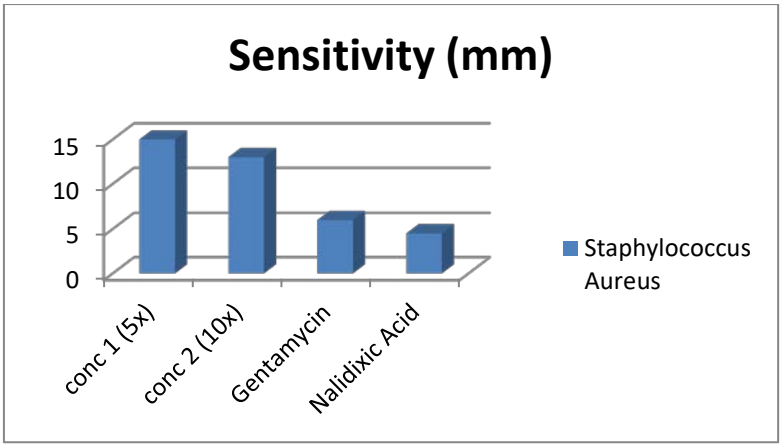

Figure 10: Comparison between SnO_2 nanoparticles and conventional antibiotics in Staphylococcus Aureus.

The antibacterial activity of silver and copper can be explained by *oligodynamic effect* .The *oligodynamic effect* (Greek oligos = few, dynamics = force) was discovered in 1893 by the Swiss Karl Wilhelm von Nägeli as a toxic effect of metal-ions on living cells, algae, molds, spores, fungus, virus, prokaryotic and eukaryotic microorganisms, even in relatively low concentrations. This antimicrobial effect is shown by ions of: mercury, silver, copper, iron, lead, zinc, bismuth, gold, aluminum and other metals. Bacteria (Gram-positive and Gram-negative) are in general affected by the oligodynamic effect, The toxic effect is fully developed often only after a long time (many hours). Elucidation of the mechanism of bactericidal action of nanoparticles is still underway. It is difficult to distinguish between the bactericidal activities of nanoparticles from that of the ions released by the nanoparticles [27]. The nutrient media facilitated the release of Sn^{4+} ions. The presence of nanoparticles in solution would ensure continuous release of ions into the nutrient media. Sn^{4+} released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [28]. Cho and his colleagues reported that the surface of the cell walls of E. coli treated with SnO_2 nanoparticles were severely damaged compared to untreated *E. coli*. Cell wall rupture due to Sn^{4+} nanoparticles was reported by Lok and his colleagues The attachment of Sn^{4+} ions or nanoparticles to the cell wall caused accumulation of envelope protein precursors, which resulted in dissipation of the proton motive force. SnO_2 nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP. The mode of action of SnO_2 nanoparticles and Sn^{4+} ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentrations than that of the ions. However, Morones and his colleagues proposed that the bactericidal mechanism of SnO_2 nanoparticles and Sn^{4+} ions are distinctly different. The activity of biocatalysts like colloidal SnO_2 is directly proportional to the adsorption power upon a biological surface .The nanoparticles were found to penetrate through the cell wall. For E. coli SnO_2 nanoparticles demonstrated greater bactericidal efficiency compared to conventional antibiotics such as Gentamycin, Nalidixic Acid etc. for bactericidal effects on E. coli.

4. Conclusion

The antibiotic sensitivity of SnO_2 nanoparticles solution and conventional antibiotics Gentamycin and Nalidixic Acid against Escherichia coli (E. Coli) and Staphylococcus Aureus (S. Aureus) have been examined. SnO_2

shows a strong antibiotic activity against Staphylococcus Aureus where SnO_2 shows comparatively less antibacterial activity against E. Coli. As the concentration decreases, antibacterial activity has been found to decrease gradually to describe that antibacterial activity has concentration dependence. SnO_2 nanoparticles show almost equivalent sensitivity like the conventional Gentamycin antibiotics and Nalidixic acid antibiotic shows no resistance in E. Coli. SnO_2 nanoparticles show more sensitivity than the conventional Gentamycin and Nalidixic acid antibiotics in S. Aureus.

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