**In vitro** study of the effect of zinc oxide nanoparticles on *Streptococcus mutans* isolated from human dental caries

Arshad Mahdi Hamad¹* and Qanat Mahmood Atiya²

¹Department of Biology, College of Science, Tikrit University, Iraq.

*E-mail: arshadmnh1995@gmail.com

**Abstract.** Dental caries is a public health concern worldwide for which *Streptococcus mutans* has been reported Known as the potential etiology of infection. In recent years, nanotechnology has applied to Creation of novel material properties. The research was studying the effects of Nanoparticles of Zinc Oxide on *Streptococcus mutans* isolate from dental caries. In this research, Different ZnO NPs concentrations were studied as its anti-bacterial effects on *Streptococcus mutans* was isolated from dental caries cases. 27 *Streptococcus mutans* isolates were obtained, which is equivalent to 20% of the total number of 135 isolates. This nanomaterial was chosen because it has a high affinity for human cells and does not cause harm compared to other nanoparticles. The nanoparticles are those very fine materials that can be produced so that their dimensions or the dimensions of their grains range from (1-100) nanometers, and because of the small size of the nanomaterials, which made them behave differently from large-sized materials whose size exceeds 100 nanometers, and because of the small size of the particles. Nanoparticles, which gave them electrical and magnetic properties that differ from large particles of the same compounds. The well diffusion method and the MIC experiment were conducted, and the results were good, as in the well diffusion method the efficacy increased with increasing the concentration of ZnO NPs. In the MIC experiment, it was observed that MIC in the sixth tube, meaning that the MIC of ZnO NPs towards *Streptococcus mutans* equals 0.312 mg/ml.

**Keywords.** ZnO NPs, *Streptococcus mutans*, MSB agar, Inhibition Zone, Dental caries.

1. Introduction

The Nanoparticales effects on human health are quite different from the macromolecules they are made from [1]. The increased nanoparticles activity may have a beneficial advantage or harmful effect, or both at the same time[2,3]. In recent times, Nanotechnology has attracted the world's attention greatly due to its characteristics compared to the giant molecules from which it is derived. The nanoparticles of zinc oxide, copper oxide and silver are used extensively industrially, including textile industries, dyes, motor oils, cosmetics, detergents and sprays [4]. A common feature of these nanocomposites is antimicrobial activity[ 5]. The antimicrobial effects of nanoparticles on pathogenic bacteria such as *E. coli, S. aureus* have been extensively studied[6]. The antimicrobial behavior of inorganic oxide metals particles such as magnesium oxide, titanium oxide, silicon oxide, titanium oxide and zinc oxide indicate that they can be used as antimicrobial treatments as possible. Use them
surgically under the heading of so-called nanomedicine [7]. One of the advantages of using inorganic oxide nanoparticles as antimicrobial agents is that they are highly effective against antibiotic-resistant pathogenic strains and are more heat resistant and less toxic [8]. ZnO NPs have a very good safety and non-toxicity profile was watched while taking different nanoscale sizes of the particles of zinc oxide [9]. Tooth decay is among the most famous oral diseases in the oral cavity in humans [10]. It is one of the main oral problems that affect the health of all ages (children, adolescents, adults, the elderly) [11]. Streptococcus mutans, one of the natural flora in the mouth, is the main factor causing tooth decay and has the ability to form biofilm, and it is one of the most popular types of bacteria in this aspect [12]. The aim of this study is to investigate the direct effect of nanoparticles of zinc oxide on Streptococcus mutans that cause tooth decay.

2. Materials and Methods

2.1. Samples collection and isolation of bacteria

The samples were collected from the surfaces of the decaying teeth of the 135 patients attending the dental caries clinics at Tikrit city from both sexes, with different age stages between 8 to 65 years. The samples were collected using a sterile cotton swab, where the focus of this research was on isolating and diagnosing two genera of bacteria: Staphylococcus and Streptococcus, where a portion of the bacterial culture for each sample was transferred to Mannitol salt agar and MSB agar base in a plate by streaking on the surface of agar. The plates were then incubated for 24 hours at 37°C, with leaving one plate from each medium as a control plate to detect the presence of contamination in the preparation of the medium. All the plates were diagnosed on the second day and the colonies growing on the agricultural media were diagnosed according to their phenotypic characteristics in terms of diameter, color, texture, shape, and edge, then the characteristics of the cells and their shapes after staining them with a Gram stain as belonging to the genera Staphylococcus and Streptococcus [13]. Special biochemical tests were performed for genus discrimination Staphylococcus and Streptococcus bacteria at the age of 18-24 hours, according to the method used in [13] to differentiate between both genus and tests ability to produce Catalase, Coagulase, Carbohydrate fermentation and Hemolysis. 27 Streptococcus mutans isolates were obtained, which is equivalent to 20% of the total number of isolates, which 135 isolates. As for other isolates, they are other bacterial types.

2.2. Preparation of Mitis Salivarius Bacitracin Agar (MSB) medium

MSB medium which observed in Figure 1 is the specific Selective Medium for Streptococcus mutans. This medium was prepared according to Hi-media's guidance which contained 20(w/v)% total percentage of sucrose in the medium with the addition of 200IU/L of the antibiotic Bacitracin and this ratio was prepared based on that each gram of Bacitracin it contains a number of international units and it’s installed on the counter box according to the manufacturer. 0.0001 Potassium tellurite solution and 1% agar was added to ensure that the medium remained solid [14, 15, 16]. The method of preparation is by dissolving 90 grams of the medium in 1000 milliliters of distilled water and mixing well using hotplate with magnetic stirrer (zhongxing, taiwan, china) to ensure that solubility occurs well and then, to increase the selectivity of the medium for Streptococcus mutans, 150 grams / liter of substance was added. Sucrose for the medium so that the final percentage of sucrose is 20% in 1 liter of MSB, and after that, when the color of the medium is noticed to transparent blue, the conical flask is transferred to the autoclave to be sterilized at a temperature of 121 Celsius for 15 minutes and at a pressure of 15 pound per square inch psi, and when the medium is cooled to a temperature of 45 Celsius, potassium tellurite 0.0001, which has been sterilized by millipore filter 0.20 mm (Fisher Scientific, Wien, Austria), is added[14]. As for the solution containing the antagonist bacitracin, 1 ml of it was added after preparation as follows: By dissolving 0.364 of the powder in 100 ml of D.W, the stock solution of bacitracin was prepared. and mixed well by magnetic stirrer without any heat, and
after mixing it well, sterilize with a millipore filter (0.20Mm). The bacitracin solution is sterile at a concentration of 200IU / L. 1 ml of this solution was added to each liter of prepared medium and cooled at 45 °C to ensure that the bacitracin is not damaged so that the ratio of this antagonist in the medium is 200 IU / L [14].

**Figure 1. MSB Agar plate.**

2.3. **Muller Hinton Agar (MHA) preparation**

Muller Hinton Agar (MHA) (Hi-media) was prepared according to the manufacturer's instructions [17] by dissolving 38 grams of powder in 1000 ml of sterile distilled water and then placed on a hot plate with magnetic stirrer until the powder completely dissolves in distilled water and the medium is clear. After that, the medium is transferred to an autoclave for sterilization at a temperature of sterilization at 121°C and a pressure of 15 pis for 15 minutes, finally it is poured into the sterilized Petri dishes , after it cools to 45-50 °C then left to harden until it is ready to cultivate the bacteria and test its sensitivity to antibiotics and zinc oxide on it.

2.4. **Isolation of Streptococcus mutans**

*Streptococcus mutans* was isolated according to method of [18] which is summarized as follows: -

- Samples were collected using cotton swabs by passing them into the caries area and in the saliva surrounding the gums.

- The swabs were mapped on a selective culture medium (MSB) that was previously prepared.

- The plates were incubated under anaerobic conditions for 48 hours at 37°C.

- After the emergence of growth, the loop campaign of the growth appears on the surface of the medium (MSB), specifically the colonies to which the morphological characteristics are applied, are transferred and then streaked over the blood agar in order to activate the *Streptococcus mutans* bacteria, and the incubation was done at 37°C and in an anaerobic jar for a period of 24 hours.

- After the growth appeared on the medium of the blood agar, the single colony was transferred again to the MSB medium, and was streaked on the surface of the medium and then incubated in an Anaerobic jar at a temperature of 37°C for 48 hours. The benefit of last two steps is the activation of *Streptococcus mutans* bacteria as well as purification through Repeat the implantation on the selective medium (MSB).
2.5. *Streptococcus mutans* identification

2.5.1. Morphological feature

*Streptococcus mutans* was diagnosed phenotypically directly under light microscope with magnification level x15, the phenotypic diagnosis was based on colony shape on (MSB) agar plates, characteristics and based on Explanation cited by [18, 19]. Also, Hemolysis on blood agar plate, which must be (α) alpha-type, has been observed in *Streptococcus mutans* [20].

2.5.2. Gram Staining

Under aseptic conditions 1or 2 colonies were transferred from the surface of MSB Agar and exposed to Gram stain (SYR BIO, England) according to the known and scientific methods of work [21].

2.5.3. Biochemical tests

2.5.3.1. Catalase test

Colonies of *Streptococcus mutans* were taken from the MSB Agar plate by a sterile loop and transferred onto a sterile glass slide(Citotest ,Nantong , China) on the surface of which there is a drop of 3% H$_2$O$_2$ (Panreac, Barcelona, Spain). These colonies were mixed with a drop of hydrogen peroxide and the positive result of this test is bubbles [22, 23].

2.5.3.2. Test for carbohydrate fermentation (mannitol fermentation test)

Cystine trypticase agar CTA-Mannitol medium was used to the detect *Streptococcus mutans* ability to ferment mannitol sugar and convert the medium from red to yellow, which represents the positive result of the test [24]. The procedure of the test and media preparation was done according to [24]. Mannitol was added to medium cystine trypticase agar (Hi-media, India) at a concentration of 1% to form cystine trypticase manitol agar, which is used to detect the capacity of *Streptococcus mutans* bacteria to ferment manitol, where this medium after its preparation and autoclave sterilization at 121°C and a pressure of 15 psi for 15 minutes and after it gets cold it pours in screw capped bottles with a volume of 10 ml and keeping in the refrigerator at a temperature of 2-8 °C until use. Later Inoculate each bottle of this medium by adding 0.1 ml of *Streptococcus mutans* bacteria samples grown on Brain Heart Infusion Broth and incubate at a temperature of 37 °C for 48 hours. The coloring change of media from red to yellow, was the indicator that the bacteria have the ability to ferment mannitol and produce acid in this reaction.

2.5.4. Diagnosis of *Streptococcus mutans* by VITEK-2

The diagnosis of *Streptococcus mutans* isolates was done using VITEK-2 after growing the samples on MSB Agar medium, using VITEK-2 (BIO meriex) according to the company's instructions [25].

2.6. Zinc oxide nanoparticle characterization

Zinc oxide nanoparticle was obtained from U.S Research Nanomaterial, Inc. (USA) Diameter 20-30nm, purity 99.98%, and the stock solution was made at a concentration of 10mg / ml and was diluted according to $c_1v_1 = c_2v_2$ [18]. The nanocomposite test was performed using scanning Electron Microscope (SEM) as shown in Figure 2.
In order to ensure the effectiveness of the zinc oxide nanoparticles solution, an examination was done with the UV-V device, as shown in Figure 3, and the absorption must be at its highest value at the wavelength (400–500 nm), and this is what was observed when examining these particles with UV/visible spectroscopy.

2.7. Determination of Streptococcus mutans susceptibility to various concentration of solution ZnO NPs and de-ionized water (In vitro)

The effectiveness of ZnO NPs was evaluated against Streptococcus mutans which was isolated from cases of dental caries according to method of NCCLS [26], which included the following: Greening of a bacterial suspended from S. mutans isolates, which we want to treat with ZnO NPs solution of different concentrations, and the bacterial suspension was made by transferring 3 colonies by sterile loop to 5 ml of Normal Saline and then compare the bacterial suspension with standard McFarland solution (0.5) to stabilize the cell numbers at 1.5×10^8 cells/ml, then 100 μl of the prepared bacterial suspension was withdrawn and placed on a surface of Muller Hinton agar plates and were spread over the surface of MHA by a sterile swab and were planned length and width to ensure that the suspension was completely spread on the surface of the agar plate. Then, make holes in MHA by using the cork puncher (HI-Media, India) with a diameter of 5 mm and drilling serial concentrations of ZnO...
NPs nanoparticles were placed. Prepared (w/v) with as a negative control, de-ionized water and the concentrations were as follows: 10 mg /ml, 5 mg /ml, 3 mg /ml, 1 mg /ml, 0.5 mg /ml, 0.1 mg /ml.

2.8. Measuring the Minimum Inhibitory Concentration (MIC) of ZnONPs

The lowest inhibitory concentration is important in the process of determining the lowest inhibitory concentration of zinc oxide in the nanoscale method by method of broth dilution. The bacterial suspension was prepared by taking 4-5 colonies from the MSB plate and transfer to the broth for 15 minutes. After mixing the bacterial suspension well, 0.1 ml of the bacterial suspension was added to each test tube containing 4.8 ml of the nutrient broth and 0.1 ml of zinc oxide solution that was prepared and added as the fellow of the concentrations 10, 5, 2.5,1.25, 0.625, 0.312, 0.156 mg / ml with a positive control tube containing broth and inoculum of bacteria to detect the ability of bacteria to grow on the nutrient broth, and a negative control tube containing only the nutrient broth and ZnONPs without bacteria was mixed well. The inoculated tubes were incubated for 24 hours at a temperature of 37°C. After that, the growth in the tubes was compared with the control tube not containing Nano-Zinc Oxide, where the lowest inhibitory concentration is the first tube with no turbidity that can be seen by eye [27].

3. Results and Discussion

3.1. Morphological Characteristic

*Streptococcus mutans* were examined morphologically using a light Microscope lenses (Olympos, Japan), where the colonies appeared under the microscope in blue color, 1-2 mm in diameter and have a spherical or oval shape with a little height, meaning they are convex and adherent to the surface of MSB Agar and in its center is a pool of polysaccharides, as shown in the Figure 4.

![Figure 4. Shape growth colonies of *S. mutans* on MSB Agar plate.](image)

3.2. Microscopic examination

The microscopic inspection in the oily lens Indicated that bacteria *Streptococcus mutans* Gram Positive, avoid edge and Spherical in shape and short chains to medium in length, as shown in the Figure 5.
3.3. Biochemical tests

Biochemical tests include the following:

3.3.1. Catalase test

All *Streptococcus mutans* are negative for catalase, meaning that they do not form bubbles when tested with \( \text{H}_2\text{O}_2 \) 3%, which means that they do not have the ability to produce the catalase enzyme. It was noticed that *Streptococcus mutans* when performing the examination in the laboratory did not form any bubbles as shown in Figure 6.

![Catalase test](image)

**Figure 6.** Catalase test, shows that *S. mutans* are negative for catalase test.

3.3.2. Carbohydrate fermentation test (mannitol fermentation test)

Each *Streptococcus mutans* has the ability to ferment mannitol. The experiment was conducted in the laboratory and the result was positive, where the color of the medium changed from red to yellow, and negative control and positive control were used, and the result was positive as in Figure 7.

![Carbohydrate fermentation test](image)

**Figure 7.** Carbohydrate fermentation test (mannitol fermentation test)
3.4. Diagnosis of Streptococcus mutans by VITEK-2

VITEK2 tests showed that the Probability of *Streptococcus mutans* is 96%, and this is an excellent percentage that confirms that the bacteria isolated from caries are *Streptococcus mutans*, as noted in Figure 8.

3.5. Determination of *Streptococcus mutans* susceptibility to various concentration of solution Zn ONPs and de-ionized water (In vitro)

The results of the *Streptococcus mutans* susceptibility test to ZnO NPs appeared at a concentration of 0.5 mg / ml. This was the lowest inhibitory range, and it was highest at a concentration of 10 mg /ml. The highest inhibition zone appeared in Figure 9.
### Figure 9. *Streptococcus mutans* sensitivity to different concentrations of ZnO NPs.

#### 3.6. Measuring the Minimum Inhibitory Concentration (MIC) of ZnO NPs

MIC was determined after 24 hours of tube incubation, and where it was observed that the lowest concentration in which the growth disappeared and the tube became clear is Tube No.6, which contains a concentration of 0.312 mg/ml of ZnO NPs. For further clarification, the Table 1 below can be noted.

| Tube number | C+  | C   | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Concentration | mg/ml | mg/ml | mg/ml | mg/ml | mg/ml | mg/ml | mg/ml | mg/ml | mg/ml |
| ZnO NPs     | +   | -   | -   | -   | -   | -   | -   | -   | +   |

= There is no growth , + = There is growth , - C = Negative Control , + C = Positive Control

#### 3.7. Statistical research check for Inhibition zone of ZnO Nanoparticle on *Streptococcus mutans*.

The arithmetic mean and arithmetic Median of inhibition zone for each conc. The inhibition zone by ZnO NPs was calculated for each of the concentrations that were used on *Streptococcus mutans* isolates. 25 isolates were selected from the diagnosed isolates and the different concentration of ZnO NPs which tested on them. The arithmetic mean of the inhibition zone was calculated for each concentration (0, 0.1, 0.5, 1, 3, 5, 10) mg/ml which used on the 25 samples and the results were as in Table 2.

| ZnO NPS conc. | 0mg/ml | 0.1mg/ml | 0.5mg/ml | 1mg/ml | 3mg/ml | 5mg/ml | 10mg/ml |
|---------------|--------|----------|----------|--------|--------|--------|--------|
| Arithmetic Mean of inhibition zone for each conc. Of ZnO NPs | 0mm | 0mm | 8mm | 11mm | 14mm | 17mm | 23mm |
| Arithmetic Median of inhibition zone for each conc. Of ZnO NPs | 0mm | 0mm | 7.6mm | 12.1mm | 13.5mm | 15.4mm | 21mm |

#### 4. Discussion

The quantitative evaluation of the antibacterial nanoparticle was observed by diffusion in the pits and it was observed that, the area of inhibition depends mainly on the concentration and agreement with results by the [28] which showed that the bacterial inhibition was increased with increasing the concentration of ZnO NPs and ,also the results was an agreement with [18] who are observed that zinc particles Nanoparticles have the highest effective against Gram-positive and Gram-negative bacterial activity, and ZnO NPs have excellent antibacterial activity. The increase in the zone of inhibition with the increase in concentration may be attributed to ZnO NPs the higher surface to volume ratio is due to which gives better ion exchange. The lowest concentration of ZnO NPs was 0.1 mg / ml and this concentration did not show any efficacy against *S. mutans* bacterial isolated from dental caries, many mechanisms of anti-microbial effect by ZnONPs and the activity of these molecules by generating harmful oxygen compounds to the living cell, which is H$_2$O$_2$, and when increasing the concentration of zinc oxide nanoparticles, it leads to an increase in the production of H$_2$O$_2$ and thus increase the anti-
bacterial effect. This effect occurs because of damage to the cell membranes and also causes a defect in the components of the cell, which were resulted in the cell’s death permanently [18]. Another study showed that the anti-bacterial ability of ZnO NPs is due to their small size, which is 250 times smaller than a bacterial cell, and this facilitates for these particles to bind to the microorganisms' wall, which leads to their destruction and the death of living cells [18]. There is another possible mechanism for ZnO NPs activity against the bacteria through the release of Zn$^{2+}$ ions that have the ability to break down cell membranes and interact with intracellular components [18]. The electron microscopy images showed that the effect of ZnO NPs was on the bacterial cell wall and also increased the permeability of the cell membranes as well as changing the cell morphology [18]. This was presumed to be caused by the interaction of ZnO NPs with the bacterial cell membrane, leading to a defect in the membrane function [18]. Which leads to a change in the permeability of the membrane and the leakage of intracellular components [18], then leads to cell death [18]. This interaction was likely due Electrostatic consequences attributable to the contrary charges of the nanoparticles and the cell membrane. A study by [18] showed that when ZnO NPs come into contact with bacteria, the toxic behaviour of ZnO NPs leads to the rupture of the bacterial bilayer lipid layer, which leads to leakage of the cytoplasmic contents, and there is another possible mechanism to inhibit the bacterial isolates of S.mutans. A weak DNA damage was observed in the treated bacteria [18]. It is believed that micro-nanostructures carry a negative charge while metal oxides carry a positive charge, and this leads to the modulation of the electromagnetic attraction between the microbe and the surface of the zinc nanoparticles. Once this attraction occurs, the microbe is oxidized and dies immediately, and in general it is believed that the production of ions by nanomaterials that interact with the dependent SH-groups. For proteins on the surface of the bacterial cell [29].

5. Conclusion

The findings indicate that nanoparticles of zinc oxide have an excellent anti-bacterial benefit., and the effectiveness of these particles increases by increasing the solution concentration that contains the nanoparticles.

6. References

[1] Albrecht MA, Evans CW, Raston CL 2006 Green chemistry and the health implications of nanoparticles J. Green Chem. 8 417.
[2] Koziara JM, Lockman PR, Allen DD and Mumper RJ 2003 In situ blood-brain barrier transport of nanoparticles Pharmac. Res. 20 1772.
[3] Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R and Kreyling, W 2004 Translocation of inhaled ultrafine particles to the brain Inhal Toxicol. 16 437.
[4] Ibrahim NA and Muhammad AA Z 2020 Nanomaterials in detergents and cosmetics products: the mechanisms and implications Handbook of Nanomaterials for Manufacturing Applications Elsevier, 23.
[5] Baler SA, Scheringer M, Macleod M and Hunger BK 2008Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles Sci. Total Environ. 390 396.
[6] Jones N, Ray B, Ranjit KT and Manna AC 2008 Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms FEMS Microbiol. Lett. 279 71.
[7] Mohsen J and Zahra B 2008 Protein nanoparticle: A unique system as drug delivery vehicles Afr. J. Biotechnol. 7 4926.
[8] Zakaria ZA, Matpesa A, Ramasamy K, Ahmat N, Mohamad AS, Jsraf DA and Sulaiman MR 2010 Lack of antimicrobial activities of Dicranopteris linearis extracts and fractions Afr. J. Microbiol. Res. 4 71.
[9] Jiang W, Mashayekhi H and Xing B 2009 Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environ. Poll.* 157 1619.

[10] Damle S 2009 *Textbook of pediatric dentistry*. 3rd ed. Arya.

[11] Patini R 2020 Cyturomics: A New Approach for the Diagnosis of the Oral Microbiota *Dental Hypoth.* 11 72.

[12] Lévesque CM, Voronejskaia E, Huang YC, Mair RW and Ellen RP 2015 Involvement of sortase anchoring of cell wall proteins in biofilm formation by *Streptococcus mutans* *Infec. Immun.* 73 3773.

[13] Brown AE 2015 *Benson’s Microbiological Applications Laboratory Manual in General Microbiology* (13th ed., pp. 230-280). McGraw-Hill Companies, Inc., New York. 60.

[14] Hailan SY and Al-Khatieeb MM 2019 Antimicrobial efficacy of silver, zinc oxide, and titanium dioxide nanoparticles incorporated in orthodontic bonding agent *J. Baghdad Coll. Dent.* 31 10.

[15] Tale V, Jadhav A and Kulkarni K 2020 Biofilm forming ability of bacteria isolated from dental caries: with reference to *Streptococcus* species *Fut. Dent. J. Egypt* 5 2.

[16] Nagamine Y 2020 D-Tagatose Effectively Reduces the Number of *Streptococcus mutans* and Oral Bacteria in Healthy Adult Subjects: A Chewing Gum Pilot Study and Randomized Clinical Trial *Acta Medica Okayama* 74 307.

[17] http://himedialabs.com/TD/M173.pdf.

[18] Abd ST and Abbas FA. 2016 The Effect of Zinc Oxide Nanoparticles on *Streptococcus mutans* of Human Saliva (In Vitro Study) *J. Baghdad Coll. Dent.* 28 158.

[19] Edwardsson S 1970 The caries inducing property of variants of *Streptococcus mutans* *Odontologisk Revy* 21 154.

[20] Luis M, Pezzlo MT, Bittencourt CE and Peterson EM 2020 *Color atlas of medical bacteriology* John Wiley & Sons.

[21] Procop GW 2020 *Koneman's color atlas and textbook of diagnostic microbiology* Jones & Bartlett Publishers.

[22] Wu Y, Jiang S and Fu Z 2020 Employment of teicoplanin-coated magnetic particles for quantifying gram-positive bacteria via catalase-catalyzed hydrolysis reaction of H$_2$O$_2$ *Talanta* 211 120728.

[23] Ahmad F, Muhmood T and Mahmood A 2020 *Deciphering the mechanism of hafnium oxide nanoparticles perturbation in the bio-physiological microenvironment of catalase Nano Express.*

[24] Finegold S and Baron E 2017 *Methods for identification of etiologic agents of infectious disease*. In: *Bailey and Scott’s Diagnostic microbiology* 14th ed. St. Louis: The CV Mosby Co.

[25] Jubair HH 2015 The Relationship Between Biofilm Forming and Antibiotics Resistance of *Streptococcus mutans* Isolated From Dental Caries *Int. J. Curr. Microbiol. App. Sci.* 4 568.

[26] National Committee for Laboratory Standards 2004 *Performance standards for antimicrobial susceptibility testing, 14th Informational supplement* M,100-513. Vol. 24 No,1,NCCLS, Wayne, P A, USA.

[27] Owuama CI 2017 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method *Afr. J. Microbiol. Res.* 978.

[28] Negahdary M, Arabi F, Imandar M, Imandar M, Noughabi M T, Akbari-dastjerdi HM and Fazilati F 2012 Investigation anti-bacterial effect of zinc oxide nanoparticles upon life of *Listeria monocytogenes* *Ann. Biol. Res.* 3 3679.

[29] Thi, TUD 2020 Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities *RSC Adv.* 10 23899.