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Antimicrobial Activity of Titanium Dioxide Nanoparticles Biosynthesized by *Enterococcus hirae*

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ABSTRACT

The present study included the biological synthesis of titanium dioxide nanoparticles by lactic acid bacteria (*Enterococcus hirae* ATCC 9790). The biosynthesized nanoparticles have been characterized by using ultraviolet visible (UV- vis) spectroscopy, Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analysis (XRD). The UV- Vis spectrum showed a well-defined surface plasmon resonance peak (SPR) at 326nm.TEMfigure showed pure nanoparticles with mean average size 24.9 nm. FTIR spectroscopy confirmed the presence of bio-components which were responsible for the synthesis of TiO_2NPs.The XRD pattern showed main peak of $2\theta=27.4°$ matches the (110) crystallographic plane of rutile of TiO_2 nanoparticles. The biosynthesized TiO_2NPs have high antimicrobial activity against pathogenic Gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), Gram negative bacteria (*Salmonella*) and fungi (*Penecillium* spp.) that causing food poisoning. Results of the present study recommended green synthesis of TiO_2NPs by *Enterococcus hirae* that would play an important role in future concerning industrial, therapeutic and food technology applications.

Keywords: Biological synthesis, Titanium dioxide nanoparticles, Enterococcus hirae, Antimicrobial activity.

1. Introduction

Many researchers believe that nanotechnology will provide us with great developments in many fields, advance the world and quality of life. Nanotechnology involves the production, characterization and use of material that have dimension of about 1-100 nm. When particle size is reduced to this dimension the resulting nanoparticles exhibit different physical and chemical properties (Duncan, 2011).

Several physical and chemical methods have been used for the synthesis of metal nanoparticles in short period of time. Chemical procedures lead to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications. Biological synthesis of nanoparticles that called green synthesis is gaining importance due to its simplicity, environmental friendly in nature, cost effective and extensive antimicrobial activity which make them better than physical and chemical synthesis (Gunalan *et al*., 2012; Chatterjee *et al*., 2016).

TiO_2 is the most preferred nanoparticles because of its high photocatalytic activity, photochemically stable, high specific area, non-toxic and relatively inexpensive (Macwan *et al*., 2011). TiO_2 nanoparticles are of great interesting in a wide range of applications such as dye sensitized solar cells, photocatalysis, gas sensors, photo degradation of organic compounds, organic synthesis, cells culture and deactivation of microorganisms (Banerjee, 2011; Arya *et al*., 2021).

Titanium dioxide nanoparticles is non-toxic and has been approved by the FDA for use in food technology, cosmetics, drugs, paints pigment, toothpaste, ointmentsand food contact material not more than 1% by weight (Awati *et al*., 2003). Titanium dioxide nanoparticles have been studied for its

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antimicrobial activity against wide spectrum of microorganisms due to generation of highly reactive oxygen species (ROS) that are toxic to pathogenic microorganisms (Makwana *et al*., 2015; Ziental *et al*., 2020).

It has been demonstrated that highly reactive TiO_2NPs exhibit bactericidal activity against gram positive and gram negative bacteria (Stoimenov *et al*., 2002). On view of mycoses, TiO_2NPs can be considered as potential antifungal agents (Pana *et al*., 2009).

Salmonella and *Staphylococcus* are food borne pathogens which associated with acquired and nosocomial infections and may be life threatening in immunodeficient conditions (Tayel *et al*., 2011). These pathogens acquired resistance to antibiotics which cause health care problems that lead to treatment failure for a large number of drugs. So there is an urgent clinical need to develop new antibacterial therapies to reduce infections and this achieved by using nanoparticles especially TiO_2NPs (Jesline *et al*., 2015; Albukhaty *et al.,* 2020).

The objective of this workwas to study the biosynthesis of TiO_2NPs by lactic acid bacteria *Enterococcus hirae* ATCC 9790 and study their antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* and *Penecillium* that causing food poisoning.

2. Materials and Methods

2.1. Biosynthesis of TiO_2 nanoparticles

The tested lactic acid bacterium *Enterococcus hirae* ATCC 9790(which identified and obtained from previous work) was allowed to grow in sterile MRS broth media at 37 °C for 36-48 hrs. 25 ml of microorganism culture was diluted four times by adding 75 ml of sterile distilled water. This diluted culture solution was again allowed to grow for another 24 hrs. Then 20 ml of 0.025 M $TiO(OH)_2$ solution was added to the culture solution and it was heated on steam bath up to 60 °C for 10–20 min until white deposition starts to appear at the bottom of the flask, indicating the initiation of transformation of titanium ions to nanoparticles. The culture solution was cooled and allowed to incubate at room temperature in the laboratory ambience. After 12–48 hrs, the culture solution was observed to have distinct coalescent white clusters deposited at the bottom of the flask as TiO_2NPs **(Jha *et al*., 2009)**.

2.2. Characterization of titanium dioxide nanoparticles

2.2.1. Ultraviolet visible (UV- vis) spectroscopy

UV-Vis spectrum was measured on BEAM UVS-2700, a spectrophotometer operated at a resolution of 1 nm. The electronic absorption technique outlined above has proved to be very useful for the analysis of nanoparticles as illustrated earlier by Wang *et al*. (2004); Gaikwad *et al*. (2008).

2.2.2. Transmission Electron Microscopy (TEM)

The size and morphology of the biosynthesized TiO_2NPs of tested bacterium was studied using a transmission electron microscope, model JEOL-JEM-100 SX electron microscope, Japan, with AMT digital camera. The specimen was dispersed ultrasonically to separate individual particles, and one or two drops of the suspension was deposited onto holey carbon coated copper grids and dried under infrared lamp prior to photographing. The TEM technique enables the identifying of nanoparticles shape and size.

2.2.3. Fourier transform infrared spectroscopy (FTIR)

The presence of functional groups in microorganism extract and the synthesized nanoparticles were identified by FT-IR spectroscopy model TENSOR27.

2.2.4. X-ray powder diffraction analysis (XRD)

Glass slide coated with TiO_2 nanoparticles was checked by X-ray powder diffraction (XRD) technique using (GNR X-ray Differactometer model APD 2000PRO generator, diffractometer, one line reactor) with CuKα radiation line of λ=1.5405A over a wide range of 2Θ Bragg angles(20-80°).
The average crystalline size of TiO_2NPs was calculated by scherrer̓ s formula (Goutam *et al*., 2018; Esfahani *et al*., 2020):

$$D=0.9\lambda/\beta\cos\theta$$

Where D: is the crystalline size, β: is the full width of half maximum (FWHM) of a specific phase in radians, λ: is the wave length of incident rays (λ= 1.54) and θ: is Bragg's angle (the center angel of the peak in radian).

2.3. Antimicrobial activity of titanium dioxide nanoparticles

The tested pathogenic bacteria isolated from food (*Staphylococcus aureus, Bacillus cereus* and *Salmonella*) were isolated from mozzarella cheese, shipsy and tuna respectively. The tested bacteria with 10^{6}cfu ⁄ ml were inoculated into flasks of nutrient broth media (100 µl into 100 ml) then 100 µl of biosynthesized TiO_2NPs from *E. hirae* ATCC 9790with different concentrations (500 µg⁄ml, 1 mg⁄ml and 2 mg⁄ml) were added to each flask. The flasks were incubated in shaker incubator 150 rpm at 37 °C for 24 hrs. One flask with bacteria only used as a control. All flasks were sent to electron microscope unit of faculty of science Alexandria University to be examined and photographed by Transmission electron microscope.
The tested pathogenic fungus *Penecillium* was isolated from orange juice. The plates with the largest inhibition zone (which tested by well agar diffusion assay) and the control plate were sent directly to be examinedand photographed by Transmission electron microscope.

2.4. Preparation of samples

- Cells were collected after 24 hours of incubation and washed three times with saline solution.
- Fixation of cells in 2.5% buffered glutaraldehyde which prepared in 0.1 M phosphate buffer solution (PBS) at pH 7.4 for 2 hours at 4°C.
- Washing three times with PBS for 10 min. each time, and then centrifuged for 15 min at 3000 rpm.
- Cells were post fixed in 1% Osmic acid for 30 min.
- Washing three times with PBS for 10 min. each time, then centrifuged for 5 min at 2000 rpm.
- With ascending series of ethyl alcohol (30, 50, 70, 90% and absolute alcohol), these cells were dehydrated with each concentration for 30 min and then infiltrated with acetone for 1 hour.
- In TEM, after dehydration samples were embedded in Araldite 502 resin.

The plastic molds were cut in the LEICA Ultracut UCT ultra-microtome, stained with 1% toleudine blue. After examinationof semi-then sections ultra-thin sections were cut, stained with uranyl acetate, then counter stained with lead citrate and examined and photographed using JEOL-JEM-100 SX electron microscope, Japan.

3. Results and Discussion

3.1. Characterization of titanium dioxide nanoparticles

3.1.1. UV-Vis spectroscopy

The biosynthesis of TiO_2 nanoparticles was confirmed by UV- Vis spectrophotometer. The UV-Vis spectrum showed a well-defined surface plasmon resonance peak (SPR) as presented in Figure 1. The single and strong absorbance peak was observed at 326nm.According to Mie's theory, only a single surface Plasmon resonance band demonstrated small and spherical nanoparticlesbut anisotropic particles showed two or three bands (Rajesh *et al*., 2009).

3.1.2. Transmission electron microscopy (TEM)

The size of TiO_2 nanoparticles biosynthesized by *E. hirae* ATCC 9790was indicated by TEM was shown in Figure 2, where pure nanoparticles with mean average size 24.9 nm at 500 nm with magnification10000X.All the nanoparticles were separated and no agglomeration was noticed, indicating stabilization of the nanoparticles by a capping agent. The difference in size may be as a result of formation of nanoparticles at different times that limit the size due to constraints related to the particles nucleating in the micro-organisms (Jha *et al*., 2009). The results obtained from TEM in our studies were similar to the results of Prasad *et al*.(2007); Singh, (2016); Hariharan *et al*. (2017).

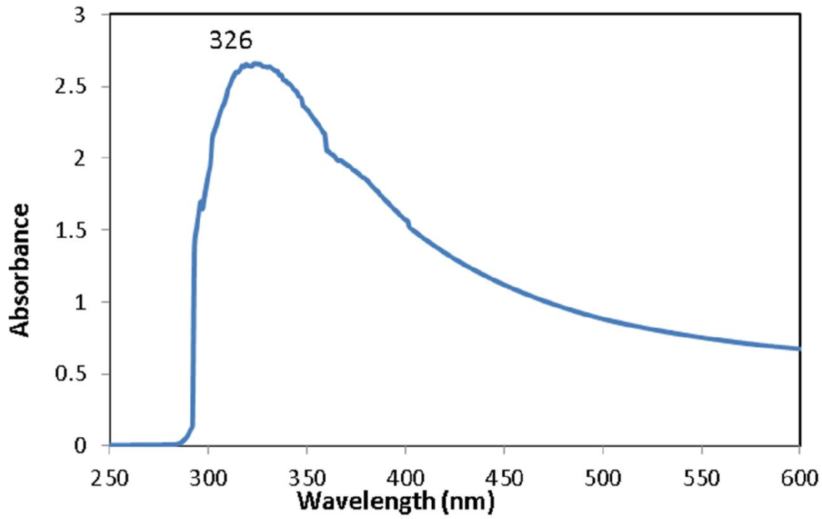

Fig. 1: UV-Vis spectroscopy of TiO_2NPs produced by *E. hirae*.

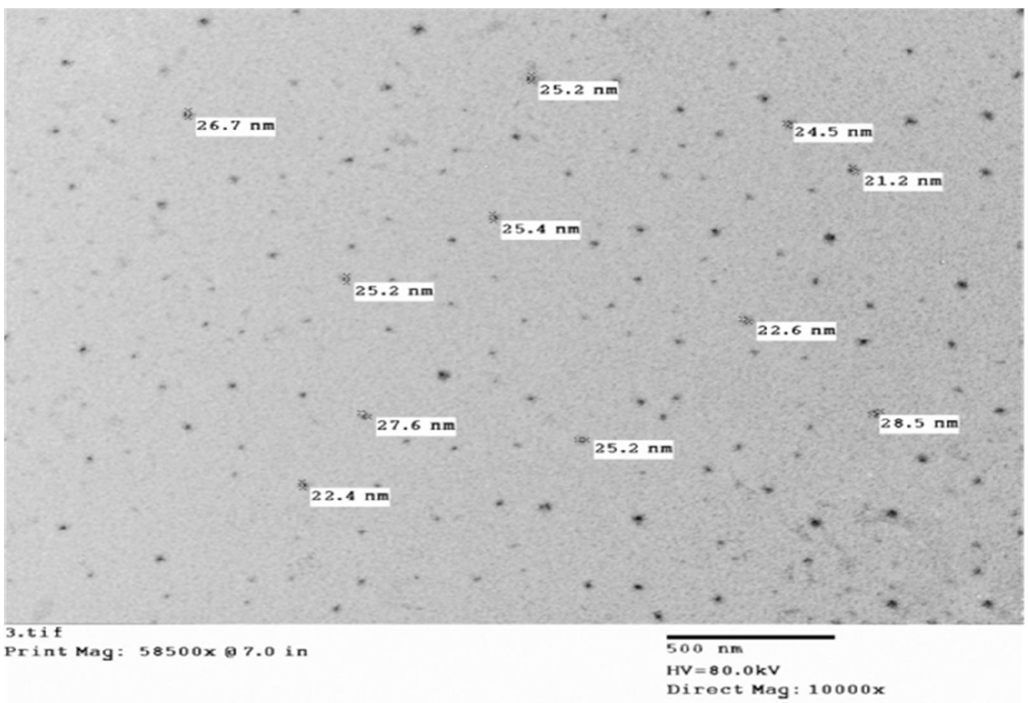

Fig. 2: Photomicrograph of Transmission electron microscopy (TEM) of TiO_2NPs produced by *E. hirae*.

3.1.3. Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of TiO_2 and the capping of the bio-reduced TiO_2NPs synthesized. Figure 3 (a & b) showed the functional groups of bacterial extract and their nanoparticles.

In the FT-IR spectrum of TiO_2NPs abroad peak appearing at 3100–3600 cm^{-1} was assigned to fundamental stretching vibration of O–H hydroxyl groups (Gaoa *et al*., 2004). The band around 2925 cm-1 is assigned to C-H vibrations. The C-H could be attributed to the organic residues, which remained in TiO_2 (Yodyingyong, 2011).

Peaks observed at 1630-1660 cm^{-1} indicates O-Ti-O bond (Shahab *et al*., 2013). Ti-O stretching vibration is confirmed by the beak at the region of 1400-1460 cm^{-1} (Vetrivel *et al*., 2015).The amide linkage between the bacterial proteins and the TiO_2 during the reaction was confirmed by the presence of a peak at 1246 cm^{-1}. The peak observed around 500-600cm^{-1} is due to the vibration of the Ti-O-O bond (Babitha and Korrapati, 2013).

Sastry *et al*. (2003) and Sanghi and Verma, (2009) have reported that bonds or functional groups such as –C-O-C-, -C-O- and –C=C- are derived from heterocyclic compounds like proteins which are present in the bacterial extract and are the capping ligands of the nanoparticles.After bio-reduction, there is a shift obseved in the absorption band than of *E. hirae* due to the binding (NH), C-N, OH and (CH) groups with the nanoparticles of titanium dioxide.The results obtained from FTIR in our studies were similar with the results of Hardy *et al*. (2007); Chatterjee *et al*. (2016).

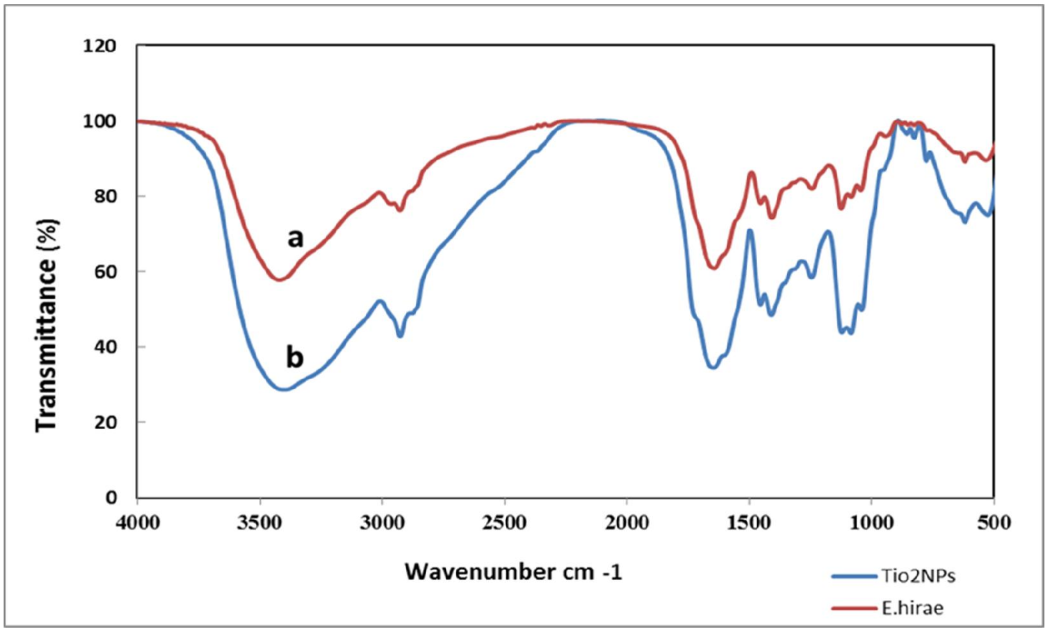

Fig 3: FTIR of **a)** bacterial extract of *E. hirae* **b)** TiO_2NPs biosynthesized by *E. hirae.*

3.1.4. X-ray diffraction analysis

The XRD patterns of TiO_2NPs indicated the presence of intense peaks of nanoparticles at miller indices 110, 101, 200, 111, 210, 211, 220 and 301. The corresponding Bragg's angles (2θ) for *E. hirae* ATCC 9790were 27.19°, 35.84°, 39.17°, 40.98°, 43.76°, 54.05°, 56.35° and 68.64° as presented in Figure 4.

The peaks of sample were identified by comparison with ICDD (International Centre for Diffraction Data) according to 2θ which was an indicator of the biologically synthesized nanoparticles TiO_2 crystallites. The main peak of 2θ=27.4° matches the (110) crystallographic plane of rutile of TiO_2 nanoparticles, indicating that nanoparticles structure dominantly correspond to rutile crystalline. There is a slight increase in the peaks which may be the result of nanoparticles biosynthesized by bacteria (Byranvand *et al*., 2013).

These results confirm the tetragonal structure of the biosynthesized TiO_2NPs as indexed with the card of ICDD file no. 01-078-4185.The size of the nanoparticles was determined from the Debye Scherer formula was 20.38 nm. This result was nearly matched with the size estimated by the TEM analysis.

Some of the unassigned peaks were observed, it may be due to the fewer biomolecules of stabilizing agents as enzymes or proteins in bacterial extract (Philip, 2009). The diffraction peaks were broadened around their base indicating that titanium dioxide nanoparticles were in the nanometer range (Rajakumar *et al*., 2012). These results matched well with the reports of Jha and Prasad, (2010); Kirithi *et al*. (2011); Holm *et al*. (2019).

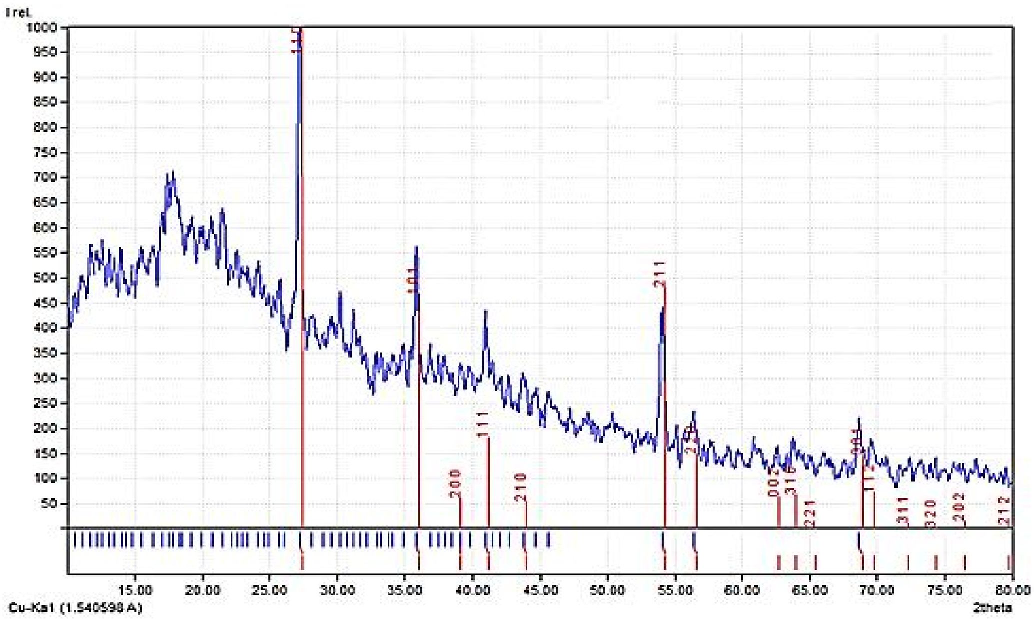

Fig. 4: XRD patterns of TiO2NPs synthesized by *E. hirae*

3.2. Antimicrobial activity of titanium dioxide nanoparticles

TiO_2 nanoparticles biosynthesized by *E.hirae* ATCC 9790had antibacterial activity against gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and gram negative bacteria (*Salmonella*). Also TiO_2NPs had antifungal activity against pathogenic fungus (*Penecillium*). The antimicrobial activity observed by the formation of inhibition zone with diameter (17 nm, 16 nm, 17 nm and 21 nm respectively). The antimicrobial activity was confirmed by transmission electron microscope (TEM) as presented by Figures (5:12). Electron microscopy was used to investigate the mechanism part of action of TiO_2NPs on microbial cells by determining if there was a differentiation in the cells morphology as a result of TiO_2NPs.

TiO_2NPs causing rupture of the cell wall, entering the microbial cells and releasing their internal components, which resulted in abnormal shape of cells and finally cell lysis.
Similar results were detected by Ahmad *et al*., (2015); Sundrarajan *et al*., (2017); Subhapriya and Gomathipriya, (2018); Razzaq *et al*., (2021) in which TiO_2NPs showed the ability to destroy bacterial cells by adhering to the cell wall, this resulting in the leakage and damage of intracellular components, presence of hydroxyl radicals, the generation of reactive oxygen species, and the release of $Ti4^+$. Also the mechanism of action of TiO_2NPs on microbial cells was illustrated by Rajakumar *et al*. (2012).

Zhang and Chen, (2009) have suggested the mechanism involving the interaction of nanoparticles with microbial cells. They documented that microorganisms carry a negative charge while nanoparticles (metal oxides) carry a positive charge which createsan electromagneticattraction between them and once the contact is made, the microbesare oxidized and dead.

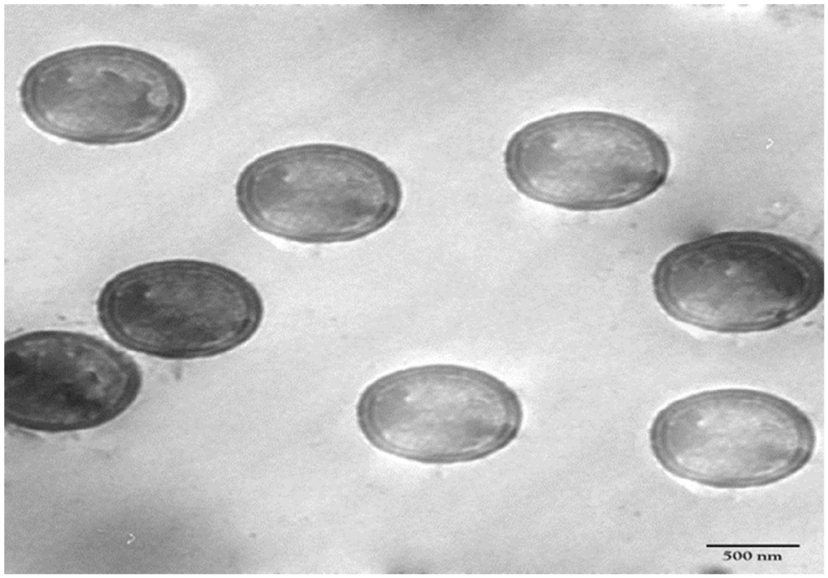

Fig. 5: Photomicrograph of transmission electron microscope at 500 nm with magnification 40000 X reveals control cells of *Staphylococcus aureus*. All cells are most probably normal with intact cell wall.

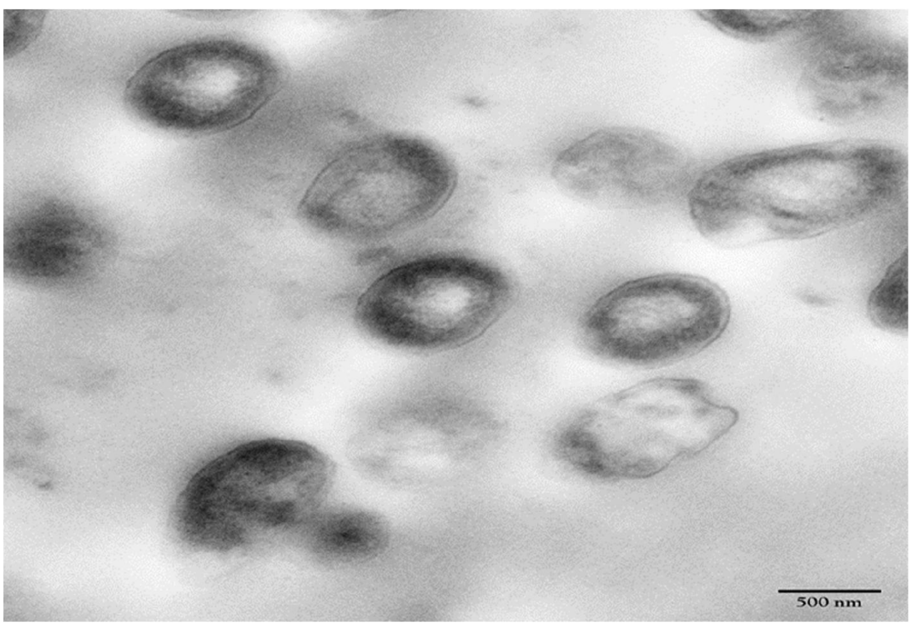

Fig. 6: Photomicrograph of transmission electron microscope at magnification 55000 X reveals the effect of TiO_2NPsof *E. hirae* ATCC 9790 on *Staphylococcus aureus*. The Figure showed cytoplasmic vaculation of bacterial cells, distortion and irregularity of bacterial cell wall, rupture of bacterial cell wall and releasing their internal components, complete lysis of most of them and elongation of two bacterial cells compared to control cells.

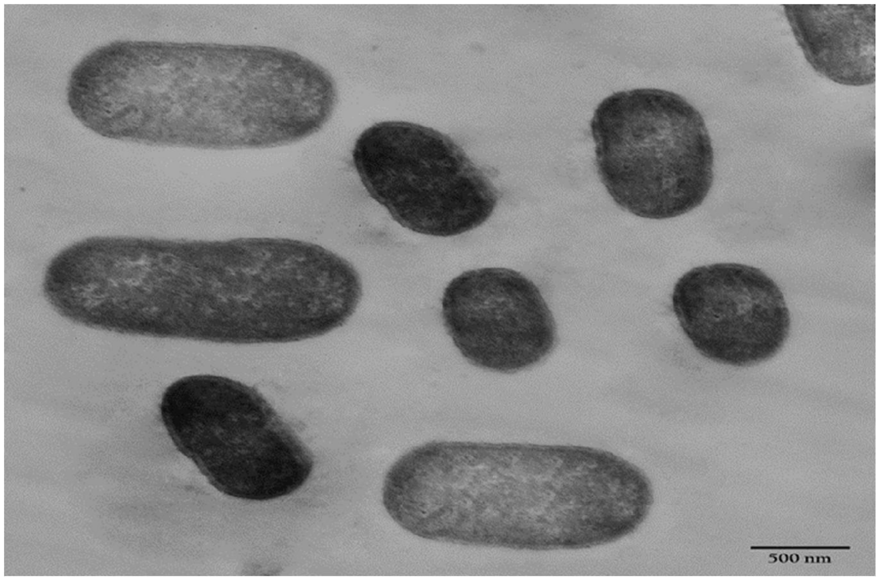

Fig.7: Photomicrograph of transmission electron microscope reveals control cells of *Bacillus cereus*. Control Figure of bacilli cells showing normal cells with thick cell wall and homogenous cytoplasm at 500 nm with magnification 40000 X.

Fig. 8: Photomicrograph of transmission electron microscope reveals the effect of TiO_2NPs of *E. hirae* ATCC 9790 on *Bacillus cereus*. Transverse and longitudinal sections of *Bacillus cereus* at 500 nm with magnification 55000X. In which TiO_2NPs entering the bacterial cells and causing rupture of the cell wall, releasing their internal components which resulted in abnormal shape of cells. Partial lysis of most of bacterial cells was observed and presence of little debris of cells refers to the complete damage caused by titanium dioxide nanoparticles.

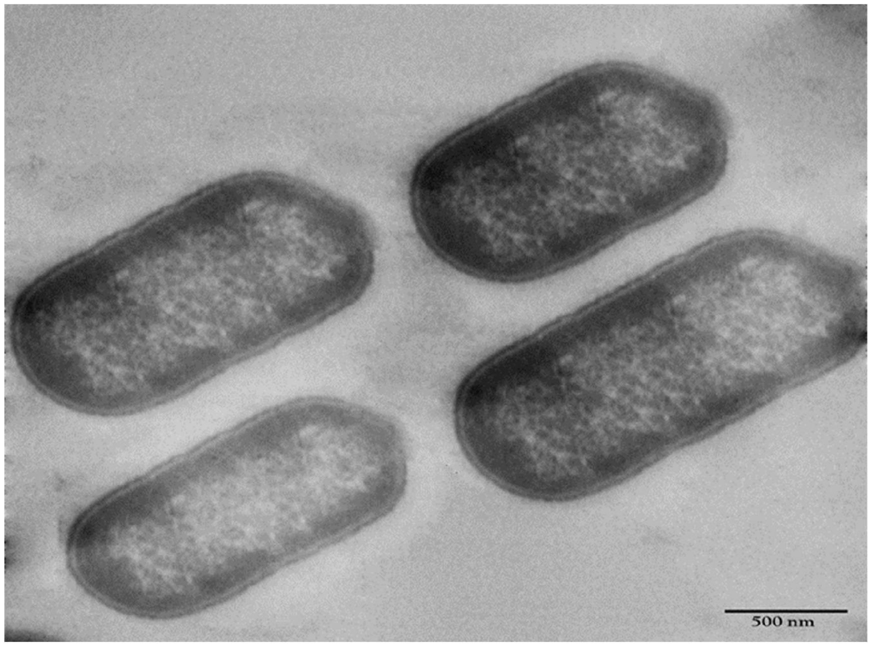

Fig. 9: Photomicrograph of transmission electron microscope reveals the control cells of *Salmonella*. Normal bacilli cells with regular cell wall and homogenous cytoplasm at 500 nm with magnification 55000 X.

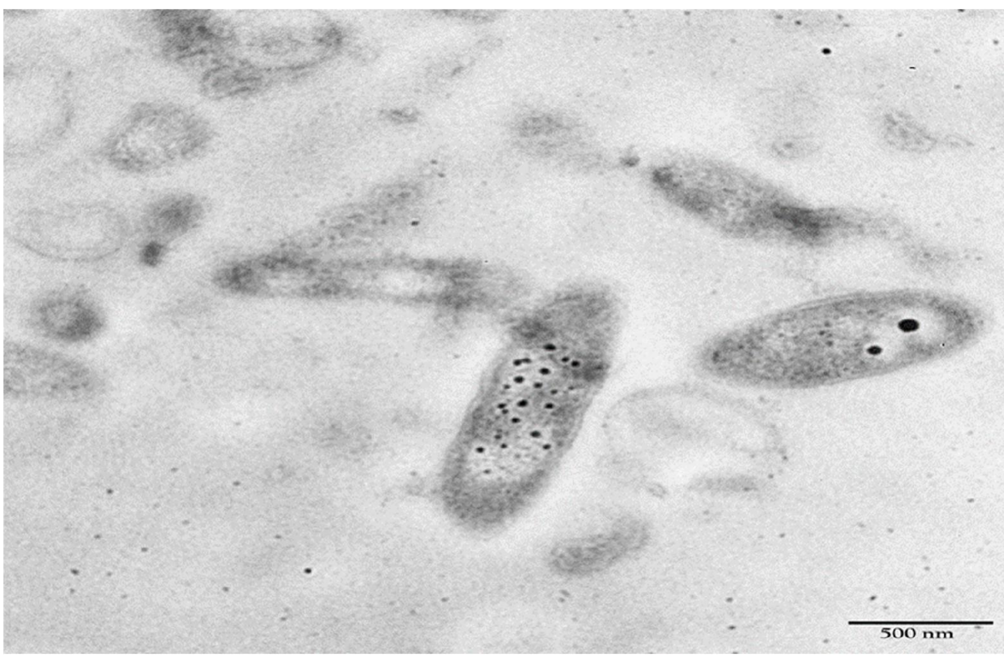

Fig. 10: Photomicrograph of transmission electron microscope reveals the effect of TiO_2NPs of *E. hirae* ATCC 9790 on *Salmonella*. Transverse and longitudinal sections of bacilli cells *(Salmonella)* at 500 nm with magnification 55000X. The Figure showed rarification of internal components, aggregation of nanoparticles inside the bacterial cells. Also focal area of debris of bacterial cells aggregated with nanoparticles in the surrounding.

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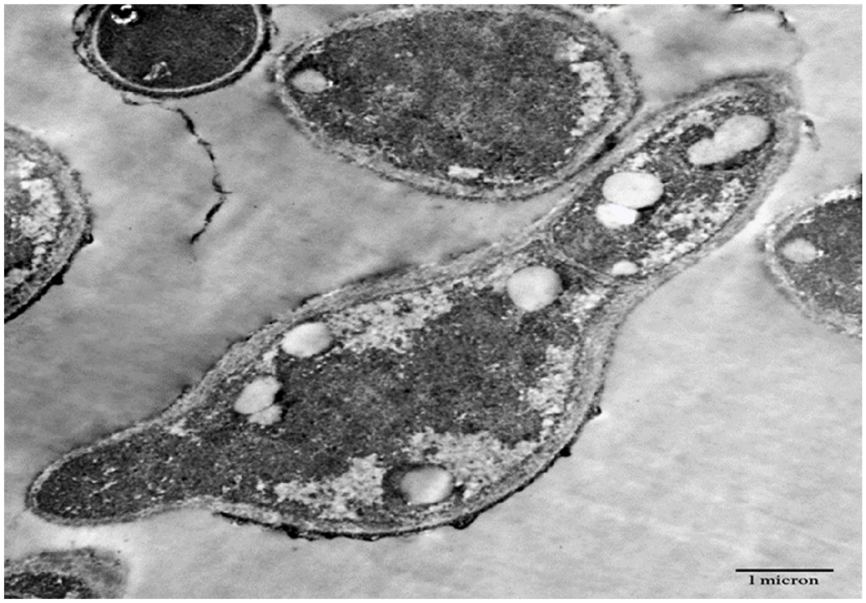

Fig. 11: Photomicrograph of transmission electron microscope reveals the hyphae of *Penecillium spp*. Transverse section of hyphae with magnification 20000 X showed normal hyphae with intact, continuous and regular septa. Internal components are most probably normal. Hyphae attached with conidiophore. Thick wall of hyphae surrounded with protective slime layer.

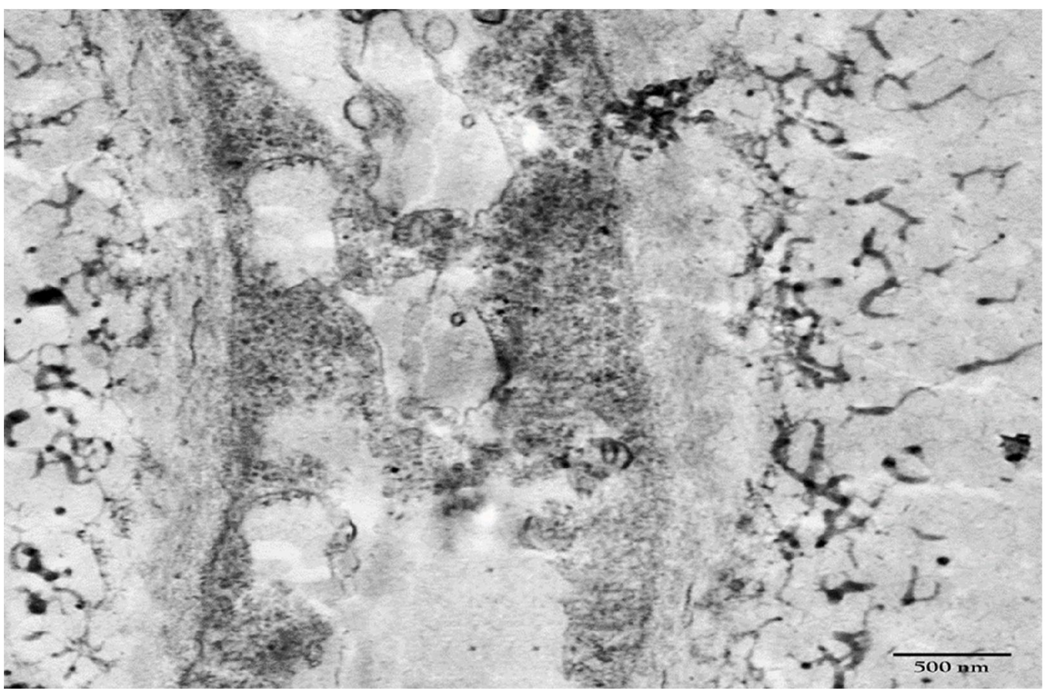

Fig.12: Photomicrograph of transmission electron microscope at 500 nm with magnification 40000 X reveals the effect of TiO_2NPs of *E. hirae* ATCC 9790 on *Penecillium spp*. TiO_2NPs showed a great antifungal effect against *Penecillium spp*, there was a complete lysis of hyphae, disruption of cell wall, and exfoliation of internal components, collapsing of internal components and presence of nanoparticles inside and in between the hyphae debris resulted inabnormal feature of hyphae compared to control.

4. Conclusion

The present study explained the use of nontoxic, inexpensive and ecofriendly easily available bacteria (*Enterococcus hirae* ATCC 9790) for the rapid synthesis of titanium dioxide nanoparticles. The biosynthesized TiO_2NPs were characterized by UV- vis, TEM, FTIR and XRD. This bacterial biosynthesis (green synthesis) provides a fast and purest form of producing nanoparticles than any other methods. TiO_2NPs showed inhibitory effect on the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* and a *Penecillium spp*. So TiO_2NPs proved to have potent antimicrobial properties and can be considered as good candidate to be used in various applications, as alternative to antibiotics, food industries and as biopreservative.

Reference

Ahmad, R., M. Mohsin, T. Ahmad, and M. Sardar, 2015. Alpha amylase assisted synthesis of TiO_2 nanoparticles: Structural characterization and application as antibacterial agents. Journal of Hazardous Materials, 283:171–177.

Albukhaty, S., L. Al-Bayati, H. Al- Karagoly, and S. Al- Musawi, 2020. Preparation and characterization of titanium dioxide nanoparticles and in vitro investigation of their cytotoxicity and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.Anim Biotechnology. 28: 1-7.

Arya, S., H. Sonawan, Math *et al.,* 2021. Biogenic titanium nanoparticles TiO_2NPs from *Tricodermacitrinoviride* extract: synthesis, characterization and antibacterial activity against extremely drug-resistant *Pseudomonas aeruginosa*, International Nano Letters, 11: 35-42.

Awati, P.S., Awate, S.V., P.P. Shah, and V. Ramaswamy, 2003. Photocatalytic decomposition of methylene blue using nanocrystalline anatasetitania prepared by ultrasonic technique. Catalysis Communications. 4 (8):393-400.

Babitha, S., and P.S. Korrapati, 2013. Biosynthesis of titanium dioxide nanoparticles using a probiotic from coal fly ash effluent. Materials Research Bulletin, 48: 4738-4742.

Banerjee A.N., 2011. The design, fabrication, and photocatalytic utility of nanostructured semiconductors: focus on TiO2-based nanostructures. Nanotechnology Science Application. 4: 35–65.

Byranvand, M.M., A.N. Kharat, L. Fatholahi, and Z.M. Beiranv, 2013. A review on synthesis of nano-TiO_2 via different methods.Journal of Nanostructure. 3:1-9.

Chatterjee, A., D. Nishanthini, N. Sandhiya, and A. Jayanthi, 2016. Biosynthesis of titanium dioxide nanoparticles using *Vigna Radiata*. Asian Journal of Pharmaceutical and Clinical Research, 9: 85-88.

Duncan, T.V., 2011. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. Journal of Colloid and Interface Science, 363(1): 1-24.

Esfahani, R.N., S. Khaghani, A. Azizi, F. Mortazaeinezhad, and M. Gomarian, 2020. Facile and eco-friendly synthesis of TiO_2NPs using extracts of *Verbascumthapsus* plant: an efficient photocatalyst for reduction of Cr (VI) ions in the aqueous solution. Journal of Iran Chemistry Society, 17 (1): 205–213.

Gaikwad, A., P. Verschuren, S. Kinge, G. Rothenberg, and F. Eiser, 2008. Matter of age: growing anisotropic gold nanocrystals in organic media. Physical Chemistry Chemical Physics. 10 (7): 951-956.

Gaoa, Y., G. Yanfeng, M. Yoshitake, W. SeonSeo, H. Ohta, and K. Koumoto, 2004. TiO_2 nanoparticles prepared using an aqueous peroxotitanate solution. Ceramics International, 30: 1365–1368.

Goutam, S.P., G. Saxena, V. Singh, A.K. Yadav, R.N. Bharagava, and K.B. Thapa, 2018. Green synthesis of TiO_2 nanoparticles using leaf extract of *Jatrophacurcas*, for photocatalytic degradation of tannery wastewater. Chemical Engineering Journal, 336: 386–396.

Gunalana, S., R. Sivaraja, and V. Rajendran, 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International, 22(6): 693–700.

Hardy, A., J.D. Haen, M.K.V. Bael, and J. Mullens, 2007. Sol-gel Science. Technol., 44: 65–74.

Hariharan, D., K. Srinivasan, and L.C. Nehru, 2017. Synthesis and characterization of TiO_2nanoparticles Using Cynodon Dactylon leaf extract for antibacterial and anticancer (A549 Cell Lines) Activity. Journal of Nanomedicine Research, 5 (6): 00138.

Holm, A., M. Hamandi, F. Simonetb, B. Jouguetb, F. Dappozze, and C. Guillard, 2019. Impact of rutile and anatasephase on the photocatalytic decomposition of lactic acid. Elsevier. (29).

Jesline, A., P. Neetu, P.M. John, C. Narayanan, and S.M. Vani, 2015. Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant *Staphylococcus aureus*. Applied Nanoscience. 5:157–162.

Jha, A., and K. Prasad, 2010. Biosynthesis of metal and oxide nanoparticles using *Lactobacilli* from yoghurt and probiotic spore tablets. Microbial Biotechnology Journal, 5 (3):285-291.

Jha, A.K., K. Prasad, and A.R. Kulkarni, 2009.Synthesis of TiO_2 nanoparticles using microorganisms. Colloids Surf B Biointerfaces, 71 (2): 226–229.

Kirthi, A.V., A. Abdul Rahuman, G. Rajakumar, S. Marimuthu, T. Santhoshkumar, C. Jayaseelan, G. Elango, A. AbduzZahir, C. Kamaraj, and A. Bagavan, 2011. Biosynthesis of titanium dioxide nanoparticles using bacterium *Bacillus subtilis*. Materials Letters. 65: 2745–2747.

Macwan, D.P., P.N. Dave, and S. Chaturvedi, 2011. A review on nano TiO_2 sol-gel type synthesis and its applications. Journal of Materials Science. 46: 3669–3686.

Makwana, S., R. Choudhary, and P. Kohli, 2015. Advances in antimicrobial food packaging with nanotechnology and natural antimicrobials. International Journal of Food Science and Nutrition Engineering, 5(4):169-175.

Pana, C.A., K. Milan, V. Renata, P. Robert, S. Jana, K. Vladimir, H. Petr, Z. Radek, and K. Libor, 2009. Antifungal activity of silver nanoparticles against *Candida* spp., Biomaterials, 30: 6333–6340.

Philip, D., 2009. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy, 73(2): 374–381.

Prasad, K., A.K. Jha, and A.R. Kulkarni, 2007. *Lactobacillus* assisted synthesis of titanium nanoparticles. Nanoscale Research Letters, 2: 248–250.

Rajakumar, G., A. Abdul Rahuman, S.M. Roopan, V.G. Khanna, G. Elango, C. Kamaraj, A. Zahir, and K. Velayutham, 2012. Fungus-mediated biosynthesis and characterization of TiO_2 nanoparticles and their activity against pathogenic bacteria. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. Part A, 91: 23– 29.

Rajesh, R.W., L. Jaya, S.K. Niranjan, D.M. Vijay, and B.K. Sahebrao, 2009.Phytosynthesis of silver nanoparticle using *Gliricidiasepium* (Jacq.). Current Nanoscience, 5 (1): 117-122.

Razzaq, Z., A. Khalid, P. Ahmad, M. Farooq, M.U. Khandaker, A. Sulieman, I.U. Rehman, S. Shakeel, and A. Khan, 2021.Photocatalytic and Antibacterial Potency of Titanium Dioxide Nanoparticles: A cost-effective and environmentally friendly media for treatment of air and wastewater. Catalysts. (11): 709.

Sanghi, R. and P. Verma, 2009. Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresource Technology, 100: 501-504.

Sastry, M., A. Ahmad, M.I. Khan, and R. Kumar, 2003.Biosynthesis of metal nanoparticles using fungi and actinomycete. Current Science, 85:162-170.

Shahab, M., T. Tabish, B. Zaman, Z. Tariq, and M. Kamran 2013. Characterization and synthesis of nanosized TiO_2 particles. Hunedoara International journal of Engineering, 11(3):313-6.

Singh, P., 2016. Biosynthesis of titanium dioxide nanoparticles and their antibacterial property. International journal of chemical, molecular, nuclear, materials and metallurgical engineering.10 (2).

Stoimenov, P.K., R.L. Klinger, G.L. Marchin, and K.J. Klabunde, 2002. Metal oxide nanoparticles as bactericidal agents. Langmuir. 18: 6679-6686.

Subhapriya, S. and P. Gomathipriya, 2018. Green synthesis of titanium dioxide (TiO_2) nanoparticles by Trigonellafoenum-graecum extract and its antimicrobial properties. Microbial Pathology. 116: 215–220.

Sundrarajan, M., K. Bama, M. Bhavani, S. Jegatheeswaran, S. Ambika, A. Sangili, P. Nithya, and R. Sumathi, 2017. Obtaining titanium dioxide nanoparticles with spherical shape and antimicrobial properties using M. citrifolia leaves extract by hydrothermal method. Journal of Photochemical Photobiological B Biology. 171: 117–124.

Tayel, A.A., W.F. EL-Tras, S. Moussa, A.F. EL-Baz, H. Mahrous, M.F. Salem, and L. Brimer, 2011. Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. Journal of Food Safety, 31: 211–218.

Vetrivel, V., K. Rajendran, and V. Kalaiselvi, 2015.Synthesis and characterization of pure titanium dioxide nanoparticles by Sol-gel method. International Journal of Chemistry Technology Research, 7 (3):1090-1097.

Wang, W., Z. Wang, C.M. Wang, *et al*., 2004. Synthesis of rutile (a-TiO_2) nanocrystals with controlled size and shape by low-temperature hydrolysis: effects of solvent composition. Journal of Physical Chemical B, 108:14789–14792.

Yodyingyong, S., 2011. Physicochemical properties of nanoparticles titania from alcohol burner calcination bull. Chemical Society, 25(2): 263-272.

Zhang, H., and G. Chen, 2009. Potent antibacterial activities of Ag/TiO_2nanocomposite powders synthesized by a one-pot sol–gel method. Environ Journal Science Technol. 43(8): 2905–2910.

Ziental, D., B.C. Goslinska, T. Dariusz, Mlynarczyk, Glowacka-Sobotta, A., B. Stanisz, T. Goslinski, and L. Sobotta, 2020.Titanium dioxide nanoparticles, prospects and applications in medicine. Nanomaterials, f10: 387.