World Journal of Science and Research. 1(2): 31-37 (2016)

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research

Research Article Chemistry

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY OF TITANIUM NANOPARTICLES

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ABSTRACT

In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. The world of nanochemistry is a vast world from individual molecules to continual systems that constitute a phase. In the present study aimed to Synthesis, characterization and evaluation of its antibacterial activity of titanium nanoparticles. Titanium nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in Titanium nanoparticles. The appearances of creamy colour in the reaction vessels suggest the formation of Titanium nanoparticles. UV-Visible and FTIR spectroscopy are further confirmed the structural characterization and functional group identification of titanium nanoparticles. The SEM analysis showed the particle size 58.28 nm as well the spherical structure of the nanoparticles. Titanium nanoparticles might be useful for the development of newer and more potent antibacterial agents. All the above data's represented in our study contribute to a novel and unexplored area of nanomaterials as medicine.

***Citation*:** R. Kanagaraj and S. Halimabi Alias Shakila Banu (2016) Synthesis, characterization and antimicrobial activity of titanium nanoparticles. World Journal of Science and Research. 1 (2): 31-37.

Article Info:

Received on 15 April 2016
Accepted on 29 May 2016
Online May 2016

Keywords:

Nanoparticle, Titanium dioxide, Antimicrobial activity, Antioxidant,

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INTRODUCTION

Nanotechnology is becoming a new area of increasing research and industrial interest since the 1980. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually working by the use of physical or chemical principles to develop a myriad of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others (Contescu and Putyera, 2009).

Nanochemistry or Nanotechnology are related with the production and the reactions of nanoparticles and their compounds. It is concerned with the unique properties associated with assemblies of atoms or molecules on a scale between that of the individual building blocks and the bulk material (from 1 to 1000 nm). This science use methodologies from the synthetic chemistry and the material's chemistry to obtain nanomaterials with specific sizes, shapes, surface properties, defects, self-assembly properties, designed to accomplish specific functions and uses. Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology (Prabhu *et al*., 2010).

Different methods for metallic nanoparticle synthesis

Several methods are used for synthesis of nanoparticles (NPs) such as physical, chemical, enzymatic and biological

Types of nanoparticles

Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs (Xu, *et al*.,2005).

Methods for titania nanoparticle synthesis:

Titanium dioxide, also known as titania, is a naturally occurring oxide of titanium. The properties of titanium dioxide includes high refractive index, light absorption, non-toxicity, chemical stability and relatively low-cost production. Titanium dioxide nanoparticles have attracted attention in the fields of environmental purification, solar energy cells, photocatalysts, gas sensors, photo electrodes and electronic devices. It has been widely used as a pigment in paints, ointments, toothpaste etc. Surface area and surface to volume ratio increase dramatically as the size of material decreases (Kusumawardani *et al*., 2010; Djouadi *et al*., 2011).

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes Kruis, *et al*., (2000). It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source (Jung, *et al*.,). The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This makes possible the formation of small nanoparticles in high concentration. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment Kruis, *et al*., (2000).

Titanium nanoparticles could be synthesized by laser ablation of metallic bulk materials in solution. The ablation efficiency and the characteristics of produced nano Ti depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique Tsuji *et al*., (2002).

Synthesis of titanium dioxide nanoparticles by different methods

Titanium dioxide has received great attention due to its unique photocatalytic activity in the treatment of environmental contamination. But for practical application, the photocatalytic activity of TiO_2 needs further improvement. An efficient way to improve the TiO_2 photoactivity is to introduce foreign metal ions (surface modifications) into TiO_2, which is also called heterogeneous photocatalysis. The sol-gel process is the most attractive method to introduce foreign metal ions into TiO_2 powders and films. Several different methods have been developed for generating titania nanoparticles.

Following are the methods:

1. Titania particles are often synthesized in industries by digesting or eliminate with sulfuric acid, followed by thermal hydrolysis of Titanium (IV)-ions in a highly acidic solution and eventually carrying out a dehydration of the Titanium (IV) hydrous oxide (X. Jiang, Herricks *et al*. 2003). The particles obtained with this method are often irregular in shape and exhibit broad distribution in size. Recently, several techniques have been reported for synthesizing monodispersed powders through controlled nucleation and growth processes in dilute Titan(IV)-oxide solutions (Masaru Yoshinaka, Ken Hirota *et al*. 1997; Jean and Ring 2002).
2. The most common procedures have been based on the hydrolysis of acidic solutions of titanium (iv) salts, gas-phase oxidation reactions of $TiCl_4$ (Matijevic, Budnik et al. 1977) and hydrolysis reactions of titanium alkoxide (Jean and Ring 2002) . However, powders produced by these methods have generally lacked the properties of uniform size, shape and unagglomerated state desired.
3. Monodispersed spherical titania oxide particles were prepared by controlled hydrolysis of titanium tetraethoxide in ethanol (Eiden-Assmann, Widoniak et al. 2003). In some cases, the titania nanoparticles can be made by reaction in aerosols (Salmon and Matijevic 1990). The TiO_2 aerogels were obtained by using a supercritical drying gel method (Novak, Knez *et al*. 2001).
4. Using a variation of this approach, (Yaacov Almog, Shimon Reich *et al*. 1982) have successfully prepared monodispersed polymer particles in the range of 1-6 microns. Their method involves the use of a polymeric steric stabilizer in combination with a quaternary ammonium salt which, the authors claimed acts as an electrostatic co-stabilizer. Production of titania particles from an alcoholic solution of titanium tetra alkoxide using

an amine-containing additive and water to hydrolyze said titanium alkoxide solution is another alternative method.

5. Another approach to preparing micron size particles is by dispersion polymerization. This method has been very thoroughly reviewed by (Barrett 1997) and it has been shown to produce particles with a very narrow size distribution. The process involves the polymerization of a monomer dissolved in a medium in the presence of a graft copolymer dispersant (or its precursor) to produce insoluble polymer dispersed in the medium.

6. The TiO_2 occurs in three different crystalline polymorphs: rutile (tetragonal), anatase (tetragonal), and brookite (orthorhombic). These phases of TiO_2 has been studied widely because of its potential applications mainly in photoelectric conversion in solar cells (O'Regan and Gratzel 1991; Bach, Lupo et al. 1998). The dye-sensitized TiO_2 was used for solar energy conversion in photoelectrochemical cells (Nazeeruddin,Kay *et al*. 2002).

7. Several works have been carried out for the synthesis of TiO_2 nanoparticles, such as microemulsion-mediated hydrothermal (Wu, Long *et al*. 1999), hydrothermal crystallization (Yang and Gao 2005; Zhu, Lan *et al*. 2005).

8. Hydrothermal synthesis is a soft solution for chemical processing which provides an easy route to prepare a well-crystalline oxide under the moderate reaction condition, i.e. low temperature and short reaction time (Pookmanee, Rujijanagul *et al*. 2004). By switching to sol-gel precursors with significant lower hydrolysis rate, it is possible to produce titania spherical colloids with narrow distribution in size. Spherical monodispersed particles have been synthesized in this regard by using a precursor, $Ti(OPr)_3$ (acac), derived from the modification of $Ti(OPr)_4$ with acetyl acetone (acac) (X. Jiang, T. Herricks *et al*. 2003).

Titanium dioxide

Titanium dioxide (TiO_2) has become part of our everyday lives. It is found in various consumer goods and products of daily use such as cosmetics, paints, dyes and varnishes, textiles, paper and plastics, food and drugs, and even paving stones. 4.68 million tons of titanium dioxide were produced worldwide in 2009 (PR web. com (NE) 2010); 1,5 million tons/year are produced in the European Union (Stand letzer zugang, 2011). Production was even higher before the financial crisis in 2007 and 2008.

Applications of titanium oxide photocatalyst:

Photocatalysis refers to the chemical reaction that occurs when light strikes a chemical compound that is light sensitive, such as titanium oxide. When light strikes titanium dioxide, a chemical reaction repeated in the immediate region and causes the breakdown of organic toxins, odors, and more. This reaction has many valuable results; several of important applications. TiO_2 causes remove environmental pollution substances, such as NOx emitted by exhaust gas etc. from the atmosphere. SOx, a detrimental inorganic matter in the atmosphere is also broken down. In the present study aimed to Synthesis, characterization and evaluation of its antibacterial activity of titanium nanoparticles.

MATERIALS AND METHODS

Experimental

Titanium dioxide (TiO_2) and Trisodium citrate ($Na_3C_6H_5O_7$) [Loba Chemi, India] have been used in the synthesis of titania nano particles.

Experimental procedure

Titania nanoparticles synthesized by the method of Hema *et al*., 2013). A quantity of 1% TiO_2 was dissolved in 50 mL of distilled water and subjected to stirring at a temperature of 45^{o}C. Then 4% trisodium citrate was dissolved in 50 mL of distilled water which was then added drop wise into the reaction media with a feed rate of 0.42 mL/min to maintain the ratio of titania and trisodium citrate as 1:4. The stirring was continued for 2 h. The temperature of 45^{o}C was maintained till the end of the reaction. Drying was carried out using a conventional oven at 110^{o}C for 24 h to obtain titania nanoparticles.

Characterization of titanium dioxide nanoparticles

UV –Visible spectra analysis

The reduction of pure Ti+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium and the absorption spectra were recorded over the range of 300-700 nm using UV-Vis spectrophotometer (VARIAN CARY EL06023680).

Fourier transform infrared Spectroscopy

To determine Fourier transform infra-red (FTIR) pattern of the TiO2 nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FTIR Spectrum BX (Wellesley, MA, USA).

SEM analysis of silver nanoparticles

The scanning electron microscopy (SEM) analysis of freeze dried sample was performed by mounting nanoparticles on specimen stubs with double-sided adhesive tape and coated with platinum in a sputter coater and examined under JEOL 63861 SEM (Japan) at 10 kV.

In vitro antioxidant activity

DPPH radical-scavenging activity (2,2-diphenylpicrylhydrazyl) DPPH radical-scavenging activity was determined by the method of Shimada, *et al*., (1992).

Determination of antibacterial activity

The antibacterial activity was performed by nutrient agar plate method (Cormican *et al*., 1996).

Antibacterial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007). Petri plates were prepared by pouring 30 ml of NA medium for bacteria The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar. Briefly, inoculums containing bacteria were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample (30µl and 30µl for standard) was laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

Synthesis of Titanium dioxide nanoparticles

The synthesis of titanium dioxide nanoparticles through trisodium citrate were carried out. Titanium is used as reducing agent and has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous Titanium when exposed to trisodium citrate was reduced in solution, there by leading to the formation of Titanium hydrosol. The time duration of change in colour varies from chemical to chemical. It is well known that Titanium nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in Titanium nanoparticles. The appearances of creamy colour in the reaction vessels suggest the formation of Titanium nanoparticles (Shankar *et al*., 2004).

Fig 1: Photographs of Titanium nanoparticles colour formation

Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of titanium nanoparticles. It is quite sensitive to the presence of titanium colloids because these nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation. UV-Vis spectra from synthesized TiO_2 nanomaterials show the absorbance peak to be below 350 nm. (Hayle *et al*., 2014). With increasing particles size, the plasmon absorption shifts toward red. The adsorption spectra of the yellow titanium solution (Figure 2) prepared by trisodium citrate reduction shows the surface plasmon resonance at about 330 nm, indicating the presence of spherical and roughly spherical Ti nanoparticles with an average size of 58.28 nm as confirmed by SEM photographs.

UV/Visible spectroscopy can be used as a characterization technique that provides information on whether the nanoparticle solution has destabilized over time. The optical properties of titanium nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighbouring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions. It is observed that the maximum absorbance of titanium nanoparticles occurs at 330 nm. As the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates). The rapid and irreversible change in the extinction spectrum clearly demonstrates that the nanoparticles are agglomerating. In the present investigation the peak was decreased due to destabilization of nanoparticles (Fig 2).

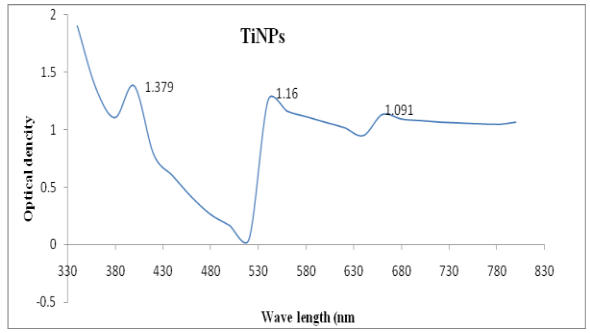


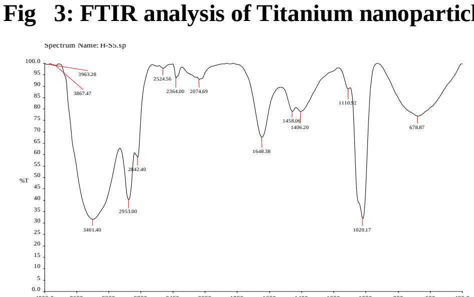
Fig 2: The UV Visible spectrum of TiNPs

FTIR Analysis

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. Fourier Transform Infrared (FTIR) spectroscopy can effectively be used to measure the particle formation. It is found that the width and intensity of peaks in an IR spectrum have explicit dependence on the particle size. As particle size increases, the width of the peak decreases and intensity increases (Pacios *et al*., 2007).

The FT-IR spectra of TiO2 nanoparticles as prepared and given in Fig. 4. Many absorption bands belong to the organic groups such as OH and alkane were appeared. In TiO_2 as prepared sample, between 3800 to 3000 cm-1 a broad band was observed which related to stretching hydroxyl (O-H), representing the water as moisture. The other peaks at 1648 cm-1 were indicated to stretching of titanium carboxilate, which formed from trisodium citrate as precursors. The peak between 800 and 450 cm-1 was assigned to the Ti- O stretching bands. Only the strong absorption between 800 and 450 cm-1 was remained, which attributed to formed of TiO_2 nanoparticles (Li Chena *et al*., 2010; Nasr *et al*., 1996; Music *et al*., 1997).

Fig 3: FTIR analysis of Titanium nanoparticles



SEM analysis

SEM analysis was carried out to understand the topology and the size of the Ti-NPs, which showed the synthesis of higher density polydispersed spherical Ti -NPs of various sizes. The SEM image showing the high density Titanium nanoparticles synthesized by the trisodium citrate further confirmed the development of titanium nanostructures. Most of the nanoparticles were scattered, as observed under SEM. The SEM analysis showed the particle size 58.28 nm as well the spherical structure of the nanoparticles (Fig 4).

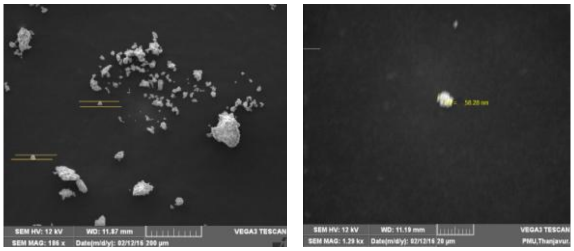


Fig 4: High resolution scanning electron microscopic (SEM) image of Titanium nanoparticles (TiNPs). Spherical TiNPs size 58.28 nm.

DPPH Assay

DPPH radical scavenging activity of TiNPs and standard as ascorbic acid. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al*., 2003). Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). The antioxidant activity was increased with increase the concentrations. Antioxidant activity of TiNPs is close to the standard as ascorbic acid.

Table 1: DPPH radical scavenging TiNPs

Concentrations	TiNPs	Ascorbic acid (Standard)
20µl/ml	17.78 ±1.24	25.6±2.04
40µl/ml	37.78 ±2.64	61.26±4.90
60µl/ml	68.18 ±4.77	88.98±7.11
80µl/ml	87.98 ±6.15	99.34±7.94
IC_{50} (µl/ml)	35.03	45.56

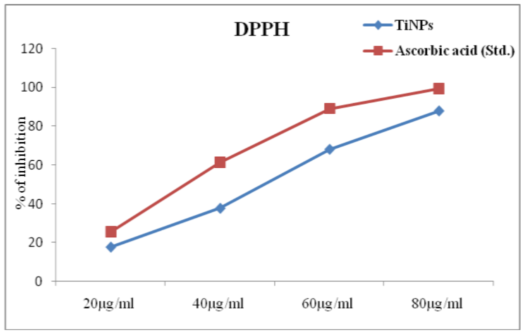


Fig 5: DPPH radical scavenging TiNPs

Antibacterial activity

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of TiNPs has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Torresdey *et al*., 2003). The biologically synthesized Titanium nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The TiNPs shows highest antibacterial activity was observed against Escherichia coli and Staphylococcus aureus. The inhibitory activities in culture media of the Ti nanoparticles reported in table were comparable with standard antimicrobiotic viz. chloromphenical.

In this study, to evaluate the antibacterial effects Ti nanoparticles against various bacterial strain such as Escherichia coli and Staphylococcus aureus.. There were distinct differences among them. When Ti nanoparticles were tested they effectively inhibited bacterial growth. The results show that Ti nanoparticles having antibacterial activity against *E. coli* that was similar to that found by Sondi and Salopek-Sondi (2004).

Escherichia coli can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In some cases, virulent strains are also responsible for haemolyticuremic syndrome, peritonitis, mastitis, septicaemia and pneumonia. Staphylococcus aureus is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections, such as cellulitis, impetigo, and folliculitis (Todar, 2007).

In contrast, the inhibitory effect of Ti nanoparticles was mild in *S. aureus* as compared with other microorganisms; these results suggest that the antibacterial effects of Ti nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms was inhibited by the NPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less titanium to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al*., 2011).

The efficacy of the Ti nanoparticles against *Escherichia coli* and *Staphylococcus aureus* may derive from the difference as a point of membrane structure. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ti nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ti nanoparticles as an antibacterial agent. The TiNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications.

Table 2: Antibacterial activity of TiNPs, TiO_2 and Control

Microorganism	Titanium dioxide (30µl)	Titanium dioxide nanoparticles (30µl)	Standard (Chloramphenical) (30µl)	Control (Distilled water - 30µl)
Escherchia coli (mm)	0.78 ±005	3.73 ±0.26	6.21 ±0.43	0
Staphylococcus auerus (mm)	0.42 ±0.02	2.97 ±0.20	6.11 ±0.42	0

Values were expressed as Mean ± SD

Escherichia coli ***Staphylococcus auerus***

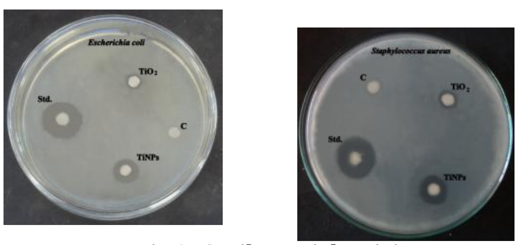


Fig 6: Antibacterial activity

Titanium dioxide (TiO_2) nanoparticles is a promising material, widely used in many applications due to its high photo catalytic activity, excellent gas-sensitive properties, dielectric properties, high stability, low cost and non-toxicity. The unique optical property and chemical stability of titania makes it well suited in the splitting of water and in the photo-oxidation processes. As nanosized particles, these materials exhibit broad band UV absorption, a benefit that currently has been exploited in sunscreen applications. Also, the addition of nanoparticles would likely enhance the stiffness, toughness and service life of polymeric materials. The present study included the chemical reduction of titanium ions through trisodium citrate and testing for their antibacterial activity.

It is confirmed that titanium nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. Applications of Ti nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

CONCLUSION

The present study exhibit a simple method of synthesis of titanium nanoparticles from a novel primitive chemical source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. Since the nanoparticles thus synthesized shows antimicrobial activity, they can be used in the field of pharmaceutical industry. Titanium nanoparticles might be useful for the development of newer and more potent antibacterial agents. All the above data's represented in our study contribute to a novel and unexplored area of nanomaterials as medicine.

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Source of support: Nil;

Conflict of interest: None declared