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Research Article

BIOSYNTHESIS OF TITANIUM DIOXIDE NANOPARTICLES USING *VIGNA RADIATA*

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ABSTRACT

Objectives: Nanotechnology holds an important area in recent research due of its immense use in the different field of sciences. The small size, large surface area, orientation, and physical properties make them appropriate to be used in medical sciences, physics, and chemistry. Physical and chemical processes of synthesizing nanoparticles are continued for ages. The biological approaches to preparing nanoparticles have drawn the attention of researchers due to eco-friendly nature, low cost, and easier steps for synthesis. To synthesize titanium nanoparticles, *Vigna radiata* legumes were powdered and used, and the effectiveness of biologically synthesized titanium nanoparticles against clinical pathogens and anticancer activity were checked.

Methods: Green synthesis of titanium dioxide nanoparticles is carried out in simple steps. The extract of *V. radiata* legumes was used for the biological synthesis of the titanium dioxide nanoparticles which was characterized by Fourier transform infrared spectroscopy and scanning electron microscopy. Antibacterial activity of the titanium dioxide nanoparticles was checked against nine clinical pathogens. 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay was performed to determine the antioxidant activity of the nanoparticles. Cytotoxic activity against osteosarcoma cell lines was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Results: Oval shaped titanium nanoparticles were biologically synthesized. The particles showed effectiveness against both Gram-positive and Gram-negative bacteria. The particles exhibited antioxidant nature. Cytotoxicity assay revealed the nanoparticles were capable of inhibiting proliferation of osteosarcoma cell lines.

Conclusions: *V. radiata* has been confirmed to be a good source for biosynthesis of titanium nanoparticles. The cytotoxicity effect of the nanoparticles hints that it can be further used for treatment and medicine purposes.

Keywords: Green synthesis, Titanium dioxide, *Vigna radiata*, Fourier transform infrared spectroscopy, Scanning electron microscopy, 2,2-diphenyl-1-picryl-hydrazyl-hydrate, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

INTRODUCTION

An overwhelming interest on the studies with nanomaterials has been noticed in the recent days. Nanoparticles exhibit remarkable physicochemical and optoelectronic properties due to bounding electrons within their 1 nm dimensions. The applications of nanomaterials are now extended to areas of optoelectronics, catalysis, reprography, light emitters, and single electron transistors [1]. Due to increased surface area of nanoparticles, the area of interaction with pathogenic bacteria increases resulting in making them suitable as antimicrobial agent. The small size of the particles enables them to easily enter bacterial surface and capable of harming them [2].

Many researches on biosynthesis of silver and gold have been done over the years. Both gold and silver nanoparticles are found to have immense applications in various fields. Silver nanoparticles synthesized using chili seeds extracts were found to be very effective against pathogenic bacteria as well as showed antioxidant characteristics which were capable of inhibiting the proliferation of MG 63 osteosarcoma cell lines [3]. The applications of gold nanoparticles in the generation of biosensors, DNA labeling is well known [4]. Not many works have been reported on the biosynthesis of rare metal nanoparticles such as lanthanum and titanium. Titanium dioxide nanoparticles are used in the field of photocatalysts, cosmetics, and pharmaceuticals [5]. The titanium nanoparticles have the capability of reacting with O_2 and -OH, which are adsorbed on the surface for obtaining oxygen and hydroxyl free radical [6].

Green synthesis of nanoparticles from plant extracts and microbes has attracted attention of researchers in recent years. Biosynthesis of nanoparticles is considered better than chemical synthesis because of formation of toxic chemical species which is adsorbed on the particle surface after chemical synthesis. This makes the nanoparticles improper for medical applications. Moreover, they are cost effective and environmental friendly in nature due to a biological process, which makes them superior than chemical and physical process of synthesis [7]. Fruits such as blackberry, blueberry, and pomegranate are used in synthesizing gold and silver nanoparticles [8]. Titanium nanoparticles are biosynthesized using bacteria such as *Lactobacillus crispatus* [9] and *Bacillus subtilis* [5]. Among plants, usage of *Nyctanthes* arbor-tristis leaves extract [10] and *Psidium guajava* leaves extract [11] in preparation of titanium nanoparticles has been recorded.

The study deals with the synthesis of titanium dioxide nanoparticles using *Vigna radiata* (green gram) legumes extract and evaluation of the characteristics of nanoparticles. *V. radiata* seeds were chosen for the experiment because sprouted green gram seeds have strong reducing capacity with dynamic antioxidants properties [12].

METHODS

Collection of green gram *(V. radiata)*
The green gram seeds were purchased from Vellore market, Tamil Nadu.

Chemicals used
All chemicals used including titanium oxide were purchased from Sigma-Aldrich.

Collection of bacterial pathogens
Nine clinical pathogens were collected from Microbial Biotechnology Laboratory, VIT University, Vellore. The pathogens included *Escherichia*

coli, *Staphylococcus aureus*, *Serratia marcescens*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Proteus mirabilis,* and *Shigella* sp. The pathogens were used to check the antimicrobial activity of the titanium oxide nanoparticles synthesized using *V. radiata* seed extracts.

Preparation of the extract

The green gram seeds were washed and soaked in water. The sprouted green gram was then taken from water and dried in shade. 10 g of dried sprouted green grams were crushed and boiled with 100 ml of distilled water for 30 minutes. The extract was brought to room temperature, filtered and stored for biosynthesis of titanium dioxide nanoparticles [13].

Biosynthesis of titanium dioxide nanoparticles using green gram seeds

About 1 mM titanium dioxide aqueous solution was prepared and kept in orbital shaker for 2 hrs. 20 ml of filtered extract of green gram was mixed with 80 ml of 1 mM TiO_2 solution at room temperature and stirring for 24 hrs [11]. After 24 hrs of incubation, the solution was filtered, and the residues were dried and calcined to obtain the titanium dioxide nanoparticles. The nanoparticles were powdered and characterized and further analysis was carried out.

Characterization of titanium oxide nanoparticles

The nanoparticles were analyzed using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The presence of functional groups and the binding property of the titanium oxide nanoparticles were determined by FTIR. The powdered titanium oxide nanoparticles were added to potassium bromide and recorded for FTIR. The dried nanoparticles were sputter coated with gold before analyzing the structure under SEM. The images of the coated samples were further recorded for analysis [3].

Antibacterial activity of titanium nanoparticles

The effect of biosynthesized titanium nanoparticles was determined by well-diffusion technique against nine clinical pathogens. 6 mm wells were cut on Mueller-Hinton agar swabbed with individual pathogenic bacteria. 4 wells were cut in each plate where 50 µl, 75 µl, and 100 µl of dissolved nanoparticles were added. One well was maintained as control by adding sterilized distilled water. The plates were incubated for 24-48 hrs and checked for the zone of inhibition [14].

Antioxidant assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate [DPPH] assay)

DPPH assay was performed to determine the antioxidant activity of the biologically synthesized titanium nanoparticles. 100 µl DPPH solution prepared using methanol along with 100 µl of titanium nanoparticles were added to 2.8 ml acetate buffer followed by incubating in the dark for 15-20 minutes. The change in color was observed, and absorbance was measured at 517 nm. Ascorbic acid was taken as standard [15].

$$\text{DPPH scavenging percentage} = \frac{\left[\begin{array}{l}\text{Absorbance}(\text{control}) \\ -\text{Absorbance}(\text{sample})\end{array}\right]}{\text{Absorbance}(\text{control})} \times 100$$

Cytotoxicity assay of the titanium nanoparticles

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out for the cytotoxic assay of the synthesized nanoparticles. Cell lines obtained from National Centre for Cell Science, Pune, were grown in Eagles minimum essential medium containing 10% FBS. Optimum conditions of maintaining the cells were followed such as 37°C, 5% CO_2, 95% air, and 100% humidity. The cell dilution was done in a medium containing 5% FBS until the density reaches 1×10^5 cells/ml. 100 µl cell suspension was added to each well of a 96 well plate at a plating density of 10000 cells/well. After 24 hrs of incubation, the cells were treated with serial concentrations of the test samples. Dimethyl sulfoxide (DMSO) was used initially for dissolving sample, and the nanoparticles were dispersed in phosphate-buffered solution (PBS). After adding the sample, the plates were incubated for 48 hrs. Control was maintained without nanoparticles.

About 15 µl MTT in PBS was added to each well and incubated at 37°C for 4 h. The medium with MTT was removed, and formed formazan crystals were solubilized in 100 µl of DMSO. The absorbance was measured at 570 nm.

$$\text{Percentage of cell viability} = \left(\frac{A}{A_0}\right) \times 100$$

Where, A=Absorbance of sample

A_0=Absorbance of control.

Nonlinear regression graph was plotted between cell inhibition percentage and log concentration and IC50 were determined using Graph Pad Prism software [16].

RESULTS AND DISCUSSIONS

Biosynthesis of titanium nanoparticles using green gram seeds extracts

The nanoparticles were synthesized, dried, and crushed to powder.

Characterization of biosynthesized titanium nanoparticles

FTIR

The FTIR spectrum was measured in a range of 500-4000/cm. Peaks observed at 1631.78/cm and 1641.42/cm which is prominent in Fig. 1 indicates O-Ti-O bond [17]. The peak around 3000/cm appeared due to the -OH stretching and the Ti-O stretching vibration is confirmed by the peak at the region of 1400-1460/cm [18]. The peaks present at the range of 1020-1250/cm depicts the existence of aliphatic amines and a small peak at 1552.70/cm represents nitro compounds in the biosynthesized nanoparticles.

SEM

SEM analysis was performed with the titanium nanoparticle to learn the structure of the nanoparticles. Small amount of sample was taken on a copper grid coated with carbon and dried under mercury lamp [19]. The oval shaped smooth surfaced nanoparticle was observed as shown in Fig. 2.

Antibacterial assay

The titanium dioxide nanoparticles were effective against all the clinical pathogens used for the antibacterial tests. Zone of inhibition were observed in the plates and recorded in Table 1. Titanium nanoparticles are capable of dissolving the outer membranes of bacteria due to the presence of hydroxyl groups leading to the death of the organisms [20].

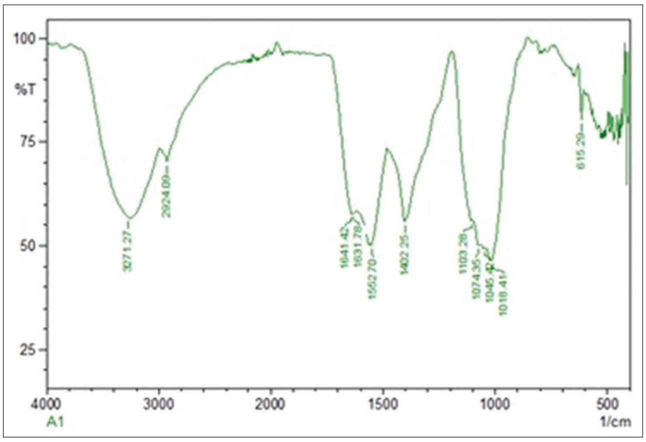


Fig. 1: Fourier transform infrared spectroscopy spectrum of biosynthesized titanium dioxide nanoparticles

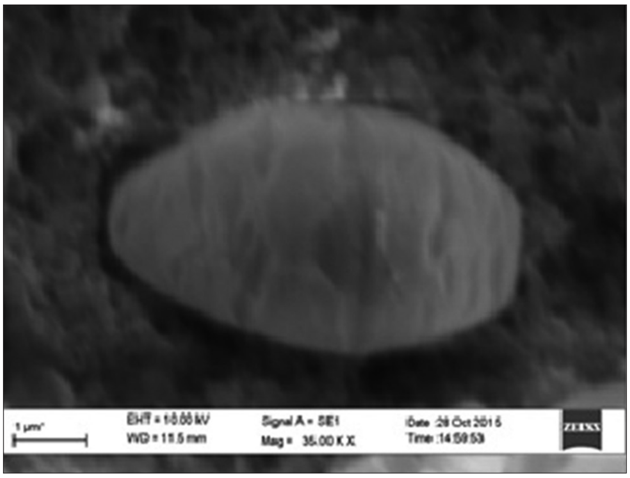


Fig. 2: Scanning electron microscopy image of the biosynthesized titanium dioxide nanoparticles

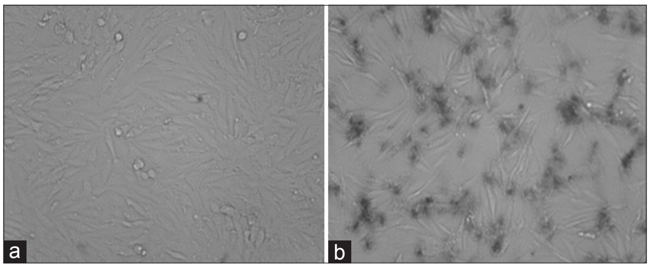
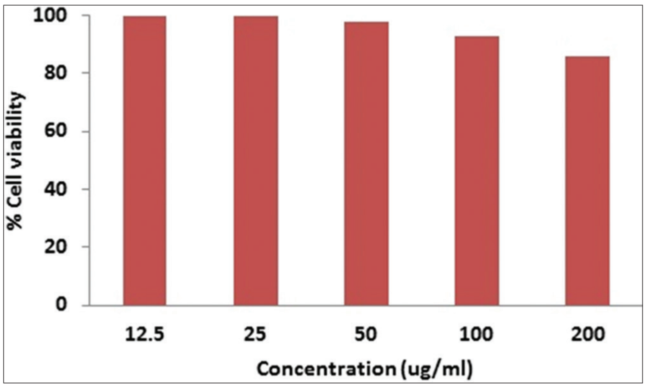


Fig. 3: Cytotoxicity activity of biologically synthesized titanium dioxide nanoparticles against osteosarcoma cell lines (Mg 63): (a) Control, (b) test



Graph 1: The cell viability of Mg63 cell lines when treated with biosynthesized titanium dioxide nanoparticles

Table 1: Antibacterial activity of titanium dioxide nanoparticles against bacterial pathogens

Sl. No	Organisms	Zone of Inhibition (cm)		
		100 µl	75 µl	50 µl
1	*Staphylococcus aureus*	2.8	2.5	2.4
2	*Escherichia coli*	3.3	3	2.9
3	*Enterobacter* sp.	3.2	2.8	2.8
4	*Serratia marcescens*	3.6	3.4	3
5	*Salmonella* sp.	2.8	2.5	1.8
6	*Pseudomonas aeruginosa*	3.2	2.8	2.2
7	*Klebsiella pneumonia*	3	2.8	2.6
8	*Proteus mirabilis*	3.4	3.4	3.1
9	*Shigella* sp.	3.6	3.2	2.9

Antioxidant assay

Free radicals are unstable atoms which are always a hassle for the formation of stable bonds by receiving or donating an unpaired electron. The stable compound DPPH gets reduced by gaining a hydrogen or electron. The change in color in the test sample after 15 minutes incubation indicates the nature of the nanoparticles to be antioxidant and hence the reducing activity of the nanoparticles [21]. The antioxidant activity of the proteins presents in green grams legumes are studied in many previous researches, which result in stronger reducing the activity of the titanium nanoparticles synthesized using green gram legumes extract [22].

Cytotoxicity activity

The titanium dioxide nanoparticles showed cytotoxic activity against the Mg 63 osteosarcoma cell lines. At IC50 value of 200 µg/ml, the osteosarcoma cells proliferation was inhibited at a significant rate. Fig. 3 clearly indicates the difference in osteosarcoma cell lines before and after treatment with titanium dioxide nanoparticles. The cell viability of the osteosarcoma cell lines decreased with increasing concentration of titanium dioxide nanoparticles. Graph 1 shows the changes in percentage of cell viability with concentrations of titanium dioxide nanoparticles. The synthesized nanoparticles can be further incorporated in studies for anticancer drugs. In previous studies, titanium dioxide nanoparticles proved to be cytotoxic on rat embryo fibroblast cell lines [23].

CONCLUSIONS

Titanium dioxide biosynthesized using *V. radiata* seed extracts were characterized by FTIR and SEM, which demonstrates the nature and structure of the nanoparticles. The particles were found to be effective against clinical pathogens due to their hydroxyl bonds present and can be further used in antibiotic drugs. The reducing activity of the particles qualifies them as good antioxidant compounds. The titanium dioxide nanoparticles synthesized were of more importance due to the proliferation of Mg 63 cell lines.

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