CAREER POINT UNIVERSITY HAMIRPUR

CPUH-Research Journal: **2018,** *3(2),* 52-56
ISSN (Online): 2455-6076
http://www.cpuh.in/academics/academic_journals.php

Green synthesis characterization and antimicrobial activity of titanium dioxide nanoparticles from *Azardiachta indica* leaf extracts

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ABSTRACT: Metal nanoparticles are being widely used in various biomedical applications due to their small size to volume ratio and extensive thermal stability. Because of this, working for developing new materials and methods for synthesis and application of nano materials find more important. Among all the methods green synthesis of nano materials is finding much attention because of its simplicity fastness, chemical free nature, nontoxicity, economical approach. The present study synthesized titanium dioxide from the ethanolic neem extracted by reflux method using of titanium isoproxide precursor. The current study was designed to test the antimicrobial potential of Titanium dioxide (400,600 & 800) that is synthesized from plant extract. It is used against the bacterial strains such as *Staphylococcus aureus,Streptococcus pneumonia, Pseudomonas putida, Proteus vulgaris* and fungal strain *Candida albicans*. The antimicrobial activity was tested by Kirby Bauer method. Themost susceptible Gram positive bacterial strains was *Streptococcus Pneumoniae* while Gram negative bacteria was *Pseudomonas aerogenosa* and is also very effective against *Candida albicans.* The antimicrobial activity of this titanium oxide (800) nanoparticle was found to be higher than the standard antibiotic (Tetracycline). These results show the effect of titanium oxide (800) NP's against the tested pathogens. Thus, the nanoparticles showed considerably good activity against the isolates, and it can be concluded that they may act as promising, antibacterial and antifungal agents in the coming year

Keywords: Nanoparticle; titanium dioxide; tetracycline; a*zardiachta indica*

INTRODUCTION

Nano materials find wide applications due to their behaviour at nano scale. Among all the methods of synthesis, biosynthesis of nano materials is finding much attention because of its simplicity, chemical free nature and nontoxicity. In the present study the TiO2 nano particles are synthesized from titanium tetra isopropoxide solution using neem leaves extract. This was calcinated at 3 different temperatures to obtain different structures or forms of TiO2. This then used against the microbes like *Staphylococcus aureus, Streptococcus pneumonia, Bacillussubtilis, Proteus vulgaris, Pseudomonas aerogenosa, E coli* and the fungus *Candida albicans.*

MATERIAL AND METHODS

Material: The plant species *Azardiachta indica* leaf was collected from the plant of 15 m height and semi-straight trunk, in the month of February, 2015 from Mundur village of Palakkad district. (Fig -1). The precursor for the synthesis of Titanium dioxide nanoparticles, Titanium tetra isoproxide was obatained from Sigma Alrich, through The Precision Scientific and Co, Coimbatore. (Fig-4)

Preparation of Extract: Fresh leaves (100 g) of *Azardiachta indica* was weighed and air dried in the shade until disappearance of moisture. The dried leaves grounded to fine powder using a tabletop mixture. 15g air dried powered leaves extracted with 250 ml of ethanol using Soxhlet apparatus. The ethanol evaporates continuously and was recycled, thereby extracting the compounds present in the samples. They were continuously extracted until the extraction is complete (4 hours). After extraction, the mixture was filtered and the extract was stored in airtight containers in refrigerator for future use. (Fig-3)

Synthesis of Nanoparticles: 50 ml of leaf extract was taken and centrifuged at 5000 rpm for 5 minutes and is filtered using Whatman No1 filter paper to obtain a clear solution. 0.4 M Titanium tetra isoproxide was added to the leaf extract and is reacted on a magnetic stirrer under 50^0 C. After 4 hours of continuous stirring, the titanium dioxide nanoparticles were formed. (Fig-5) And the nanoparticles are obtained by the centrifugation of the mixture at 10,000rpm for 15 minutes. The centrifuged particles were washed with ethanol, and are again centrifuged at 5000 rpm for 10 minutes. Separated Titanium dioxide particles were dried and grinted to calcinate at different temperatures (400, 600 & 800^0C) in muffle furnace for 3 hours.

Characterization: Several techniques have been reported worldwide for the characterization of herbal and medicinal based nanoparticles.Green synthesized TiO2 nanoparticles characterization was done at Nanoscience department of Bharatiar University.

1. FTIR

Fourier transform infrared (FTIR) spectral measurements were carried out to identify the potential biomolecules in *Azardiacta indica* leaf extract which is responsible for reducing and capping the bioreduced TiO2 nanoparticles were subjected to FTIR analysis by Potassium Bromide pellet method in 1:100 ratios and spectrum was recorded in Nicolet Impact 500 FT-IR spectrophotometer using diffuse reflectance mode. The FTIR spectra of synthesized TiO2 NPs taken were analyzed, which discussed for the possible functional groups for the formation of nanoparticles.

2. SEM

Scanning Electron Microscope (SEM) analysis was done at Nanoscience department of Bharathiar University,Coimbatore. The analysis done by preparing thin films on a gold coated copper grid by dropping a very small amount of the sample on a grid. Extra solution was removed using a blotting paper and thin films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. TiO2 Nanoparticles were examined with SEM (JEOL JSM 6390) at an accelerating voltage of 20kV and a working distance of 15mm.

3. Raman Spectroscopy:

Raman spectroscopy (The scheme of preparation of TiO2 nanoparticles by Shilpa., 2013). FRA 106/S Raman spectrometer) with an excitation line of 785 nm.

Antimicrobial Study of Green Synthesized TiO2 NP's

1. Source of Microorganisms: Microbial strains obtained from the Microbial Type Culture Collection (MTCC), Chandigarh. Stock cultures of different Gram-negative : *E.coli* (MTCC No :443), *Proteus vulgaris* (MTCC No : 1771) and *Pseudomonas aerogenosa* (MTCC No : 424) and Gram-positive :*Bacillus subtilis* (MTCCNo:10619) , *Streptococcus pneumonia* (MTCC No:2672) and *Staphylococcusaureus* (MTCC No; 96) bacteria and fungus *Candida albicans* (MTCC No : 227)were sub cultured and maintained in nutrient broth / Brain heart Infusion broth & Todd's Hewitt Broth.

2. Screening of Antibiotics: The screening of antibiotics was done to identify the standard antibiotic against the pathogenic organisms like Gram negative (*E. coli, Proteusvulgaris* and *Pseudomonas aeruginosa*) and Gram-positive (*Bacillus subtilis, Streptococcus pneumoniae* and *Staphylococcus aureus*) bacteria and fungus *Candida albicans.* For that; Tetracycline, Erythromycin, Streptomycin andAmpicillin are selected. And that study showed that, Tetracycline is found sensitive against gram positive and gram negative bacteria. For fungus Chloramphenicol showed sensitivity than Amphotericin and thus Tetracyclin is selected against bacteria and Chloramphencol is selected as the standard for fungus in antibiotic sensitivity testing.

Preparation of Titanium Dioxide Samples: Titanium dioxide 400, 600, 700 were weighed separately at different concentrations (150,200 and 250µg) and is dispersed in 1 ml of distilled water by continous heating and vortexing. And these samples were added to the wells after tested to low concentrations (50 and 100µg).

Antimicrobial Assay (Kirby Bauer): The disc diffusion method was used for the antimicrobial assay. Using a sterile cotton swab lawn cultures of the test organisms were made on nutrient agar plates under aseptic conditions. Plates were left standing for 10 minutes to let the culture get absorbed. Then 8 mm wells were punched into the Muller Hinton agar (MHA) plates for testing the antimicrobial activity of titanium dioxide nanoparticles synthesized at various temperatures (400, 600 and 800). 20 µl of each sample (400, 600, 800) at different concentrations (150, 200 & 250) was poured into each wells and is closed using 0.8 % agar. For comparing the activity, standard disks (Tetracycline) are used. The plates are incubated at 27^0C for 12 – 24 hours. After incubation the zone diameter was measured. As control tetracycline against bacteria & Chloramphenicol for fungus were utilized after the screening test.

RESULTS AND DISCUSSION

The formation of browinish colour (Fig-5) in the aqueous extract shows the presence of Titanium dioxide nanoparticles after reacting with titanium tetra isoproxide on the magnetic stirrer for 4 hours at 50^0C and then subjected to centrifugation. The appearance of a brownish colour confirms the presence of TiO2 nanoparticles in the solution. The time duration of change in colour was 4 hours, it will change the colour at the time of stirring.This change in colour of the reaction mixture was taken as a primary evidence for the formation of s nanoparticles. The time duration of change in color and thickness of the color varies from plant to plant and the time of stirring temperature.

CHARACTERIZATION OF GREEN SYNTHESIZED TiO2 NANOPARTICLES

The obtained results of SEM and FTIR studies confirmed the fact that sample powder consists of green synthesized Titanium dioxide nanoparticles from the leaf extract *Azardiachta indica.*

FTIR

FTIR spectroscopy analysis was conducted to identify the biomolecules responsible for capping of the bioreduced TiO2 NPs synthesized using plant extract. Fig. 10 shows the synthesized TiO2 NPs using neem leave extract. Where the absorption peaks were located at 880.91 (Ti–O–O bond), 1,087.13 & 1,045.96 (C–N stretch aliphatic amines) 1,329 & 1,381 (C–O stretching vibrations in alcoholic groups), 1,662 (N–H bend bond), and 3,327 (O– H stretching due to alcoholic group).

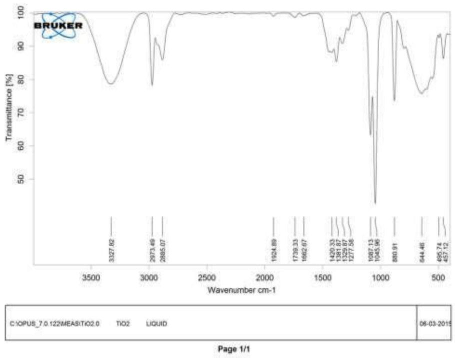

Figure 1: FTIR spectra of green synthesized TiO_2 nano particles

SEM: The formation of titanium dioxide nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the appearance for the pre ence of TiO_2 nanoparticles synthesized from *Azardiachta indica*. The synthesized nanoparticles show below 4µm in size. The SEM image shows cluster of nanoparticles at different sizes. The SEM studies cofirmed the formation of TiO_2 nanoparticles

Raman Spectroscopy: The intensity variation for an irradiated sample is attributed to the recombination of self-trapped excitons, which is a combined effect of defect centers generated by oxygen vacancies and small particle size, leaving behind unpaired charges to enhance photoactivity. Moreover, the high energy electron beam irradiation of suitable dose rate can promote the photoactivity of TiO_2 nanoparticles. Several factors like phonon confinement strain defects and nonstoichiometry due to EB irradiation can contribute to changes in the peak position, intensity and width of Raman bands in anatase phase TiO_2 nanoparticles, which in turn promote the photo activity of TiO_2 nanoparticles. Raman spectroscopic studies of TiO_2 nanoparticles are of great significance because of the band broadening and shift of Raman bands with particle size variation. The Raman spectra of TiO_2 were (Narayanan.,1950) Since then, a number of papers have reported in the literature discussing the lattice dynamics and the assignment of the observed Raman bands for the structure.

The variation in the Raman bands with a phonon confinement model. As the particle size decreases, the phonon is increasingly confined withinthe particle and the phonon momentum distribution increases. This broadening of the phonon momentum leads to a broadening of the scattered phonon momentum according to the law of conservation of momentum. This phonon dispersion causes asymmetric broadening and may lead to a shift of the Raman bands (Narayanan.,1950). On the basis of factor group analysis, the anatase has six Raman active modes.Raman spectra for anatase TiO2 nanoparticles of pure and irradiated samples are shown in Fig- 11. All the six Raman modes were observed in the range 100–1000 cm^{-1} at room temperature. The most intense band observed at 147 cm^{-1} arises from the optical vibration mode *Eg* . Other five clear modes at 198 cm^{-1}, 400 cm^{-1}, 514 cm^{-1}, 519 cm^{-1} and 641 cm^{-1} arise from the optical vibration modes E_g , *Big* and E_g respectively.

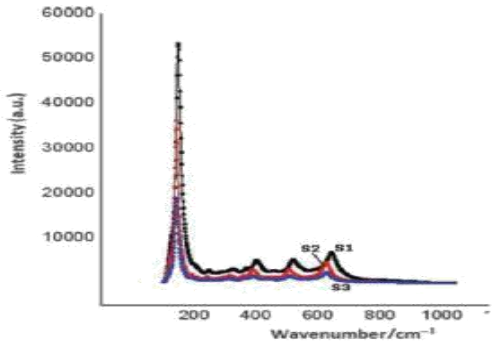

Figure 2: Raman spectra of pure and irradiated anatase TiO_2 samples

The Raman peaks at 147 and 198 cm−1 areconsistent with Ti Ti bonds present in the octahedral chains. The observed Raman bands at 514, 519, 400 and 641 cm−1 are originated from Ti O bonds. Present results are slightly higher than the reported values for bulk sample, and this variation might occur due to the nano-scale size of the samples.The Raman peaks shift towards a higher wavenumber due to the increasing force constants.It is also noticed that the general features of the Raman spectra of irradiated samples have a strong resemblance to those of the pure sample, with a reduction in the intensity of the peaks. This intensity decrease could be due to the reduction in particle size caused by the EB irradiation. The volume contraction effect, due to the particle size reduction, reduces the vibrational amplitudes of the nearest neighbour bonds due to increase of the mean square relative displace ment, (Kim., 2010) which can be interpreted as a measure of the static disorder and thermal vibrational disorder of the material caused by the EB irradiation.

This decreasing vibrational amplitude affects the intensity of the Raman peaks.

Table 1: Comparison of Raman modes of Anantase TiO_2 samples with literature

Mode	Present values			Literature Values [18]	Calculated Values [24]
	S1	S2	S3		
Eg	147	143	143	143.4	147
Eg	198	193	193	196.2	197
Big	400	389	390	396.5	397
AIg	514	-	-	-	-
Big	519	515	515	517.7	517
Eg	641	626	627	639.4	640

The Titanium dioxide synthesized at three different temperatures was used for checking the antibiotic sensitivity at three different concentrations (150 µg, 200µg & 250µg). The TiO2 400 shows the sensitivity to *Streptocouus pneumonia* (2.3 cm) and *Pseudomonas aerogenosa* (2.2 cm), TiO2 600 shows sensitivity to *Staphylococcus aureus* (2.2 cm), *Streptococcus pneumonia* (2.3 cm) and *Pseudomonas aerogenosa* (2.1 cm) The antimicrobial activity of TiO2800 wasfound to be higher sensitivity zone than the standard antibiotics (Tetracycline & Chloramphenicol) for all pathogens (Table-1). The nanoparticles showed considerably good activity against *Streptococcus pneumonia* (2.1 cm) (Fig-12) and *Pseudomonas aerogenosa* (2.4 cm) (Fig-15) and *Candida albicans* (2.1 cm) (Fig-it can be concluded that they may act as promising, antibacterial and antifungal agents in the coming years. TiO2 NPs are the mostly studied for their photocatalytic antimicrobial activity among various NPs.

Table 2:

Organisms	**TiO_2 400**				**TiO_2 600**				**TiO_2 800**			
	Control	**Concentrations (µg)**			**Control**	**Concentrations (µg)**			**Control**	**Concentrations (µg)**		
		150	200	250		150	200	250		150	200	250
Streptococcus pneumoniae	3.5	-	2.1	2.3	3.4	-	1.9	2.3	3.4	-	1.8	2.1
Staphylococcus aureus	3.1	0.6	1.9	2.1	3.2	0.6	1.8	2.2	2.7	-	1.6	2.1
Bacillus subtilis	3.3	-	1.4	1.6	3.2	-	1.6	1.8	32	-	1.4	1.8
Proteus vulgaris	2.1	-	1.5	1.9	1.6	-	1.7	2	1.7	-	1.6	1.7
Pseudomonas putida	2.1	1.1	2.0	2.2	2.4	-	1.8	2.1	2.1	-	2.2	2.4
E coli	2.3	-	1.6	1.8	2.3	-	1.4	2	2.2	-	1.3	1.6
Candida albicans	3.4	-	2.2	2.5	4	0.6	2.4	2.6	3.2	-	2	2.1

In their study suggested that TiO2 NPs failed to exhibit antibacterial activity, but upon combination with antibiotics they were able to inhibit the growth of microorganisms. But here, TiO2 NPs without any kind of combination inhibited the growth of MRSA isolates with a maximum zone of 14mmat 500 lg/ml and a minimum zone of 11 and 12 mm at 100 lg/ml against strong and weak isolates. (Zhang et al., 2009) have suggested the possible mechanisms involving the interaction of nanomaterials with the biological molecules. The author documented that microorganisms carry a negative charge while metaloxides carry a

positive charge which creates an ‘‘electromagnetic’’ attraction between the microbe and treated surface concluding that once the contact is made, the microbe is oxidized and dead instantly (Zhang and Chen., 2009).

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