Synthesis and Characterization of TiO₂ Nanoparticles Using Cynodon Dactylon Leaf Extract for Antibacterial and Anticancer (A549 Cell Lines) Activity

Abstract

The synthetic techniques, for the synthesis of Metal oxide nanoparticles involve toxic solvents, high pressure, high-energy and high temperature which may be efficient but not eco-friendly. The synthetic techniques for metal oxide nanoparticles using plants, has several advantages over the chemical synthesis such as cost effectiveness, simplicity as well as compatibility for medical applications. In this part of the study we are using the cynodon dactylon leaf extracts to synthesize titanium dioxide nanoparticles. The properties of TiO₂ include high refractive index, light absorption, non-toxicity, chemical stability and relatively low cost production. The cynodon dactylon leaf has reduced the size of TiO₂ nanoparticles. In the present study antibacterial effect of TiO₂ nanoparticles on E. coli strain was analyzed. The well diffusion assay is used to confirm the inhibition zone of bacteria. Further the characteristics of the obtained TiO₂ nanoparticles were studied using XRD, FTIR, Laser Raman spectroscopy, Scanning Electron Microscopic and the results are presented in detail.

Keywords: TiO₂; Cynodon dactylon; Antimicrobial activity

Introduction

The nanoparticles are different from the large particles of the same composition due to their large surface area and volume ratio. The metal oxide nanoparticles have received considerable attention on medical line due to their antibacterial properties, resistance against microbes, drug delivery, antibiotics and immune chromatography, tissue / tumour image, anticancer activities and identification of pathogens in clinical specimens [1-2]. TiO₂ is the most promising material in the group of the metal oxides. In n-type semiconductor, titanium dioxide is a most important semiconductor due to its light absorption, charge transport properties [3]. TiO₂ has three crystal structures namely anatase, rutile, and brookite. In three phases, the anatase phase has got applications in photo-voltaic cells (Fujima and Donald 2000), photo catalysts and more applications for its antimicrobial properties [4].

Among all cancers, lung cancer is leading death cancer in the world wide [5]. A549 lung carcinoma non small cell lines is an adenoma lung cancer cell lines, which is widely used to study the investigation of cytotoxicity, when nanoparticles induce the cell line [6]. Recently TiO₂ nanoparticles used for the several types of cancers are MCF 7 cell lines [7], A549 cell lines [8], HeLa cell lines [9]. TiO₂ nanoparticles induce in the cell lines, and then ROS (Reactive Oxygen species) is produced. This ROS has damaged in the DNA of apoptosis and necrosis [10].

To synthesis titanium dioxide nanoparticles by chemical methods. In the prepared TiO₂ nanoparticles are toxic, flammable and not so eco-friendly. But, the green synthesis of TiO₂ nanoparticles using plant extracts has several advantages over chemical synthesis such as cost effective, simplicity, non-toxic as well as its compatibility for medical applications [11-13]. In this part of the study we are using the Cynodon Dactylon leaf extracts to synthesis titanium dioxide nanoparticles. Cynodon dactylon leaf is a member of the gramineae family. In herbal medicine, it shows a lot of medical applications such as anti diarrheal, antioxidant, anti diabetic, anti cancerous, antimicrobial, immunomodulator, and germicidal. It also contains mineral constituents, crude proteins and chemical constituents like linolenic acid, hydro quinine, and hexadecanoic acid. It also shows the DNA protective activity [14,15]. In our research report, the small sized titanium dioxide nanoparticles was obtained, using of the Cynodon dactylon leaf extract and it is found to show a good anti bacterial and anticancer activity against E. Coli and A549 cell lines.

Materials and methods

Preparation of TiO₂ nanoparticles

Fresh and healthy leaves of Cynodon dactylon were collected from the campus of Bharathidasan University, India. The leaves were washed several times with deionised water. Next 20g of thoroughly washed leaves were boiled with 150ml of deionised water. The boiled suspensions were filtered through whatman no 1 filter paper. The final extract solution was collected and stored at 4°C for the synthesis of TiO₂ nanoparticles. The Erlenmeyer...
flask containing 0.1M of Titanium tetra isopropoxide in 100 ml of leaf extract solution was reacted under stirring at 50°C for one hour. The above solution was heated on a hot plate at 80°C for two hours. The obtained product was poured into the powder from and calcinated in muffle furnace at 500°C for 5 hrs. The obtained TiO₂ powder is named as sample A. The Cynodon dactylon leaf was heated at 30°C for one hr, finally the obtained product is calcinated in a muffle furnace at 500°C for 5 hrs. The obtained powder named as sample B.

**Characterization of TiO₂ nanoparticles**

Powder X-ray diffraction analysis was performed using XPERT PRO equipped with CuKα radiation. The morphology of the titanium dioxide nanoparticles was characterized using Field Emission Scanning Electron Microscope (FEI- QUANTA – FEG 250) and HRTEM ([JED JEM 2100]). Fourier Transform Infrared Spectra were recorded over the range of 400 - 4000 cm⁻¹ using a Perkin Elmer 100 spectrophotometer. The structural property was also investigated using Raman spectroscopy, Renishaw, in the range of 100-700 cm⁻¹. UV - vis diffuse reflectance spectra were recorded on UV - visible spectra photometer (Shimadzu UV 2450).

**Well diffusion technique**

Antimicrobial activity of synthesized nanoparticles was investigated by Well diffusion method against bacterial strain, *Escherichia coli*. The 0.1ml of *E. coli* culture was inoculated into 5ml of Luria broth and incubated for 3-6 hrs to standardize the culture to McFarland standard (10⁶ CFU/ml). The concentrations of TiO₂ were ranges from 10, 20, 30 and 40μm in 50μl. The antimicrobials activities were determined by agar well diffusion assay. Under aseptic conditions, MHA medium was dispensed into pre-sterilized Petri dishes. After solidification it was then inoculated with micro-organism. A hole of diameter 8 mm was punched in the media and then filled with the different dilutions of Synthesized TiO₂. Gentamycin (10 μm) was used as positive control. After inoculation, the Petri dishes were incubated for 24 hours at 37°C. The diameter of the zone of inhibition was recorded as indicated by a clear area that is devoid of the growth of microbes.

**Cell viability assay**

A549 lung cancer cells were seeded at 1X10⁴ in the 96 well plate and allowed for attaching at over night. The next day media was aspirate with commercially available TiO₂ and green synthesized TiO₂, medium. That condition was kepted until 24 hrs. After the incubation, The yellow (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT solution (5mg/ml) was added to every well and incubates for 4 hours. After incubation with MTT, the upper part of solution is removed gently. The purple colour formazan was dissolved by adding 100µl of DMSO, finally colour formazan was dissolved by adding 100µl of DMSO, finally the plate was read at 595 nm and optical density was calculated.

\[
\text{% of cell viability} = \left( \frac{\text{Absorbance O.D by sample}}{\text{Absorbance O.D by control}} \right) \times 100
\]

**Result and Discussion**

**Powder x-ray diffraction (XRD)**

The X-Ray diffraction was done for TiO₂ nanoparticles using X-rays with wavelength of 1.54Å that is shown in Figure 1. No peaks were observed for the sample B whereas XRD peaks were obtained for Sample. The XRD pattern of Cynodon dactylon powder (Sample B) is shown on the top most column. The peaks were observed at 25.3°, 37.8°, 48.0°, 53.9°, 55.1°, 62.7°, 68.8°, 70.3° and 75.1° which corresponds to planes (101), (004), (200), (105), (211), (204), (116), (220) and (215) respectively. The experimental XRD pattern agrees with the JCPDS card No 89 - 4921. The diffraction peaks can be perfectly assigned to the anatase TiO₂. Broadening of the peaks are due to the fact that the crystalline size of sample A is very small.

**Fourier transform infrared spectroscopy**

Figure 2a shows the FTIR spectrum of TiO₂ nanoparticles in which the peaks corresponding to 3400 cm⁻¹, in the spectra are due to the stretching of H - bond of the O - H (Alcohol) group, the peaks corresponding to 2933.73 cm⁻¹ and 2255.35 cm⁻¹ were indicated as the functional group of C-H (alkane stretching and - C ≡ C – (alkynes, variable not present in symmetrical alkynes). The peaks observed at 1593.09 cm⁻¹ and 1404 cm⁻¹ corresponds to C = C (medium weak multiple bands). The obtained products are the result of the organic compounds like vitamins, enzymes, monosaccharide, polysaccharide and lignin’s present in the Cynodon dactylon leaf powder (sample B). In Figure 2b all the peaks are assigned with that of Figure 2a. That means that the leaf compounds were mixed with TiO₂ nanoparticles and is clearly seen in Figure 2a. In sample A, the peaks corresponding to 511.34 cm⁻¹, 686.81 cm⁻¹ and 773.81 cm⁻¹ show the stretching and vibration modes of O – Ti – O.

**Raman spectroscopy**

According to factor group analysis anatase has six Raman active modes which are 144cm⁻¹, 197cm⁻¹, 399cm⁻¹, 511.34 cm⁻¹, 686.81 cm⁻¹ and 773.81 cm⁻¹.
513 cm\(^{-1}\)(A\(_{1g}\)), 519 cm\(^{-1}\)(B\(_{1g}\)) and 639 cm\(^{-1}\). In this part of study, four active Raman modes of 145 cm\(^{-1}\)(E\(_{g}\)), 399 cm\(^{-1}\)(B\(_{1g}\)), 516 cm\(^{-1}\)(B\(_{1g}\)) and 639 cm\(^{-1}\)(E\(_{g}\)) for anatase TiO\(_2\) are evaluated for the sample A. Compared to the reference sample, the intensities of sample A are decreased which confirm the absorption of the bio molecules of *Cynodon dactylon* leaf extract by the sample A surface Figure 3.

**Scanning Electron Microscope**

To analyses the morphological studies of nanoparticles, the Field Effect Scanning Electron Microscopy is used. Figure 4 shows FESEM image of TiO\(_2\) nanoparticles. In the Figure 4, it can be seen that, the particles agglomerate with each other: In the SEM analysis particles were found to be irregular shape.

**Transmission Electron Microscope**

The morphology, crystallinity and size of the green synthesized TiO\(_2\) nanoparticles were also determined by TEM images. The shape of the nanoparticles was hexagonal and irregular in shape with moderate variation in size (Figure 5). The size was in the range of 13 – 34 nm. The average size of the nanoparticles was found to be 16 nm. The selected area electron diffraction pattern indicates, green synthesized TiO\(_2\) nanoparticles are in anatase phase with good crystallinity with dotted concentric rings which can be assigned to non spherical shape of TiO\(_2\), which is also assigned with XRD analysis. These are all shown in Figure 6.

**Antimicrobial activity**

The synthesized TiO\(_2\) nanoparticles range from 10, 20, 30 and 40 μm and they are using to determine the antibacterial effect by agar well diffusion method. The TiO\(_2\) showed (Figure 7) antimicrobial activity when tested against the pathogens. The antibacterial activity is found to be concentration dependent (i.e.), the antibacterial activity increased with the increase in
the concentration of TiO$_2$. The zone of minimum inhibition concentration was measured in a range of 15mm in 10μm. It is evident from the result that the cells were highly sensitive to all tested concentrations of the TiO$_2$ nanoparticles, which was confirmed from the size of the zone of inhibition.

![Figure 6: E.coli growth on the agar medium with TiO$_2$ nanoparticles and control.](image)

The TiO$_2$ nanoparticles exhibited a good antibacterial activity against Gram – negative bacteria (Table 1). The effect of antibacterial activity was minimum when the inhibition concentration was 10μm. This result is possible due to the difference in the concentration of TiO$_2$ reacting in the gram negative cell wall. The cell wall contains a thinner layer of peptidoglycan. The TiO$_2$ interacts with the membrane permeability of the bacteria and deaves the cell wall resulting in the killing of bacteria. It is evident from the result of Figure 6 that the TiO$_2$ nanoparticles possess potent bactericidal activity.

![Figure 7: Anticancer activity for A549 cell lines for (A) commercially available TiO$_2$ and (B) green synthesized TiO$_2$ NPs.](image)

### Anticancer activity (MTT assay)

Anticancer activity of A549 cell lines was studied using cell viability assay. IC$_{50}$ value for A549 cell lines is 140 μg/ml. In 100% of live cells, 50% of cell is death when our synthesized nanoparticles were injected to the cells. That concentration value is IC$_{50}$. value. In the above Figure 7 shows the commercially available TiO$_2$ and green synthesized TiO$_2$. Commercially available TiO$_2$ IC$_{50}$ value 200 μg/ml [16]. In the above result, green synthesized TiO$_2$ have good anticancer activity because in low concentration high cells are death. That means, bio molecules in Cynodon dactylon is giving excess electron to TiO$_2$ super oxide radicals O$_2^-$ are formed, the super oxide radicals produces ROS (Reactive Oxygen Species) in cancer cell lines. That ROS is used to break the cancer wall. Super oxide radicals production increased, ROS production is also increased. These are all reasons for electron production from TiO$_2$. So leaf extract is working for high amount of electron production. So our green synthesized TiO$_2$ nanoparticles efficiency is high for anticancer activity (A549 cell lines).

### Table 1: Well Diffusion of TiO$_2$ nanoparticles against E.coli.

| TiO$_2$ Nanoparticles Concentration (µm) | Diameter of Zone of Inhibition (mm) |
|-----------------------------------------|------------------------------------|
| 10                                      | 15                                 |
| 20                                      | 17                                 |
| 30                                      | 16                                 |
| 40                                      | 19                                 |
| Control                                 | 12                                 |

### Conclusion

The TiO$_2$ nanoparticles were synthesized successfully with Cynodon dactylon leaf extract by green synthesis method. The physico - chemical properties of synthesized TiO$_2$ nanoparticles were investigated by XRD, FE-SEM, HRTEM and Raman spectroscopy analysis indicating the properties of synthesized TiO$_2$ nanoparticles. Titanium dioxide nano particles show the inhibitory effect on the growth of E.coli, and enhanced anticancer activity against A549 (lung cancer) it was confirmed by the above said parameters.

### References

1. Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A (2016) Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules 21(7): E836.
2. Petkar KC, Chavhan SS, Agatonvik-Kustrin S, Sawant KK (2011) Nanostructured materials in drug and gene delivery: a review of the state of the art. Crit Rev Ther Drug Carrier Syst 28(2): 101-164.
3. Fujishima A, Rao TN, Tryk DA (2000) Titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 1(1): 1-21.
4. Syetali M, Anuratha V, Akila M, Yogananth N (2017) Anti genotic effect ofTiO$_2$ nanoparticles biosynthesized from sargassum polycystum-a marine macroalage. Journal of nanomedicine research 5(4): 0025.
5. Stewart, BW Wild, CP (2017) World cancer report 2014. IARC Nonserial Publication, France, pp. 630.
6. Akhtar MJ, Ahmed M, Kumar S, Khan MM, Ahmad J, et al. (2012) Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. Int J Nanomedicine 7: 845-857.
7. P Babji, D Roja Sri (2016) Synthesis, Characterization and Antitumor Activity of Fe₂O₃-Cu₂O-TiO₂ Nanocomposite on MCF-7 Human Breast Cancer Cells. J Chem Chem Sci 6(7): 616-23.

8. Tedja R, Marquis C, Lim M, Amal R (2011) Biological impacts of TiO₂ on human lung cell lines A549 and H1299: particle size distribution effects. Journal of Nanoparticle Research 13(9): 3801-3813.

9. Zhang S, Yang D, Jing D, Liu H, Liu L, et al. (2014) Enhanced photodynamic therapy of mixed phase TiO₂ (B)/anatase nanofibers for killing of HeLa cells. Nano Research 7(11): 1659-1669.

10. Shukla RK, Kumar A, Gurbani D, Pandey AK, Singh S, et al. (2013) TiO₂ nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. Nanotoxicology 7(1): 48-60.

11. El Rafie MH, Shaheen TI, Mohamed AA, Hebeish A (2012) Bio-synthesis and applications of silver nanoparticles onto cotton fabrics. Carbohydr Polym 90(2): 915-920.

12. Iravani S (2011) Green synthesis of metal nanoparticles using plants. Green Chemistry 13(10): 2638-2650.

13. Nadagouda MN, Varma RS (2008) Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chemistry 10(8): 859-862.

14. Sahu N, Soni D, Chandrashekhar B, Sarangi BK, Satpute D, et al. (2013) Synthesis and characterization of silver nanoparticles using Cynodon dactylon leaves and assessment of their antibacterial activity. Bioprocess Biosyst Eng 36(7): 999-1004.

15. Jananie RK, Priya V, Vijayakshmi K (2011) Determination of bioactive components of Cynodon dactylon by GC-MS analysis. NY Sci J 4(4): 1-5.

16. Wang Y, Cui H, Zhou J, Li F, Wang J, et al. (2015) Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. Environ Sci Pollut Res Int 22(7): 5519-5530.