J. Environ. Nanotechnol.
Volume 3, No. 3 pp. 73-81
ISSN (Print): 2279-0748
ISSN (Online): 2319-5541
doi: 10.13074/jent.2014.09.143098

Biosynthesized and Chemically Synthesized Titania Nanoparticles: Comparative Analysis of Antibacterial Activity

Paskalis Sahaya Murphin Kumar, Arul Prakash Francis, Thiyagarajan Devasena*

Centre for Nanoscience and Technology, Anna University, Chennai

Received:10.06.2014 Accepted:11.08.2014

Abstract

Green synthesis of nanoparticles using plant extract is the novel method to develop environmentally benign nanoparticles which can be used in numerous biomedical applications. In this study we have synthesized TiO_2 nanoparticles from Titanium Oxysulfate solution using Hibiscus flower extract. The synthesized nanoparticles were characterized using x-ray diffraction (XRD), scanning electron microscopy (SEM) and FTIR. The XRD pattern with sharp peaks describes the crystallinity and purity of titanium dioxide nanoparticles. The shape and morphology of TiO_2 nanoparticles were studied by SEM analysis, the results clearly represent that the flower extract capped titanium dioxide nanoparticles were dispersed and disaggregated. FTIR spectrum discloses the information about the interaction between the functional groups of the phytochemicals in the flower extract and the TiO_2. This report also explains the efficient antibacterial activity of biostabilized TiO_2 nanoparticles when compared to chemically synthesized TiO_2. Based on the results we confirm that the flower extract stabilized TiO_2 nanoparticles may have potential biomedical applications when compared to chemically synthesized TiO_2 nanoparticles.

Keywords: *Hibiscus; TiO_2; XRD; SEM; antibacterial.*

1. INTRODUCTION

The characteristic properties of nanoparticles such as increased surface area, size and morphology are different and improved when compared to the bulk counterparts. Metal nano particles are extensively exploited because of their unique physical properties, chemical reactivity and potential applications in various research areas such as antibacterial, antiviral, diagnostics, anticancer and targeted drug delivery (Bhumkar etal. 2007; Jain et al. 2009).

Metal nanoparticles are usually synthesized using various chemical method such as chemical reduction, solvo-thermal reduction, electrochemical techniques (Krishna, and Goia Dan, 2009 ; Saxena et al. 2010) and photochemical reaction in reverse micelles (Taleb et al. 1997). Among them, chemical reduction is the most frequently applied method. Previous studies showed that the use of a chemical reducing agent resulted in generation of larger particles and consume more energy. It was also reported that more side products were formed by chemical approaches which are not eco-friendly. Moreover, the chemically synthesized nanoparticles were reported to show less stability and more agglomeration (Mukherjee et al. 2001). Hence there is a need to develop an eco-friendly protocol that could produce stable and dispersible nanoparticles of controllable size by consuming less energy.

Alternate methods are also adopted for the synthesis of metal and metal oxide nanoparticles which utilize bacteria, fungi and plant extracts as reducing agents. These biological methods, so called green synthesis methods, are not only benign and environment friendly but also cost effective, rapid, less laborious, easily scalable to large scale and more efficient than conventional methods. It also benefits us by being compatible for various biomedical and

* **Thiyagarajan Devasena** *Tel.: +919962645151*
E-mail: tdevasenabio@annauniv.edu

pharmaceutical applications as they do not use toxic chemicals for the synthesis. Moreover, green synthesis generates nanoparticles with high dispersity, high stability and narrow size distribution (Bhainsa, KC et al. 2006; Willner et al. 2007).

Titanium dioxide (TiO_2) nanoparticles have wide environmental applications such as air purification and waste water treatment. TiO_2 nanoparticles possess potential oxidation strength, high photo stability and non-toxicity, are used in dye sensitized solar cells (Li et al. 2004; Salim et al. 2000; Ito et al. 1999). TiO_2 nanoparticles are also used in industrial applications such as pigment, fillers, catalyst supports and photocatalyst due to optical, dielectric, antimicrobial, chemical stability and catalytic properties (Barbe et al. 1997; Carp et al., 2004; Ruiz et al. 2004).

Previously the synthesis of TiO_2 nano particles using plants such as Nyctanthes arbor-tristis, Eclipta prostrata L., Jatropha curcas L, were reported (Sundararajan et al., 2011; Rajakumar et al. 2012; Manish et al. 2012). Plant phytochemicals act as capping agents and helps in synthesizing highly mono disperse nanoparticles, preventing their aggregation, thereby increasing its stability. For example, it has been reported that the phytochemicals and polyphenols coat the nano particles and act as capping agent. These polyphenol capping can lead to a synergistic effect when the capped particles are used for bio medical applications (Mohsen et al. 2011).

Hibiscus rosa sinesis (commonly called shoe flower) is rich in polyphenolic phytochemicals like tannins and phenolic proteins, triterpenoids, 2,3-hexanediol, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and squalene (Mandade et al. 2011; Anusha et al. 2011). These compounds have good antimicrobial, anti oxidative and anti proliferative activity and therefore be used to treat cancer, especially lung cancer, cardiovascular disease, asthma and pulmonary function (Tsao et al. 2003; Feskanich et al. 2000; Kim et al. 2002). In addition, the phytochemicals are believed to act as capping agent to stabilize the synthesized silver nanoparticles and to prevent its coalescence. Such nanoparticles may have the advantage of offering synergistic biomedical effects. For example, antibiotic-coated nanoparticles exhibited a synergistic antimicrobial effect (Devasena et al. 2009a; Devasena et al. 2009b) Hence it can be hypothesized that Hibiscus flower extract may result in phytochemicals stabilized nanoparticles with better bio medical activity. Hence we aimed for the first time to use *Hibiscus rosa sinesis* and standardize a protocol to synthesize TiO_2 nanoparticles. We characterized the as synthesized TiO_2 nanoparticles to study the morphology, size, crystal structure and surface capped functional groups. We also investigated the antibacterial activity of the green synthesized TiO_2 against bacterial strains such as *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus.* Evidently this is the first study to synthesize the TiO_2 nanoparticles using the flower *extract of Hibiscus rosasinensis*.

To justify the protocol optimized by us we have compared the characterization profile and the antimicrobial activities of green synthesized and chemically synthesized TiO_2 nanoparticles.

2. MATERIALS & METHODS

2.1. Materials

All the chemicals and reagents used for the preparation of TiO_2 nanoparticles were purchased from Merck India Ltd. and Hi Media. The standard strains (*Vibrio cholerae, Pseudomonas aeruginosa*, *Staphylococcus aureus*) used for the antibacterial studies were obtained from Microbial Type Culture Collection and gene bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

The flowers were collected from a village in Kanyakumari district, Tamilnadu. The petals were seperated and dried under shadow. The dried petal was identified as *Hibiscus-rosa-sinensis L.* of *Malvaceae* family, by the botanist of Plant Anatomy Research Centre, West Tambaram, Chennai based on the organoleptic macroscopic examination of the sample (Nair NC and Henry 1983).

2.2. Methodology

2.2.1. Preparation of Extract

10g of air dried petals of *Hibiscus rosa sinensis* was taken in the beaker and extracted with 200ml water at 70^{0}C for 2 hrs. The extract was filtered using whatman filter paper. The filterate was used for the synthesis of nanoparticles.

2.2.2. Preparation of TiO_2 nanoparticles

TiO_2 nanoparticles were prepared as follows.

To 0.5 M solution of Titanium Oxysulfate, 5ml of flower extract was added in dropwise under continuous stirring. The pH of the solution was adjusted to 7 by continuous washing with water. The mixture was subjected to stirring for 3 hours. After stirring, the nanoparticles formed was separated by centrifugation at 8000 rpm for 15 mins. The precipitate was washed repeatedly with water to remove the byproducts. The same procedure was used to synthesize TiO_2 nanoparticles chemically without the addition of flower extract, where the pH was adjusted to 7 using 0.1 M sodium hydroxide. The nanoparticles were dried at 100ºC for 3 hours.

2.2.3. Characterization of TiO_2 nanoparticles

The crystal nature and average crystallite size of the TiO_2 nanoparticles, was recorded using X-ray diffraction (XRD) (Rigaku) with CuKα radiation (1.5406 Å) in the 2θ scan range of 20-80°. The surface morphology and size of the particles were investigated using scanning electron microscopy (Tescan Vega3) and with an acceleration voltage of 7 kV. The FT-IR spectrum of TiO_2 nanoparticles was recorded on Perkin Elmer Spectrum Fourier Transform Infrared spectrophotometer in the region of 4000 to 500 cm^{-1}.

2.2.4. Evaluation of Antibacterial Activity

The antibacterial activities of the both bio and chemically synthesized TiO_2 nanoparticles were evaluated using the three bacterial strains such as *Vibrio cholerae, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Bacteria were grown overnight on Mueller Hinton agar plates and the activity was assessed by disc diffusion method. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. Streptomycin was used as positive control. The experiments were carried out in triplicate. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the disc.

3. RESULTS AND DISCUSSION

3.1 Nanoparticles Characterization

In this study we have synthesized TiO_2 nanoparticles from Titanium Oxysulfate solution using *Hibiscus* flower extract. The green synthesised TiO_2 nanoparticles were characterised using XRD, SEM and FTIR and investigated for antibacterial activity, in comparison with chemically synthesized TiO_2 nanoparticles. The XRD pattern of TiO_2 nanoparticles obtained using flower extract of *Hibiscus rosa-sinensis* and chemically synthesized TiO_2 nanoparticles are shown in Figure 1. A sharp diffraction peak was observed in chemically synthesized TiO_2 nanoparticles, whereas, the intensity of diffraction peak of green synthesized TiO_2 nanoparticles is less with slight broadening. The lattice parameters obtained were close and consistent with standard data for TiO_2 (JCPDS 21-1272) (Vijayalakshmi et al., 2012). We have calculated the average crystallite size of TiO_2 nanoparticles synthesized by green route and chemical route using the Scherrer's formula($d = 0.89\lambda/\beta\cos\theta$) The calculated crystallite was found to be 7 nm and 24 nm for green and chemically synthesized TiO_2 nanoparticles respectively. The results of XRD analysis confirms the presence of TiO_2 nanoparticles in the green synthesised sample. Previous reports have also used XRD as an evidence for the confirmation of TiO_2 nanoparticles (Li et al. 2006).

The XRD peaks of green synthesised TiO_2 nanoparticles obtained using flower extract of *Hibiscus rosa-sinensis* and chemically synthesized TiO_2 nanoparticles differ in the broadening and intensity. The diffraction peak of the green synthesised TiO_2 nanoparticles is broadened, whereas the peak of chemically synthesized TiO_2 nanoparticles is comparatively sharp. Naheed Ahmad et al., 2012 have reported the correlation between XRD peak broadening and the size reduction during green synthesis protocol. Thus, the broadening of XRD peak of green synthesised TiO_2 nanoparticles observed in our study confirms the size reduction. The XRD peak of chemically synthesised TiO_2 nanoparticles is sharp, thus indicating that their size is still larger than the green synthesised TiO_2 nanoparticles. The intensity of the diffraction peak of green synthesized TiO_2 nanoparticles is less when compared to chemically synthesized nanoparticles. Earlier reports on XRD data of nanoparticles have documented an inverse relation between peak intensity and surface functionalization of nanoparticles (Daizy Philip, 2009). Surface coating of the nanoparticles with functional groups (i.e, surface functionalization) results in an internal strain in the particles consequently decreasing the signal:noise ratio. As a result, the intensity of the XRD peak decreases (Kannan et al., 2008). Therefore, we suggest that the phytochemicals present in the extract of Hibiscus flower would have coated the surface of the TiO_2 nanoparticles, resulting in decreased intensity in XRD peak. This phytochemical coating may enhance the stability and the dispersibility of the nanoparticles, which in turn may enhance their bioavailability, making them suitable for biological applications. The chemically synthesized TiO_2 nanoparticles on the other hand showed comparatively high intense peaks, clearly indicating that they are bare and uncapped.

Overall, the XRD profile reveals that the green synthesis protocol that we developed using Hibiscus flower is valid for the production of bio-functionalized and bio-stabilized titania nanoparticles with potential biomedical activities.

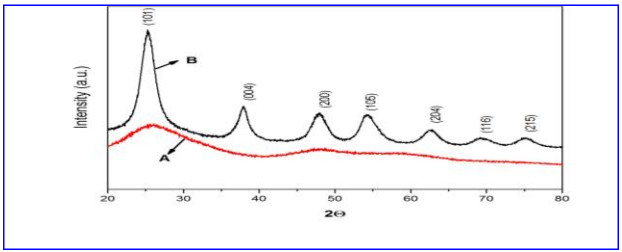

Fig. 1: XRD pattern of TiO_2 nanoparticles.

a) Biostabilized TiO_2 nanoparticles b) Chemically synthesized TiO_2 nanoparticles.

The functional groups that could cap the surface of the particles were confirmed by us through FTIR studies. Fig. 2 shows the FTIR spectrum of Flower Extract (A), green synthesized TiO_2 nanoparticles (B) and chemically synthesized TiO_2 nanoparticles (C). In green synthesized TiO_2 nanoparticles a broad band was observed between 3800 to 3000 cm^{-1} which is due to hydroxyl (O-H) stretch, representing the water as moisture. The peak at 1631 cm^{-1} explains the stretching of C-O and C=O bonds of carboxylate group present in the flower extract. The peaks at 2948 and 2897 cm^{-1} is due to the asymmetric and symmetric stretching vibrations of carbonyl groups and secondary amines. The intense peak between 800 and 450 cm^{-1} describes the Ti-O stretching bands. However in chemically synthesized TiO_2 nanoparticles a broad absorption peak around 3342 cm^{-1} is due to the stretching vibration of -OH groups on the TiO_2 surface. The peak at 1631 cm^{-1} confirmed the O–H bending of dissociated or molecularly adsorbed water molecules, respectively. The broad band from 400 to 800 cm^{-1} was ascribed to the strong stretching vibrations of Ti–O–Ti bonds. FTIR spectrum reveals the information about the interaction between the functional groups of the plant phytochemicals and the nanoparticles. Thus, we could get idea about the groups involved in the surface functionalization. In our study, the phytochemicals present in the crude extract of the flower may interact with the surface of the TiO_2 nanoparticles, thus forming a cap. A previous study reported that a shift in the absorption bands is indicative of the linkage between the nanoparticles and the corresponding compound present in the extract.

Shifts were noticed from 1053 and 619 cm-1 to 1125 and 635cm-1 respectively, which corresponds to amines and phenolic groups capping around the nanoparticles. Phenol and amide capped nanoparticles were reported to possess significantly higher biomedical activities than uncapped nanoparticles. Therefore, the green synthesized TiO_2 nanoparticles may possess potential biomedical benefits. Furthermore, FTIR results reveal the presence of carboxylate group, carbonyl groups, and secondary amines in the green synthesized nanoparticles. The intense peak between 800 and 450 cm^{-1} describes the Ti-O stretching bands. However, in chemically synthesized TiO_2 nanoparticles a broad absorption peak around 3342 cm^{-1} is obtained which may be due to the stretching vibration of -OH groups on the TiO_2 surface. The peak at 1631 cm^{-1} confirmed the O–H bending of dissociated or molecularly adsorbed water molecules, respectively. The broad band from 400 to 800 cm^{-1} was ascribed to the strong stretching vibrations of Ti–O–Ti bonds (Cheyne et al., 2011). Taken as a whole, the FTIR spectrum indicates that the TiO_2 nanoparticles synthesized using flower extract were surrounded by polyphenols, amines and proteins. From the FTIR data we suggest that the phenolic groups and amines of the extract acts as capping agent on the surface of the TiO_2 nanoparticles and prevent them from aggregation. Thus, we could justify a supportive correlation between the XRD and the FTIR report of the bio-stabilized nanoparticles, both confirming the stability and the dispersibility of the particles

Fig. 3a and 3b shows the SEM images of biostabilised TiO_2 nanoparticles obtained using flower extract of Hibiscus rosa-sinensis and chemical route respectively. The image describes the surface morphology of the TiO_2 nanoparticles. The green synthesized TiO_2 nanoparticles show monodispersity without aggregation when compared to that of

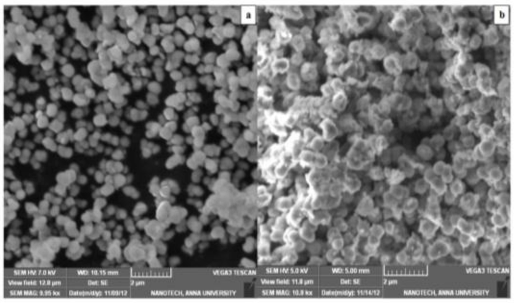

Fig. 2: SEM micrographs of TiO_2 nanoparticles.

a) Biostabilized TiO_2 nanoparticles b) Chemically synthesized TiO_2 nanoparticles.

chemically synthesized TiO_2 nanoparticles. This is due to the capping of TiO_2 nanoparticles with the compounds present in the flower extract. The SEM images reveal the surface morphology of the green synthesised TiO_2 nanoparticles. The particles were found to be spherical with distinct edges and without aggregation. Previous report that plant phytochemicalfunctionalized nanoparticles are disaggregated and stable with good dispersibility (Archana et al., 2012) supports our findings. We could therefore speculate that the phytochemicals of hibiscus flower extract coats the surface of the TiO_2 nanoparticles thus preventing their aggregation.

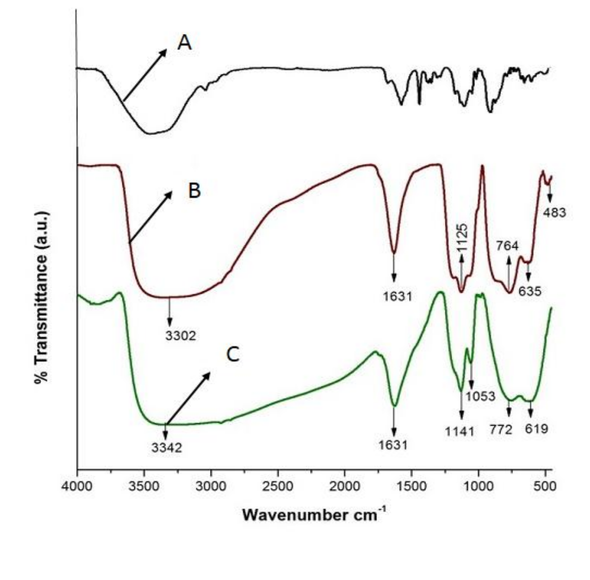

Fig. 3: FTIR spectrum of TiO_2 nanoparticles.

A) Fower Extract B) Green synthesized TiO_2 nanoparticles C) Chemically synthesized TiO_2 nanoparticles.

Taken together the XRD, SEM and the FTIR profile shows that the protocol optimized by us has generated TiO_2 nanoparticles stabilized with phytochemicals of flower extract and hence this can be referred as "biostabilized TiO_2 nano particles".

3.2. Antibacterial Studies

TiO_2 nanoparticles at various concentrations (5, 10, 15 and 20 µg/ml) were tested for antibacterial activity using *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by disc iffusion method. The antibacterial activity of TiO_2 nanoparticles was compared with the positive control, Streptomycin. The zone of the inhibition is summarized in the Table-1a and 1b and displayed in Fig 4 and 5. The bactericidal activity exhibited by 15 µg/ml of biostabilized TiO_2 nanoparticles was comparable with that of the positive control, Also the activity of biostabilized TiO_2 nanoparticles at a concentration of 15 µg/ml was found to be greater than that of the positive control. However the zone of inhibition produced by chemically synthesized TiO_2 nanoparticles is lesser than green synthesized TiO_2 nanoparticles at all the concentrations studied against *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Staphylococcus aureus.*

Table 1a. Zone of inhibition produced by biosynthesized TiO_2 nanoparticles and positive control (Streptomycin)

Pathogen bacteria	Zone of inhibition (mm)				
	Positive control (Streptomycin)	Different concentrations of biosynthesized TiO_2 nanoparticles (µg/ml)			
		5	10	15	20
Vibrio cholerae	12	8	10	15	17
Pseudomonas aeruginosa	13	10	11	13	14
Staphylococcus aureus	14	7	12	13.5	14.5

Table 1b. Zone of inhibition produced by chemically synthesized TiO_2 nanoparticles and positive control (Streptomycin)

Pathogen bacteria	Zone of inhibition (mm)				
	Positive control (Streptomycin)	Different concentrations of chemically prepared TiO_2 nanoparticles (µg/ml)			
		5	10	15	20
Vibrio cholerae	12	6	7	8	10
Pseudomonas aeruginosa	13	6	7	9	10
Staphylococcus aureus	14	7	8	10	11

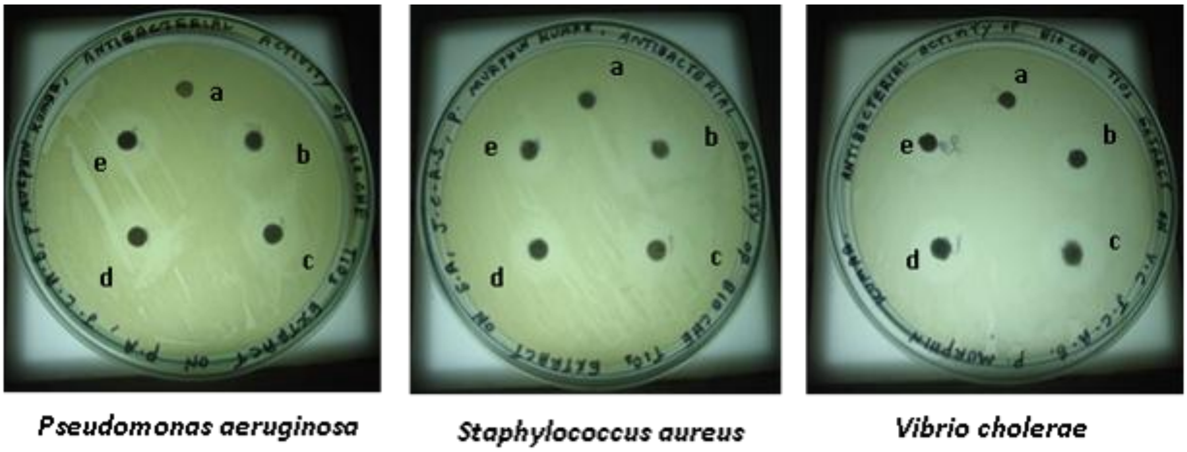

Fig. 4: Zone of inhibition produced by biostabilised TiO_2 nanoparticles.
a) 5 µg/ml; b)-10 µg/ml; c)-15 µg/ml; d)-20 µg/ml; e) Positive Control

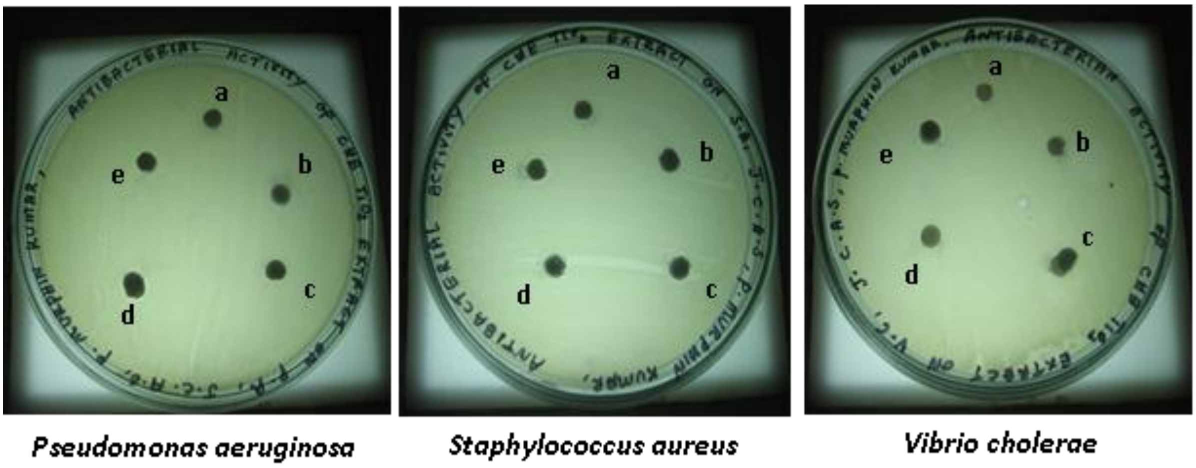

Fig. 5: Zone of inhibition produced by chemically synthesized TiO_2 nanoparticles.
a) 5 µg/ml; b)-10 µg/ml; c)-15 µg/ml; d)-20 µg/ml; e) Positive Control

The results of antibacterial studies clearly suggest that the TiO_2 nanoparticles synthesized using *Hibiscus rosa sinensis* flower extract has a far better antibacterial activity even at a low dose when compared to the chemically synthesized TiO_2 nanoparticles. The mechanism of action of TiO_2 nanoparticles has not been established yet. Nanomaterials were reported to exhibit broad-spectrum biocidal activity towards various microorganisms like bacteria, fungi, and viruses (Ikigai et al., 1990). Previous studies have reported that the membrane proteins were inactivated by nanoparticles, which decreases the membrane permeability causing the cellular death (Mohanpuria et al., 2008). Nanomaterials also produce retardation in the bacterial adhesion and development of bio-film, hence it is easy to destroy the microbes (Gou et al., 2010). Polyphenols on the other hand were reported to possess various beneficial activities like antibacterial, antiviral, antifungal and anticancer activities. Polyphenols were also reported to inhibit exotoxins found in bacteria (Tanapon et al., 2010; Toda et al., 1990). With these background data, we can suggest that the biostabilized TiO_2 nanoparticles capped by polyphenols has better activity than chemically synthesized TiO_2 nanoparticles to induce membrane damage and cell death of *Pseudomonas aeruginosa, Vibrio cholerae* and *Staphylococcus aureus.*

4. CONCLUSION

In this study we have prepared TiO_2 nanoparticles by 1) using Hibiscus flower extract as capping agent and 2) chemical method. Both nanoparticles were characterized for their size, and crystallinity using XRD, capping phytochemicals by FTIR and morphology by SEM. The TiO_2 nanoparticles which was capped and stabilized by phenolic and amine moieties of flower extract was found to be lesser in size and more dispersed than the TiO_2 nanoparticles prepared by chemical method. Moreover the flower extract-stabilized TiO_2 nanoparticles exhibited considerable antimicrobial activity against pathogenic bacteria, which is comparable with that of standard antibiotic. Based on these results we conclude that the flower extract-stabilized TiO_2 nanoparticles may have potential biomedical applications when compared to chemically synthesized TiO_2 nanoparticles due to its enhanced dispersibility, stability and surface coatings.

REFERENCES

Al-Salim, N. I., Bagshaw, S. A., Bittar, A., Kemmtt, T. and Mcquillan, A. J., Characterisation and activity of sol–gel-prepared TiO2 Photocatalysts modified with Ca, Sr or Ba ion additives, J Mater Chem., 10, 2358–2363 (2000). doi:10.1039/b004384m

Anusha Bhaskar, Nithya V, and Vidhya V. G., Phytochemical screening and in-vitro antioxidant activities of the ethanolic extract of Hibiscus rosa sinensis L, Annals of Biological Research, 2, 653-661 (2011).

Archana Maurya, Pratima Chauhan, Amita Mishra, and Abhay K. Pandey., Surface Functionalization of TiO2 with Plant Extracts and their Combined Antimicrobial Activities Against E. faecalis and E. Coli, Journal of Research Updates in Polymer Science, 1, 43-51 (2012).

Balantrapu Krishna, and Goia Dan., Silver nanoparticles for printable electronics and biological applications, Journal of materials research, 24, 2828 (2009). doi:10.1557/jmr.2009.0336

Barbe, C. J., Arendse, F., Comte, P., Jirousek, M., and Gratzel, M. Nanocrystalline titanium oxide electrodes for photovoltaic applications. J Am Ceram Soc., 80, 3157 (1997).
doi:10.1111/j.1151-2916.1997.tb03245.x

Bhainsa, K. C., and D'Souza S. F., Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus, Colloids and Surfaces B: Biointerfaces, 47, 160–64 (2006). doi:10.1016/j.colsurfb.2005.11.026

Bhumkar, D. R., Joshi, H. M., Sastry, M., and Pokharkar V.B., Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin, Pharm Res., 24, 1415-26 (2007).
doi:10.1007/s11095-007-9257-9

Carp, O., Huisman, C. L. and Reller, A., Photoinduced reactivity of titanium dioxide, Prog Solid State Chem., 32, 133 (2004). doi:10.1016/j.progsolidstchem.2004.08.001

Daizy Philip, Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 374–381 (2009). doi:10.1016/j.saa.2009.02.037

Devasena, T. and Ravimycin, T., Activity of ketoconazole coated gold nanoparticles against dandruff causing fungi, Asian J biosci., 4, 44-46 (2009a).

Devasena, T. and Ravimycin, T., Ketoconazole coated Silver nanoparticles- A potent antidandruff agent, Int J plant sci., 4, 517-520 (2009b).

Feskanich, D., Ziegler, R., Michaud, D., Giovannucci, E., and Speizer, F., et al., Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women, J Natl Cancer Inst., 92, 1812-23 (2000).
doi10.1093/jnci/92.22.1812

Gou N, Onnis Hayden A, and Gu A. Z., Mechanistic toxicity assessment of nanomaterials by whole-cell-array stress genes expression analysis, Environ Sci Technol., 44, 5964 -70 (2010). doi:10.1021/es100679f

Ikigai, H., Toda, M., Okubo, S., Hara, Y. and T Shimamura. Relationship between the anti-hemolysin activity and the structure of catechins and theaflavins, Jpn J Bacteriol., 45, 913—919 (1990).
doi:10.3412/jsb.45.913

Ito, S., Inoue, S., Kawada, H., Hara, M. and Iwaski, m., Low-temperature synthesis of nanometersized crystalline TiO2 particles and their photoinduced decomposition of formic acid, Colloid Interface Sci., 59, 216 (1999).

Jain, D., Daima, H. K., Kachhwaha, S., and Kothari, S. L., Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Digest J Nanomat Biostru., 4, 557-63 (2009).

Kannan, P. and John, S. A., Synthesis of mercaptothiadiazole-functionalized gold nanoparticles and their self-assembly on Au substrates, Nanotechnology, 18, 085602 (2008). doi:10.1088/0957-4484/19/8/085602

Kim, D. O., Lee, K. W., Lee, H. J., and Lee, C. Y., Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals, J Agric Food Chem., 50, 3713–17 (2002). doi:10.1021/jf020071c

Li L., Sun X., Yang Y., Guan N. and Zhang F., Synthesis of anatase TiO2 nanoparticles with beta-cyclodextrin as a supramolecular shell, Chem Asian J., 1, 664-8(2006). doi:10.1002/asia.200600103

Li, Y., White, T. J. and Lim, S. H., Low-temperature synthesis and microstructural control of titania nano-particles, Journal of solid state chemistry, 177, 1372 (2004). doi:10.1016/j.jssc.2003.11.016

Manish Hudlikar Shreeram Joglekar, Mayur Dhaygude, and Kisan Kodam, Green synthesis of TiO2 nanoparticles by using aqueous extract of Jatropha curcas L. Latex, Materials Letters, 75, 196–199 (2012). doi:10.1016/j.matlet.2012.02.018

Mohanpuria, P., Rana, N. K. and Yadav, S. K., Biosynthesis of nanoparticles: Technological concepts and future applications, Journal of Nanoparticle Research, 10: 507–517 (2010). doi:10.1007/s11051-007-9275-x

Mohsen Zargar, Azizah Abdul Hamid, Fatima Abu Bakar, and Mariana Nor Shamsudin, Green synthesis and antibacterial effect of silver nanoparticles using Vitex Negundo L., Molecules, 16, 6667-6676 (2011). doi:10.3390/molecules16086667

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S. and Sainkar, S. R., Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis, Nano Lett., 1, 515-519 (2001). doi:10.1021/nl0155274

Naheed Ahmad and Seema Sharma, Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus, Green and Sustainable Chemistry, 2, 141-147 (2012). doi:10.4236/gsc.2012.24020

Nair N.C. and Henry A.N., Flora of Tamil Nadu, India, 34 (1983).

Rajakumar, G., Abdul Rahuman, A., Priyamvada, B., Gopiesh Khanna, V., and Kishore Kumar, D., Eclipta prostrata leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles, Materials Letters, 68, 115–17 (2012). doi:10.1016/j.matlet.2011.10.038

Rajesh Mandade, Sreenivas, S. A., Sakarkar, D. M. and Avijit Choudhury, Radical scavenging and antioxidant activity of Hibiscus rosasinensis extract, African Journal of Pharmacy and Pharmacology, 5, 2027-2034 (2011).

Richard W Cheyne, Tim AD Smith, Laurent Trembleau and Abbie C Mclaughlin, Synthesis and characterisation of biologically compatible TiO2 nanoparticles, Nanoscale Research Letters, 6, 423 (2011). doi:10.1186/1556-276X-6-423

Ruiz, A. M., Sakai, G., Cornet, A., Shimanoe, K., and Morante, J. R., Characterisation and activity of thermally stable TiO2 obtained by hydrothermal process, Sens Actuators B Che., 103, 312 (2004). doi:10.1016/j.snb.2004.04.061

Sundrarajan, M. and Gowri, S., Green synthesis of titanium dioxide nanoparticles by Nyctanthes Arbor-Tristis leaves extract, Chalcogenide Letters, 8, 447-451 (2011).

Taleb, A., Petit, C., and Pilen, M. P., Synthesis of highly monodisperse silver nanoparticles from AOT reverse micelles: a way to 2D and 3D self-organization, Chemistry of Materials, 9, 950–59 (1997). doi:10.1021/cm960513y

Tanapon Phenrat, Jee Eun Song, Charlotte M Cisneros, Daniel P Schoenfelder and Robert D. Tilton Estimating attachment of nano and sub micrometer-particles coated with organic macromolecules in porous media: Development of an empirical model, Environ Sci. Technol., 44, 4531–4538 (2010). doi:10.1021/es903959c

Toda, M., Okubo, S., Ikigai, H., and Shimamura T., Antibacterial and antihaemolysin at activities of tea catechins and their structural relative, Jpn J Bacterio., 45, 561-566 (1990). doi:10.3412/jsb.45.561

Tsao, R., Yang, R., Ch Young and Zhu, H., Polyphenolic profiles in eight apple cultivars using high–performance liquid chromatography (HPLC), J Agric Food Chem., 51, 6347–6353 (2003). doi10.1021/jf0346298

Vijayalakshmi, R. and Rajendran, V., Synthesis and characterization of nano-TiO2 via different methods, Arch of App Sci Res., 4, 1183-1190 (2012).

Willner, I., Basnar, B. and Willner, B., Nanoparticle–enzyme hybrid systems for nanobiotechnology, FEBS Journal, 274, 302–309 (2007). doi:10.1111/j.1742-4658.2006.05602.x