www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 817-822

www.thepharmajournal.com
Received: 08-02-2019
Accepted: 09-03-2019

Neelesh Babu
Department of Botany and Microbiology Gurukula Kangri University, Haridwar, Uttarakhand, India

Vinay Mohan Pathak
ICAR-VPKAS, Almora, Uttarakhand, India

Ajeet Singh
NDRI Karnal, Division of Microbiology, Haryana, India

Navneet
Department of Botany and Microbiology Gurukula Kangri University, Haridwar, Uttarakhand, India

Correspondence
Neelesh Babu
Department of Botany and Microbiology Gurukula Kangri University, Haridwar, Uttarakhand, India

Sonchus asper leaves aqueous extract mediated synthesis of Titanium dioxide nanoparticles

Neelesh Babu, Vinay Mohan Pathak, Ajeet Singh and Navneet

Abstract
Green synthesis of metal nanoparticles has various advantages over chemical synthesis. Green synthesized nanoparticles are known for their less toxic nature. Beside the toxicity, these are also more stable and eco-friendly. This cost effective approach is utilized in present study for the green synthesis of Titanium dioxide nanoparticles using *Sonchus asper* leaves aqueous extract. Nanoparticles were synthesized via sol-gel method by using Titanium isopropoxide as a precursor. Synthesized particles were in spherical shaped measuring 9 – 22 nm in size. They were tested against three different bacterial strains *Escherichia coli* MTCC 40, *Staphylococcus aureus* MTCC 1144 and *Klebsiella pneumoniae* MTCC 4030 for antimicrobial screening. Results showed that green synthesized nanoparticles gave better result against Staphylococcus aureus than chemically synthesized nanoparticles as well as standard drugs.

Keywords: Leaves aqueous, titanium dioxide nanoparticles

Introduction

Metal and metal oxide nanoparticles have attracted considerable attention of the peoples in research field due to their unique properties i.e. surface to volume ratio as compared to their bulk counterparts [1, 2, 3]. Proprieties of nanoparticles depend upon their shape, size and crystallite structure. Titanium dioxide nanoparticles have a large number of applications due to its high photostability, oxidation strength and least toxicity [4]. It is used in various cosmetic products more precisely in sunscreens to protect skin from ultraviolet rays [5]. It also has various applications in the field of photocatalysis, gas sensors, in the treatment of water, solar cells, photo electrochemical cells, protective coatings on optical elements [6, 7]. It is widely used in paints, coatings, plastics, papers, inks, foods, medicines and several toothpastes [8]. The chemical synthesis of nanoparticles such as solvo-thermal reduction, chemical reduction and electrochemical reduction are more costly, toxic, difficult to separate, hazardous, employs high pressure and energy [9].Beside these, green synthesis of nanoparticles has been adopted as an alternate method which utilizes biological materials such as plants crude extract asa reducing agents [10]. Several works have been conducted in this scenario such as Jain *et al*., 2009 [11]; Sunderarajan and Gowri, 2011[12]; Maurya *et al*., 2012 [13]; Rajakumar *et al*., 2012 [14]; Kumar *et al*., 2014 [15]. Basically phytochemicals of plants crude extracts acts as capping agents in the green synthesis of nanoparticles. More precisely phytophenolic compounds of the crude extracts coat the particles and provide synergetic effects when these particles utilized in biomedical field [16]. Considering this present study includes the green synthesis of TiO_2 nanoparticles using the crude extract of medicinally important herb *Sonchus asper*.

Sonchus asper belongs to the family Asteraceae commonly known as Peeli dudhi in Hindi and Prickly sow thistle in English. It is an herbaceous annual or winter annual plant that contains white sticky latex with short taproot which is bushy with many lateral roots. It has erect hollow, stout, unbranched or slightly branched stem lengths 30 to 150 cm long which is most of the time reddish. It may also contain gland-tipped hairs on upper stems. Leaves of this herb are alternate, 4 to 18 cm in length, 0.5 to 5 cm in width which is crisped with 5 to 11 lobes on each side. It is found in the Himalayan region traditionally used as medicine in Pauri Garhwal in Uttarakhand. This plant has various potential bioactive chemical constituents which are useful in various human disorders such as wounds, burns, gastrointestinal infection, inflammation, cardiac dysfunction, kidney disordes liver disorder, jaundice, cancer along with impotency in humans etc. [17, 18, 19].

Experimental

Materials
Herb *Sonchus asper* was collected from the nearby land of Gurukula Kangri University, Haridwar, Uttarakhand which was further authenticated from Botanical Survey of India, Dehradun, Uttarakhand having accession number 116606. Titanium isopropoxide was used as the precursor for the synthesis of Titanium dioxide nanoparticles was purchased from Sigma Aldrich. Culture media (Muller Hilton agar and Nutrient broth) were purchased from CDH (Central Drug House) New Delhi. Three different bacterial strains *Escherichia coli* MTCC 40, *Staphylococcus aureus* MTCC 1144 *and Klebsiella pneumoniae* MTCC 4030 were used for antimicrobial screening.

Extraction
The leaves of the plant were dried in shade at room temperature for 15 -16 days. The quantity of 200 g of coarsely powdered leaves was filled in Soxhlet apparatus and extraction was carried out by distilled water [20].

Preparation of TiO_2
The sol-gel method was used for the synthesis of TiO_2 nanoparticles where Titanium isopropoxide (Sigma Aldrich >97%) was used as a titanium precursor. According to the method demonstrated by Liu *et al.*, 2003 [21], synthesis was accomplished with few modifications. Titanium isopropoxide and ethanol were mixed in a ratio of 1:2 then the suspension was slowly added to the mixture containing alcohol, hydrochloric acid and deionised water under vigorous stirring at room temperature. After the short interval of time, the solution was slowly converted into the gel with yellowish texture. The yellowish gel was further precipitated and the resulting precipitate was subjected for recovery by centrifugation with several washing by ethanol and dried at 70̊ C. The dried powder was annealing by using muffle furnace at the temperature 500̊ C. For the green synthesis of Titania nanoparticles similar procedure was employed with the addition of plant extract of *Sonchus asper*.

Characterization
Plant extract / TiO_2 composite and chemically synthesized TiO_2 nanoparticles were characterized by XRD, FTIR and FE-SEM techniques. Bruker AXS D8- Advanced diffractometer with Cu-Kα radiation (λ= 1.5406 Å) in the 2Θ range 20˚- 80̊ was used for recording X-ray diffraction pattern, Perkin Elmer spectrum two spectrometer was used in particle range 400 – 4000 cm^{-1} used for FTIR analysis and for the surface morphology Carl Zeiss Ultra plus Field-Emission Scanning Electron Microscope with an accelerated voltage of 15.00 Kv in high vacuum mode to achieve maximum magnification between 100X- 100,000X was used.

Antimicrobial activity
Antibacterial activities of nanoparticles were estimated by agar well diffusion method. Four wells of 6 mm breadth were punched by sterile borer on Muller Hilton agar plates and further seeded with stock bacterial cultures purchased from MTCC. In the concentration of 50µg/ml, powdered nanoparticle samples were dissolved in distilled water and kept under UV light for 1 hour. 45 µl of each sample was poured into the wells of Muller Hilton agar plates. Antibiotic disks of cefazolin (30mcg) and ampicillin (10 mcg) were used as a positive control. On the other hand distilled water was used as negative control.

Results
The crystallite structure of synthesized nanoparticles was analyzed by X-ray diffraction. Peaks observed by the technique confirm the presence of TiO_2. According to JCPDS card no. 21-1272 peaks correspond to the anatase phase of TiO_2. The average grain size of the particles was calculated by the Scherer formula.

$$d = \frac{0.9\lambda}{\beta cos\Theta}$$

Where,
d= Mean diameter of the particles
λ = Wavelength of the radiation.
β =Angular FWHM of peak
Θ = Diffraction angle. Figure 1 shows the XRD Pattern of the synthesized nanoparticles.

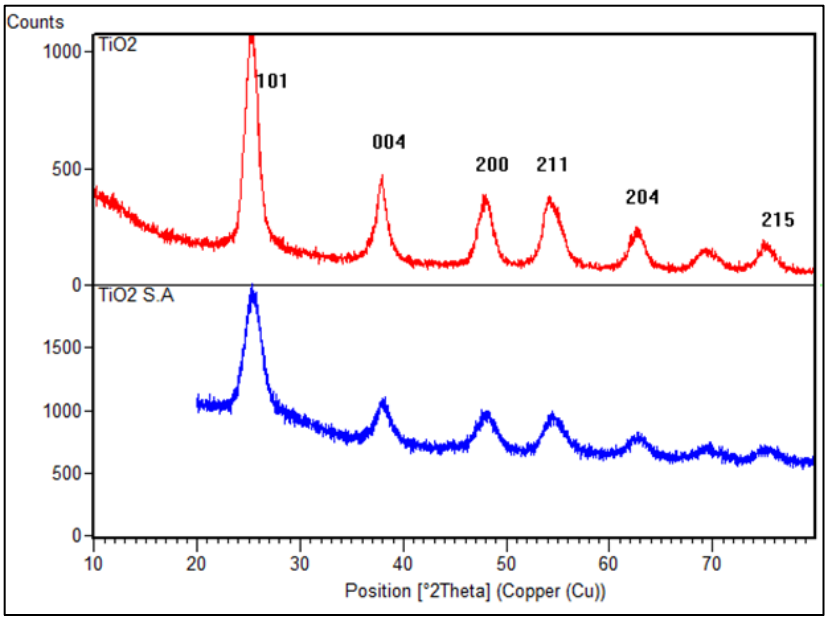

Fig 1: XRD pattern of chemically synthesized nanoparticles (TiO_2) and green synthesized nanoparticles by *Sonchus asper* (TiO_2).

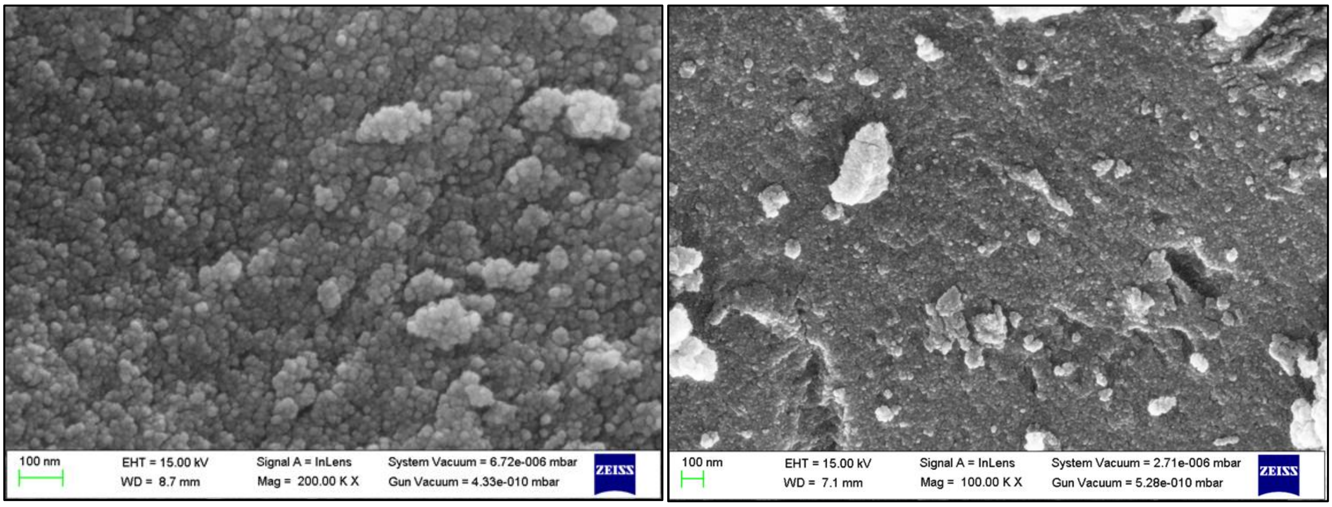

Fig 2: FESEM images of (A) chemically synthesized nanoparticles (TiO_2) and (B) green synthesized nanoparticles by *Sonchus asper* (TiO_2/SA).

The average grain size of chemically synthesized TiO_2 nanoparticles ranges between 12-22 nm whereas green synthesized particles ranges between 9-15 nm. FESEM reveal the surface morphology of the samples. It was observed that both the samples of chemically as well as green synthesized nanoparticles was spherical shaped. Figure 2 clearly shows the spherical shaped particles. Associated functional groups were determined by FTIR technique. Figure 3 and 4 shows the spectrum of FTIR peaks. Origin of the vibration bands observed from the spectrum as followes: Bands observed at from 3937 cm^{-1} - 3413 cm^{-1} shows OH stretching vibrations in TiO_2 nanoparticles [22, 23, 24]. At 3190 cm^{-1} N-H stretching bands mainly cis ordered were observed whereas from 3083 cm^{-1}- 2929 cm^{-1}shows C-H ring and C-H stretching bands. At 2851 cm^{-1} symmetric CH_2 stretching bands were observed [22]. 2600 cm^{-1} corresponds to the H bonded NH vibrations. C≡C disubstituted alkyl stretching was observed at2190 cm^{-1} [24]. At 1709 cm^{-1} C=O vibrations were observed. 1636 cm^{-1} and 1630 cm^{-1} corresponds to the bending modes of TiOH [23]. 1514 cm^{-1} shows C=C for the presence of carotenoid stretching [22]. At 1400 cm^{-1} symmetric bending of methyl group and at 1383 cm^{-1} Ti-O modes were observed [23]. 1238 cm^{-1} and 1224 cm^{-1} show asymmetric stretching of the phosphate group. At 1156 cm^{-1} stretching vibration of C-O was observed [22]. Absorption bands between 1000 cm^{-1} and 500 cm^{-1} shows absorption of Ti-O-Ti linkage in TiO_2 [25].

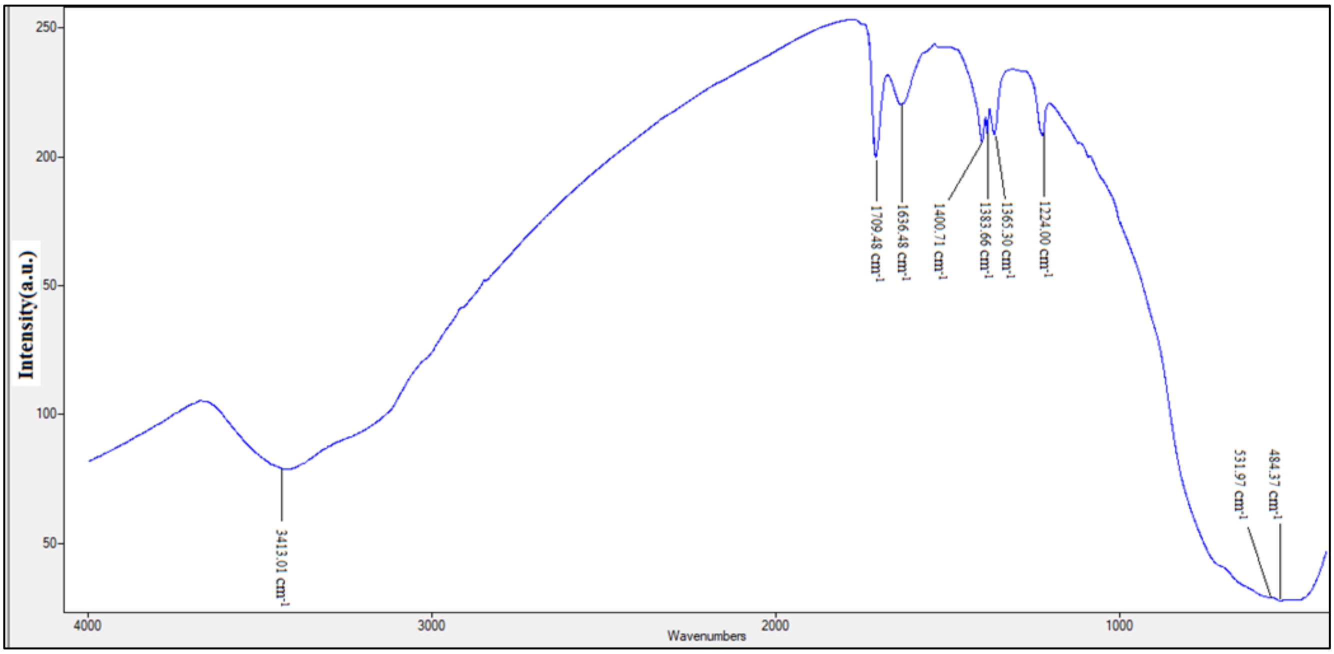

Fig 3: FTIR pattern of chemically synthesized TiO_2 nanoparticles.

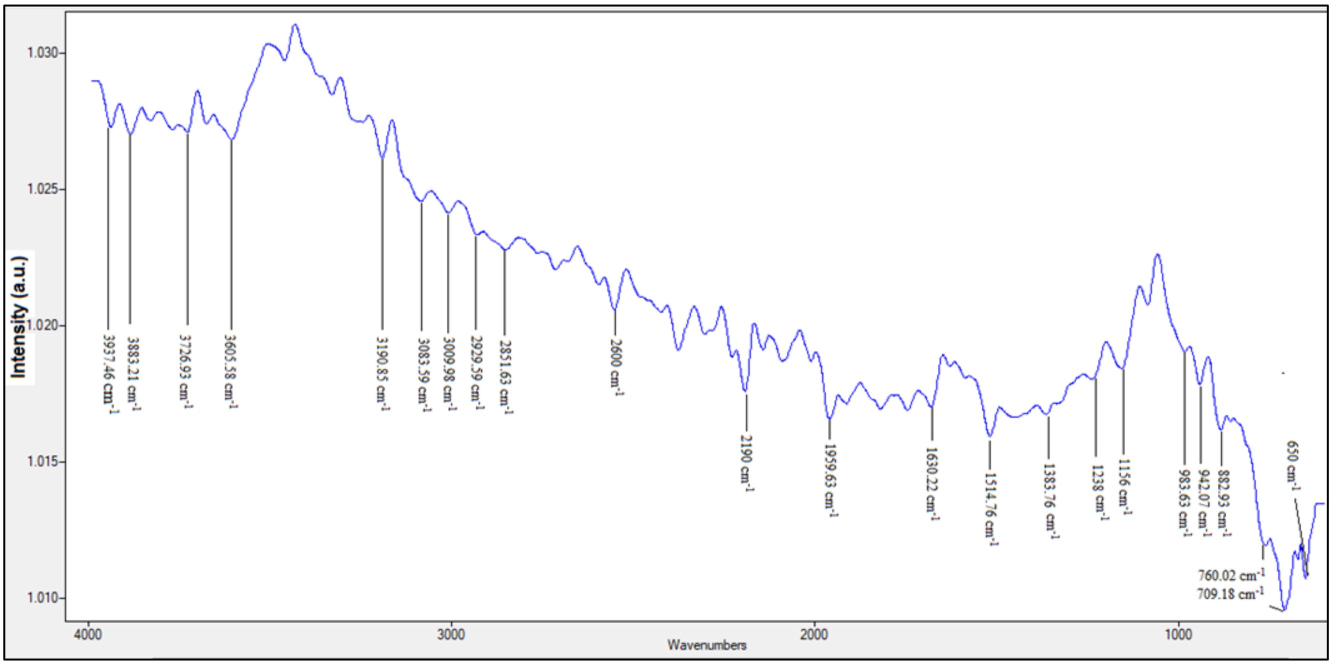

Fig 4: FTIR pattern of green synthesized nanoparticles by *Sonchus asper* (TiO_2 SA).

Chemically as well as green synthesized nanoparticles were tested against *Escherichia coli* MTCC 40 (EC), *Staphylococcus aureus* MTCC 1144 (SA) and *Klebsiella pneumoniae* MTCC 4030 (KP) at the concentration of 50µg/ml. chemically synthesized TiO_2 nanoparticles gave a maximum zone of inhibition against EC (12.66±0.57) followed by KP (8±0.57) and SA (7.33±0.33). On the other hand green synthesized nanoparticles gave maximum zone of inhibition against SA (12.33±0.33) followed by EC (12±0.57) and KP (10±0.57). Antimicrobial activities of the samples with zone of inhibitions were given in the table 1. Figure 5 shows the graphical representation of the bactericidal activities.

Table 1: Well/disk diameter 6mm; Values are the mean of three replicate, (-) shows no zone of inhibition, $P \leq 0.005$.

Samples /Antibiotics	Zone of inhibition in mm		
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*
TiO_2	07.33±0.33	12.66±0.57	08±0.57
TiO_2 SA	12±0.33	12±0.57	10±0.57
Ampicillin	09.66±0.33	20±0.57	11.66±0.57
Cefazolin	10±0.57	24±0.57	09.66±0.33

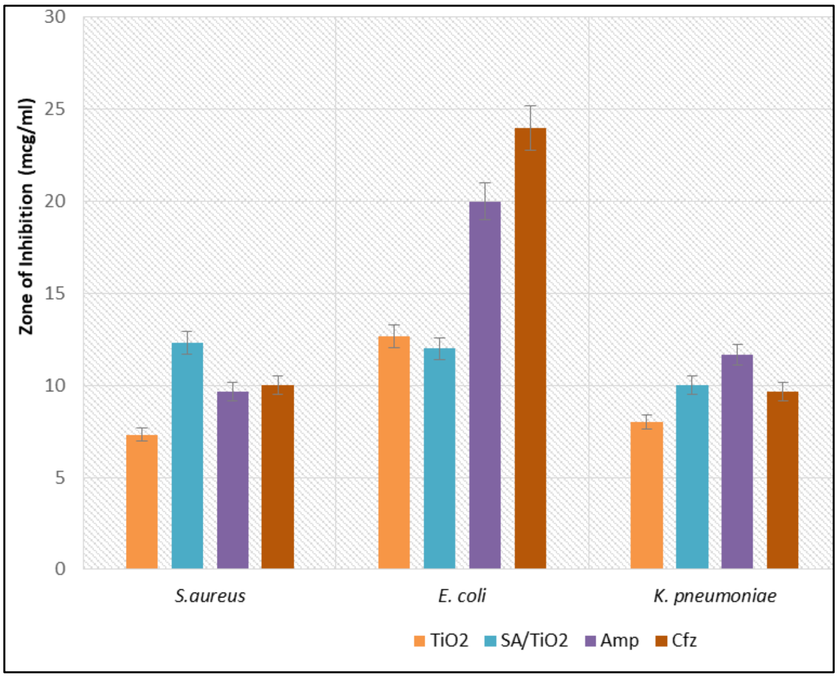

Fig 5: Graphical representation shows the comparison of the zone of inhibition between antimicrobial agents used against *Staphylococcus aureus* MTCC 1144, *Escherichia coli* MTCC 40, *and Klebsiella pneumoniae* MTCC 4030.

Discussion

The present study deals with the study of antimicrobial assay of chemically as well as green synthesized TiO_2 nanoparticles. Green synthesis has been done by using water extract of *Sonchus asper* herb via sol gel method. Titanium isopropoxide was utilized as a precursor for the synthesis and alcohol was considered as a reducing agent for the chemical synthesis. The amorphous precipitate results due to the reaction were repeatedly washed by alcohol followed by the calcination at 500º C. Synthesis of TiO_2 using Titanium isopropoxide and alcohol is due to their dehydration and hydrolysis [26]. At the intermediate of this reaction unstable Titanium hydroxide is formed which may further decompose leading to the formation of TiO_2. Phytochemicals of the plant extract may acts as capping as well as reducing agents which leads to the synthesis of stable green nanoparticles [27]. Antimicrobial activities reveal that green synthesized nanoparticles have more potential antimicrobial activity than chemically synthesized nanoparticles for all the tested pathogens. Green synthesized nanoparticles have also given more potential antimicrobial activity than all the tested antibiotics against *Staphylococcus aureus.* Whereas in the case of *Klebsiella pneumonia* it gives better activity than cefazolin. On the other hand antibiotics were more potential in the case of *Escherichia coli.* ROS (reactive oxygen species) may be the reason behind the antimicrobial potential of the nanoparticles. ROS causes the peroxidation of the bacterial lipid membranes which leads to the cell rupture causing cell death. ROS generation in the anatase phase of the TiO_2 nanoparticles is high due to the efficient production of OH group [28, 29]. FTIR results favour the presence of OH group in the particles.

Conclusion

In the present study TiO_2 nanoparticles were synthesized by chemical as well as green route. They were characterized by XRD for their size and crystallinity. Green synthesized nanoparticles were found in a smaller size than chemically synthesized nanoparticles. They have considerable antimicrobial potential against all the tested pathogens. As per results, we can conclude that green synthesized nanoparticles may have a potential biomedical application due to their antimicrobial properties, stability and enhanced dispersibility.

Acknowledgment

This research was financially supported by RGNF for SC.

Conflict of interest

We have no conflict of interest to declare.

References

1. Catauro M, Raucci MG, De Gaetano F, Marotta A. Antibacterial and bioactive silver-containing Na 2 O· CaO· 2SiO 2 glass prepared by sol–gel method. Journal of Materials Science: Materials in Medicine. 2004; 15(7):831-837.
2. Crabtree JH, Burchette RJ, Siddiqi RA, Huen IT, Hadnott LL, Fishman A. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. Peritoneal Dialysis International. 2003; 23(4):368-374.
3. Zhao G, Stevens SE. Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion. Biometals. 1998; 11(1):27-32.
4. Li Y, White TJ, Lim SH. Low-temperature synthesis and microstructural control of titania nano-particles. Journal of solid state chemistry. 2004; 177(4-5):1372-1381.
5. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer research. 2009; 69(22):8784-8789.
6. Liu S, Chen A. Coadsorption of horseradish peroxidase with thionine on TiO2 nanotubes for biosensing. Langmuir. 2005; 21(18):8409-8413.
7. Khan SU, Al-Shahry M, Ingler WB. Efficient photochemical water splitting by a chemically modified n-TiO2. Science. 2002; 297(5590):2243-2245.
8. Caballero L, Whitehead KA, Allen NS, Verran J. Photo inactivation of Escherichia coli on acrylic paint formulations using fluorescent light. Dyes and pigments. 2010; 86(1):56-62.
9. Saxena A, Tripathi RM, Singh RP. Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity. Dig J Nanomater Bios. 2010; 5(2):427-432.
10. Balantrapu K, Goia DV. Silver nanoparticles for printable electronics and biological applications. Journal of materials research. 2009; 24(9):2828-2836.
11. Jain D, Daima HK, Kachhwaha S, Kothari SL. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Digest journal of nanomaterials and biostructures. 2009; 4(3):557-563.
12. Sundrarajan M, Gowri S. Green synthesis of titanium dioxide nanoparticles by Nyctanthes arbor-tristis leaves extract. Chalcogenide Lett. 2011; 8(8):447-451.
13. Maurya A, Chauhan P, Mishra A, Pandey AK. 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. 2012; 1(1):43-51.
14. Rajakumar G, Rahuman AA, Priyamvada B, Khanna VG, Kumar DK, Sujin PJ. Eclipta prostrata leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles. Materials Letters. 2012; 68:115-117.
15. Kumar PS, Francis AP, Devasena T. Biosynthesized and chemically synthesized titania nanoparticles: Comparative analysis of antibacterial activity. J Environ. Nanotechnol. 2014; 3(3):73-81.
16. Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F *et al*. Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L. Molecules. 2011; 16(8):6667-6676.
17. Jimoh FO, Afolayan AA. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. Records of Natural Products. 2011; 5(1):29.
18. Mallik P, Mishra SK, Satapathy KB. Studies on the antibacterial and antioxidant activities of *Sonchus asper* (L.) Hill and Seseli diffusum (Roxb. Ex sm.) Sant. & Wagh. ISOR J Pharma. Bio. Sci. (ISOR-JPBS). 2014: 9:24-27.
19. Purohit P, Bohra A. Effect of some plants extracts on conidial germination of some important phytopathogenic fungi. Geobios New Report. 1998; 17:183-184.
20. Alabri TH, Al Musalami AH, Hossain MA, Weli AM, Al-Riyami Q. Comparative study of phytochemical

screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. Journal of King Saud University-Science. 2014; 26(3):237-243.

21. Liu H, Yang W, Ma Y, Cao Y, Yao J, Zhang J *et al*. Synthesis and characterization of titania prepared by using a photoassisted sol− gel method. Langmuir. 2003; 19(7):3001-3005.
22. Movasaghi Z, Rehman S, Ur Rehman DI. Fourier transform infrared (FTIR) spectroscopy of biological tissues. Applied Spectroscopy Reviews. 2008; 43(2):134-179.
23. León A, Reuquen P, Garín C, Segura R, Vargas P, Zapata P. FTIR and Raman characterization of TiO2 nanoparticles coated with polyethylene glycol as carrier for 2-methoxyestradiol. Applied Sciences. 2017; 7(1):49.
24. Chemistry Libre texts, 2013 available at: https://chem.libretexts.org/Ancillary_Materials/Reference/Reference_Tables/Spectroscopic_Parameters/Infrared_Spectroscopy_Absorption_Table
25. Lu X, Lv X, Sun Z, Zheng Y. Nanocomposites of poly (L-lactide) and surface-grafted TiO2 nanoparticles: Synthesis and characterization. European Polymer Journal. 2008; 44(8):2476-2481.
26. Gourinchas Courtecuisse V, Chhor K, Bocquet JF, Pommier C. Kinetics of the titanium isopropoxide decomposition in supercritical isopropyl alcohol. Industrial & engineering chemistry research. 1996; 35(8):2539-2545.
27. Njagi EC, Huang H, Stafford L, Genuino H, Galindo HM, Collins JB *et al*. Biosynthesis of iron and silver nanoparticles at room temperature using aqueous sorghum bran extracts. Langmuir. 2010; 27(1):264-271.
28. Dutta RK, Nenavathu BP, Gangishetty MK, Reddy AV. Antibacterial effect of chronic exposure of low concentration ZnO nanoparticles on E. coli. Journal of Environmental Science and Health, Part A. 2013; 48(8):871-878.
29. Zoltan T, Rosales MC, Yadarola C. Reactive oxygen species quantification and their correlation with the photocatalytic activity of TiO2 (anatase and rutile) sensitized with asymmetric porphyrins. Journal of Environmental Chemical Engineering. 2016; 4(4):3967-3980.