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Research Article

Biosynthesis, Antimicrobial and Cytotoxic Effects of Titanium Dioxide Nanoparticles Using *Vigna unguiculata* Seeds

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ABSTRACT
Background: Physical and chemical methods of synthesizing metal nanoparticles have been on the focus for the last decade as it has been broadly exploited by researchers. Biological synthesis of metal nanoparticles was found to be easy and economical. The wide applications of titanium dioxide in various fields have drawn attention for biosynthesis of titanium dioxide nanoparticles. Cowpea seeds are easily available and rich in protein as well as high in antioxidant which enhances the good characteristics of the nanoparticles synthesized using it. Methods: In the present investigation the nanoparticles are synthesized using *Vigna unguiculata* (cowpea) seeds extract. 0.1 mM titanium dioxide was mixed with cowpea seeds extract for the preparation of the nanoparticles. The characterization of nanoparticles was done by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Antibacterial activity of the titanium dioxide nanoparticles was checked against clinical pathogens followed by antioxidant study and cytotoxicity assay by 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay and [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] (MTT) assay respectively. Results: The oval shaped biologically synthesized nanoparticles were effective against most of the clinical pathogens. The observance of peak at 418 cm^{-1} confirms the synthesis of titanium dioxide nanoparticles. The titanium nanoparticles were highly antioxidant in nature and cytotoxic on MG63 osteosarcoma cell lines. Conclusion: The biological method of preparation of nanoparticles proved to be easy and cost effective. The nanoparticles synthesized can be further used in research for anticancer treatments.

Keywords: Biological synthesis, Titanium dioxide, *Vigna unguiculata*, Antibacterial activity, Antioxidant assay, Cytotoxicity assay.

INTRODUCTION

Biological synthesis of nanoparticles has introduced a new approach in the field of nanotechnology[1]. Green synthesis of metal nanoparticles from plant extracts is found to be more effective than physical or chemical techniques. Silver and gold nanoparticles synthesized using plant extracts have shown inhibitory effect against a range of pathogenic microorganisms[2]. Gold and silver nanoparticles from *Aloe vera* plant extracts are utilized in many fields. Neem leaves broth is used in preparation of gold and silver nanoparticles. The broth is effective due to the presence of flavonone and terpenoids[3]. Seed extract of *Capsicum sp*, commonly known as chili, are used for biosynthesis of silver nanoparticles. The particles proved to have antibacterial activity and were also highly antioxidant[4]. Apart from plants, microorganisms are also used in preparation of nanoparticles. Biosynthesis of silver nanoparticles from fungus has attracted attention of many researchers[5].

Biosynthesis using plant extracts are expanding in the field of research very widely. A genus of legumes, *Vigna*, is known for its high antioxidant characteristics. *Vigna radiata*, commonly known as green gram has been previously used in synthesis for lanthanum nanoparticles[6].

Cowpea (*Vigna unguiculata*) is an established food and animal feed crop. The crop belongs to *Fabaceae* family and sub family, *Fabiodeae*. The leguminous crop is cultivated largely in low and mid altitude parts of Africa[7] as well as tropical Asia, southern Europe and few parts of America[8]. Over the world, cowpea is grown in almost 45 countries including Nigeria, Niger, Burkina Faso, Cameroon, Mali, Uganda, Kenya, Senegal, and Tanzania in Africa. South Asian countries like Myanmar and Sri Lanka are significant producers of the crop[9]. *Vigna unguiculata* seeds are rich in protein, tannins, phenolic acids and cinnamic acid derivatives. The presence of phenolic acids is responsible for the antioxidant nature of the legumes[10]. Photocatalytic splitting of water is feasible by titanium dioxide in presence of ultraviolet radiations[11]. This indicates the usage of titanium dioxide in purification of water. The photocatalytic action of titanium dioxide is used to kill many pathogens present in water and can be regarded as an effective sterilization agent. Polarized titanium dioxide electrodes are also found efficient in killing single cancer cells[12]. Apart from titanium dioxide electrode, titanium dioxide particles are also checked for effectivity against human bladder cancer cells. The particles efficiently killed the cancer cells proving their

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potency in cancer treatment[13]. The flexibility in usage of titanium dioxide attracts the attention for biosynthesis of titanium dioxide nanoparticles. The applications of titanium dioxide range from adding the white color to toothpaste and opaqueness of paint to production of ceramic tiles. Titanium dioxide nanoparticles are used in pharmaceuticals applications as well. Lowest levels of titanium are used in various cosmetic products[14]. The wide usage of titanium in cosmetics and pharmaceuticals can be a reason for the necessity for advanced methods of synthesizing titanium dioxide nanoparticles. Titanium dioxide nanoparticles are capable of absorbing UV rays and so they are used in preparation of sunscreens. The physicochemical properties of titanium dioxide nanoparticles, especially their high refractive indices are the reason of the nanoparticles being used for this purpose[15]. The present study deals with the biosynthesis of titanium dioxide nanoparticles using cowpea seeds extracts followed by its characterization and evaluating the effect of the nanoparticles against clinical pathogens. The antioxidant and cytotoxic characters of the nanoparticles were also tested.

MATERIALS AND METHODS

Collection of Vigna unguiculata Seeds

The leguminous seeds of *Vigna unguiculata* (cowpea) were purchased from a market in Vellore, Tamil Nadu.

Chemicals used

All chemicals, including titanium dioxide were purchased from Sigma Aldrich.

Collection of Bacterial Pathogens

Twelve clinical pathogens were collected from Microbial Biotechnology Laboratory, VIT University, Vellore. The pathogens included *Escherichia coli*, *Staphylococcus aureus, Serratia marcecens, Salmonella* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Proteus mirabilis* and *Shigella* sp. All the pathogens were used for testing the antimicrobial activity of the titanium oxide nanoparticles synthesized using *Vigna unguiculata* seed extracts.

Preparation of Vigna unguiculata (cowpea) Seeds Extract

The seeds were collected, washed accurately and dried at room temperature. After drying thoroughly, the seeds were finely powdered and boiled in 100 ml of distilled water. The extract was then filtered and stored for further use[16].

Biosynthesis of Titanium Dioxide Nanoparticles using Cowpea seeds extract

0.1 mM of Titanium dioxide was freshly prepared and stirred for 2 h. 20 ml cowpea seeds extract was mixed with 80 ml of titanium oxide solution. The mixed solution was then incubated at room temperature at rotary shaker for 24 h[17]. After the incubation, the solution was filtered, collected and calcined. The dried particles were then powdered using a mortar and pestle.

Characterization of Titanium Dioxide nanoparticles

Powdered samples of the nanoparticles were analyzed for Scanning Electron Microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR)[18]. The shape of the biosynthesized nanoparticles was determined by scanning electron microscopy analysis. The functional groups present in the nanoparticles were analyzed by Fourier transform infrared spectroscopy.

Antibacterial Activity of the Titanium Dioxide Nanoparticles

The antimicrobial activity of the synthesized titanium dioxide nanoparticle was examined against various clinical pathogens. Well diffusion technique was followed for the experiment. Mueller Hinton agar plates were prepared and swabbed with each strain of pathogens. Wells were prepared on the agar followed by addition of 50 µl, 75 µl and 100 µl of nanoparticles. The plates were incubated overnight and observed for zone of inhibition[18].

Antioxidant Activity by DPPH Assay

2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was followed to check the antioxidant nature of the biologically synthesized titanium dioxide nanoparticles using cowpea seeds. Ascorbic acid was considered as standard and the stock solution was prepared using methanol. 100 µl of each titanium dioxide nanoparticles and DPPH reagent were added to 2.8 ml of acetate buffer simultaneously and incubated at dark conditions for 15-20 min. After incubation, the colour change was observed and absorbance was measured at 517 nm[19].

The percentage inhibition was calculated by the formula:

Percent inhibition of DPPH activity =

$$\frac{[\text{Absorbance (Control)} - \text{Absorbance (Sample)}]}{\text{Absorbance(control)}} \times 100$$

Cytotoxicity Assay against Osteosarcoma Cell Lines

The cytotoxic property of the synthesized nanoparticles was checked against MG 63 (osteocarcoma cell lines) by [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] (MTT) assay[20]. The cell lines obtained from National Centre for Cell Science, Pune were grown in Eagles Minimum Essential Medium containing 10% FBS. Optimum parameters to maintain the cells were 37°C, 5% CO_2, 95% air and 100% humidity. The cells were diluted with medium containing 5% FBS until the density reaches $1X10^5$ cells/ml. 100 µl cell suspension was added to each well of a 96 well plate at a plating density of 10000 cells/well. The cells were incubated at optimum parameters mentioned above. 24 hours later, the cells were treated with serial concentrations of the test samples. Dimethyl sulfoxide (DMSO) was used for initial dissolving of sample and the nanoparticles were dispersed in PBS. After addition of sample, plates were further incubated for 48 hours. Control was maintained with medium without test samples. 15µl of MTT in PBS was added in each well and kept for incubation at 37°C for 4 hours. The medium with MTT was removed and formed formazan crystals were solubilized in 100µl of DMSO. The absorbance was measured at 570 nm.

Percentage of Cell Viability = $(A/A_0) \times 100$

Where, A = Absorbance of sample

A_0 = Absorbance of control

Nonlinear regression graph was plotted between percentage of Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

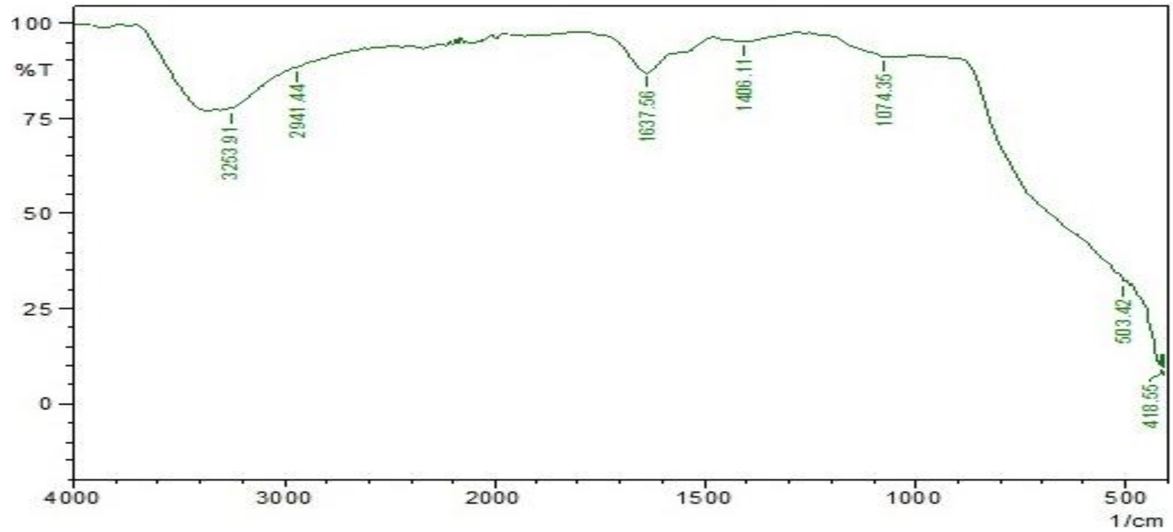


Figure 1: FTIR spectra of titanium dioxide nanoparticles synthesized using Cowpea seeds.

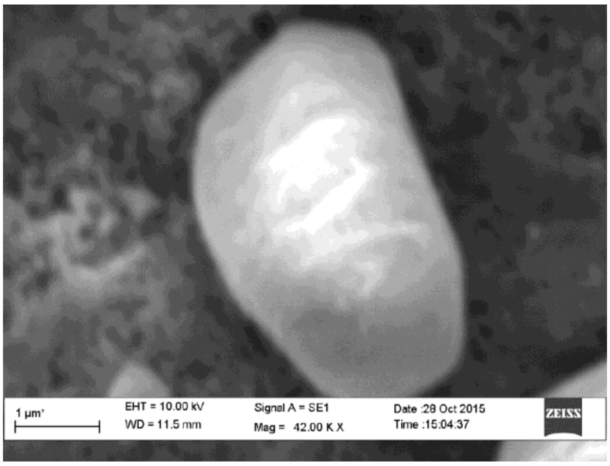


Figure 2: Titanium dioxide nanoparticle observed under SEM.

Table 1: Antibacterial activity of titanium dioxide nanoparticles synthesized using cowpea seed extracts.

Sr. No	Name of the Organisms	Zone of Inhibition (cm)		
		50 µl	75µl	100 µl
1.	*E. coli*	2	2	3.9
2.	*Salmonella* sp	-	4	4
3.	*Enterobacter*	-	4	5
4.	*Pseudomonas aeruginosa*	3	3.5	-
5.	*Serratia marcescens*	-	4	6
6.	*Staphylococcus aureus*	-	-	-
7.	*Klebsiella* sp	-	-	-
8.	*Shigella* sp	-	-	-
9.	*Proteus mirabilis*	-	-	-

RESULTS AND DISCUSSION

Biosynthesis of Titanium Dioxide Nanoparticles

The titanium dioxide nanoparticles was dried and obtained in powdered form.

Characterization of the Titanium Dioxide Nanoparticles

Fourier Transform Infrared Spectroscopy

The FTIR graph depicts the presence of N-H stretch, C-H stretch, N-H bend, C-N stretch in the synthesized nano-sized particles. The existence of these stretches determines the primary amines and alkanes functional groups of the particles. The peaks observed at 418 cm^{-1} can confirm the synthesis of titanium dioxide nanoparticles. Few similar peaks have also been recorded by Hema et al. in a study with titanium nanoparticles[21].

Scanning Electron Microscopy

The titanium nanoparticle was observed under scanning electron microscope. The nanoparticles were distinctly observed as oval shaped particles. Most of the particles observed were smooth surface with properly detectable edges. Smooth surface titanium dioxide nanoparticles have also been reported in previous studies[22].

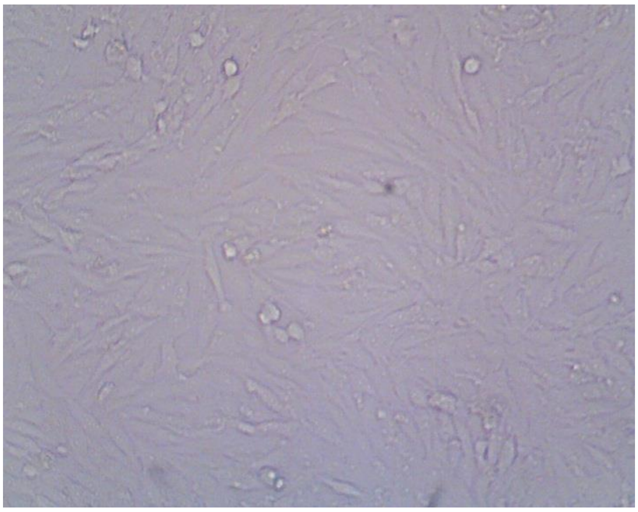
Antibacterial Activity of the Titanium Dioxide Nanoparticles

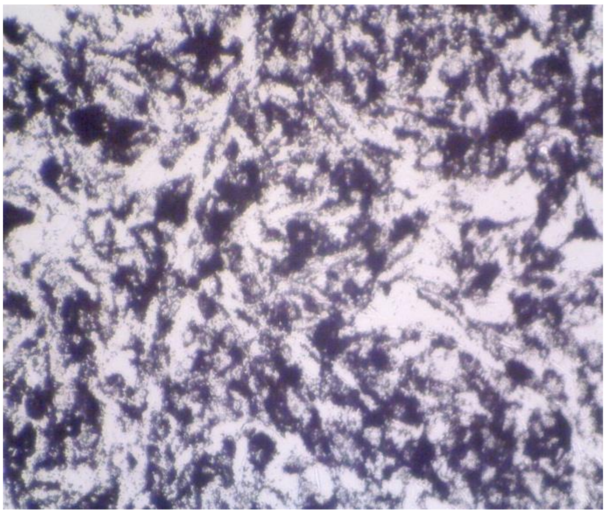
The titanium dioxide nanoparticles inhibited the growth of *E. coli*, *Salmonella*, *Enterobacter*, *Pseudomonas aeruginosa* and *Serratia marcescens*. The zone of inhibition was measured for each of the pathogens and recorded. Titanium dioxide nanoparticles are strongly effective against pathogens and hence they are often utilized as sterilization agents. The capability of the titanium dioxide nanoparticles to inhibit bacterial growth has been listed in Table I.

Antioxidant Activity by DPPH Assay

The slight change in colour after the incubation time in DPPH assay indicated the synthesized nanoparticle to be antioxidant in nature. The titanium dioxide nanoparticles were observed to have highly antioxidant which reveals their capacity of donate electrons and hence react with free radicals further converting them to form more stable products. The antioxidant nature increased with the increase in concentration of titanium nanoparticles. This suggests that the titanium nanoparticles synthesized from cowpea seeds can act against disease causing free radicals[23].

Cytotoxicity Assay against Osteosarcoma Cell lines





(a) (b)

Figure 3: Cytotoxicity activity of the nanoparticles: (a). Control, (b). Test.

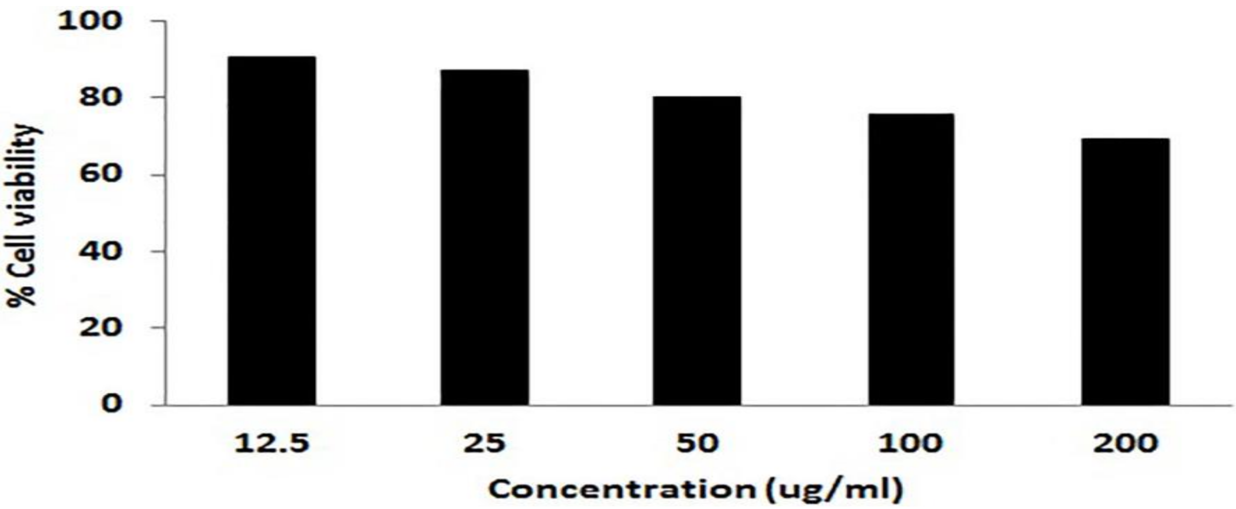


Figure 4: Graph showing cell viability after treatment with titanium dioxide nanoparticles.

The titanium dioxide nanoparticles from the cowpea seeds were found to show 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. 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 (REF 3) cell lines[24]. The cytotoxicity result as shown in Figure 3 (a and b) indicates the possibility of the biologically synthesized titanium dioxide nanoparticles can be utilized for inhibition of proliferating cancer cells. Figure 4 shows the percentage of cell viability with increasing concentration of biologically synthesized titanium dioxide nanoparticles using cowpea seeds.

CONCLUSION

The immense applications of titanium dioxide drives for the idea of neccessity and applications of titanium dioxide nanoparticles in various fields. Titanium dioxide nanoparticles were synthesized using *Vigna unguiculata* (cowpea) seeds by simple biological method. Biological method proved to be superior than physical and chemical processes of synthesizing nanoparticles because of its easy procedures,cost effectiveness and less consumption of time. The nanoparticles showed high antibacterial activity against clinical pathogenic bacteria and confirms to have potential antioxidant characteristics. Antioxidant nature of the titanium dioxide nanoparticles is enhanced by the usage of the cowpea seeds extract since the leguminous seeds are highly antioxidant in nature. The cytotoxity assay concluded that the particles were able to inhibit the proliferation of Mg 63 cell lines. This hints a scope for future use of titanium dioxide nanoparticles in research for anticancer treatments.

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CONFLICTS OF INTEREST

The authors certify that there is no conflict of interest.

REFERENCES

1. Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA. Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria*: a novel biological approach. Process Biochemistry. 2007 May 31;42(5):919-23.
2. Savithramma N, Rao ML, Rukmini K, Devi PS. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of ChemTech Research. 2011 Jan 1; 3(3):1394-402.
3. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. Journal of colloid and interface science. 2004 Jul 15; 275(2):496-502.
4. Singh N, Chatterjee A, Chakraborty K, Chatterjee S, Abraham J. Cytotoxic Effect on MG-63 Cell Line and Antimicrobial and Antioxidant Properties of Silver Nanoparticles Synthesized with Seed Extracts of *Capsicum sp*. Records of Natural Products 2016 Jan 1;10(1): 47-57.
5. Saha P, Rajkumar K, Abraham J. Comparative study on antimicrobial property of silver nanoparticles synthesized by *Fusarium equiseti* and *Fusarium solani*. Journal of Bionanoscience. 2012 Jun 1; 6(1):28-32.
6. Chatterjee A, Archana L, Niroshinee V, Jayanthi A. Biosynthesis of Lanthanum Nanoparticles using Green Gram Seeds and their Effect on Microorganisms. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016 March-April; 7(2): 1462-70.
7. Agbogidi OM, Egho EO. Evaluation of eight varieties of cowpea (*Vigna unguiculata* (L.) Walp) in Asaba agro-ecological environment, Delta State, Nigeria. European Journal of Sustainable Development. 2012 Jun 1; 1(2):303.
8. Singh U, Singh B. Tropical grain legumes as important human foods. Economic Botany. 1992 Jul 1; 46(3):310-21.
9. Abate T, Alene AD, Bergvinson D, Shiferaw B, Silim S, Orr A, Asfaw S. Tropical grain legumes in Africa and South Asia: knowledge and opportunities. Nairobi, Kenya: International Crops Research Institute for the Semi-Arid Tropics. 112 pp.
10. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chemistry. 2007 Dec 31; 101(1):10-9.
11. Chen X, Mao SS. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical reviews. 2007 Jul 11;107(7):2891-959.
12. Fujishima A, Rao TN, Tryk DA. Titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology C: Photochemistry Reviews. 2000 Jun 29;1(1):1-21.
13. Kubota Y, Shuin T, Kawasaki C, Hosaka M, Kitamura H, Cai R, Sakai H, Hashimoto K, Fujishima A. Photokilling of T-24 human bladder cancer cells with titanium dioxide. British journal of cancer. 1994 Dec;70(6):1107.
14. Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environmental science & technology. 2012 Feb 8; 46(4):2242-50.
15. Smijs TG, Pavel S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. Nanotechnology, science and applications. 2011; 4:95.
16. Prasad C, Venkateswarlu P. Soybean Seeds Extract Based Green Synthesis of Silver Nanoparticles. Indian Journal of Advances in Chemical Science. 2014; 2(3):208-11.
17. Santhoshkumar T, Rahuman AA, Jayaseelan C, Rajakumar G, Marimuthu S, Kirthi AV, Velayutham K, Thomas J, Venkatesan J, Kim SK. Green synthesis of titanium dioxide nanoparticles using *Psidium guajava* extract and its antibacterial and antioxidant properties. Asian Pacific journal of tropical medicine. 2014 Dec 31; 7(12):968-76.
18. Chauhan R, Kumar A, Abraham J. A biological approach to the synthesis of silver nanoparticles with *Streptomyces* sp JAR1 and its antimicrobial activity. Scientia pharmaceutica. 2013 Jun; 81(2):607.
19. Priyanka C, Kadam DA, Kadam AS, Ghule YA, Aparadh VT. Free radical scavenging (DPPH) and ferric reducing ability (FRAP) of some gymnosperm species. International Research Journal of Botany. 2013; 3(2):34-6.
20. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. Journal of the National Cancer Institute. 1991 Jun 5;83(11):757-66.
21. Hema M, Arasi AY, Tamilselvi P, Anbarasan R. Titania nanoparticles synthesized by sol–gel technique. Chem Sci Trans. 2013;2(1):239-45.
22. Rajakumar G, Rahuman AA, Roopan SM, Khanna VG, Elango G, Kamaraj C, Zahir AA, Velayutham K. Fungus-mediated biosynthesis and characterization of TiO_2 nanoparticles and their activity against pathogenic bacteria. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012 Jun 30;91:23-9.
23. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2, diphenyl-picryl-hydrazyl radical. African Journal of Biomedical Research. 2002; 5(1-2).
24. Suker DK, Albadran RM. Cytotoxic Effects of Titanium Dioxide Nanoparticles on Rat Embryo Fibroblast REF-3 Cell Line in vitro. European Journal of Experimental Biology. 2013;3(1):354-63.