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MICROWAVE-ASSISTED GREEN SYNTHESIS AND CHARACTERIZATION OF TITANIUM OXIDE NANOPARTICLES USING *CENTELLA ASIATICA* (L.) LEAF EXTRACT AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract

Green synthesis of titanium oxide (TiO_2) nanoparticles was successfully developed by using Centella asiatica leaf extract. Metal oxide nanoparticles have a high specific surface area and a high fraction of surface atoms. Titanium oxide nanoparticles have unique properties as a consequence of this size, distribution and morphology and are a very important component in the rapidly developing fields of nanotechnology. In this research, titanium oxide nanoparticles with leaf extract percent were characterized by instrumental analysis such as X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and UV-Vis Spectroscopy. Generated titanium oxide nanoparticles were investigated different degree of antimicrobial activity against micro-organisms and it was observed that the zone of inhibition whose diameters were estimated. This research can provide useful and comprehensive results because of their main physicochemical properties and some of their medical applications.

Keywords: *Centella asiatica leaf extract, XRD, SEM, FTIR, UV-Vis and Antimicrobial activity*

Introduction

Nanoparticles are the important role in nanotechnology that are being explored due to the diversity of potential applications in biomedical, optical, photocatalytic, sensing and electronic devices [G. Nabi et al 2018]. 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. TiO_2 is the most promising material in the group of the metal oxides [Abou-Helal,M.O et al 2002]. In n - type semiconductor, titanium dioxide is a most important semiconductor due to its light absorption, surface adsorption and charge transport properties. TiO_2 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. Metal oxide nanoparticles Nanoparticles can be made of Titanium oxide (TiO_2) by co-precipitation method [Castaneda L et al 2002]. In order to obtain these particles, titanium oxide (TiO_2) paricles is required and proposed mainly from the titanium tetra chloride, hydrochloric acid and ammonia used as the starting materials [Dhahir, T.A.AL., (May 2013)]. Titanium oxide nanoparticles prepared by various methods like sol-gel method, chemical deposition method, solid state reaction method, hydrothermal method co-precipitation method and so on [Monoharan,C & Sridhar, R, (September, 2012)]. Among these, co-precipitation method is considered to be one of the best techniques and potentially advantageous in comparison to other method to produce pure phase formation of compound, low temperature preparation, highly purity and yield nanoparticles. Thin film of Titanium dioxide (TiO_2) has generated a lot of interest because of its attractive properties such as wide band gap, high refractive index, high dielectric constant, absence of nontoxicity and also it is

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considerable interest for both scientific and technical applications [Revathi, B. P. Perumal & Nachammai.J., (September 2018)].

Materials and Methods

Experimental Preparation of TiO_2 Nanoparticles

Materials

For the preparation of experimental details, all the chemicals (Titanium chloride ($TiCl_4$) and reagents used for the preparation of TiO_2 Nanoparticles were purchased from Academy chemical store in the downtown of Yangon. This chemical was analytically pure and directly used as received without further purification.

The materials used for the synthesis of centella asiatica leaves extract were collected from market.

Methods

The synthesis method consisted of two steps (1) the preparation of the centella asiatica leaf extract and (2) the synthesis of the TiO_2 nanoparticles.

Preparation of Centella asiatica Leaf Extract

Fresh leaves of Centella asiatica were collected from the market. The leaves were cleaned thoroughly with tap water and distilled water. After cleaning the leaves, take 50 g of the leaves were crushed and grinded in mortal and pestle by adding deionized water dropwise. The paste is then mixed with 200 ml of distilled water and kept over the hot plate and boiled for 2 h at 80 °C. Then, it is set to get cool down and the extract solution was filtered by filter paper until no debris is present. The filtrate was stored for the synthesis of TiO_2 nanoparticles.

Synthesis of Titanium Oxide Nanoparticles with Centella asiatica Leaf Extact

Once the aqueous Centella asiatica leaf extract is obtained, to synthesis the TiO_2 nanoparticles, 1 g of titanium chloride ($TiCl_4$) was dissolved in 100 ml of distilled water. Distilled water was used as solvent. Different amount of leaves extract solution (30 ml and 60 ml) was added drop wise under constant stirring up to achieve the pH levels. Firstly, these solutions were subjected to stirring with magnetic stirrer for 4 h continuously. During this process, the whitish colour of the mixed solution changed into yellow with the addition of the leaf extract solution. After stirring process, TiO_2 nanoparticles were formed. The mixture solution was centrifuged and washed three times with deionised water to remove the by-product. The nanoparticles were left for drying at 100 °C for overnight and annealed at 500 °C for 1 h. Figure 1(a-d) showed the photos of experimental procedure of green synthesis of titanium oxide nanoparticles.

Characterization Techniques

The green synthesized silver nanoparticles will be characterized by XRD, SEM, FTIR and UV-vis spectroscopy for structural, morphological, chemical and optical properties. The antimicrobial activity will be studied with these titanium oxide nanoparticles using Centella asiatica leaf extract

Figure 1(a) Plant extracted solution

Figure 1(a) Titanium oxide solution

Figure 1(c) Heating Process

Figure 1(d) TiO_2 Nanoparticles

Results and Discussions

XRD Analysis of Titanium Oxide (TiO_2) Nanoparticles

The titanium oxide nanoparticles by green synthesis were obtained and XRD technique was used to examine towards studying phase analysis, particle structure, and crystallographic investigation and lattice parameters. The phase analysis of powder was determined by using an X-ray Diffractometer (Rigaku RINT 2000). The XRD profiles of TiO_2 particles of different extract solution (30 ml and 60 ml) at 500 °C, thus obtained and shown in figure 2 (a & b). There were extra peaks on pattern of all particles because the results clearly proved the influence of the concentration of extract solution on the product composition and agreed with the typical TiO_2 pattern of anatase nano particles structure. All peaks were found to be well matched with the diffracted of standard. The intensity of (101) reflection was much stronger than that of remaining TiO_2 peaks. The XRD measurement showed that all peaks of TiO_2 were consisted with that TiO_2 standard (JCPDS) file having a tetragonal structure. The broad peaks in XRD patterns indicate fine crystallite size of the TiO_2 particles. The average crystallite size of TiO_2 particles were in the range of 42.39 nm. The crystallite size was calculated from the XRD peak broadening of peaks using Scherrer's formula. By analyzing XRD measurement, all the peak heights and peak positions were in good agreement with library file of XRD machine.

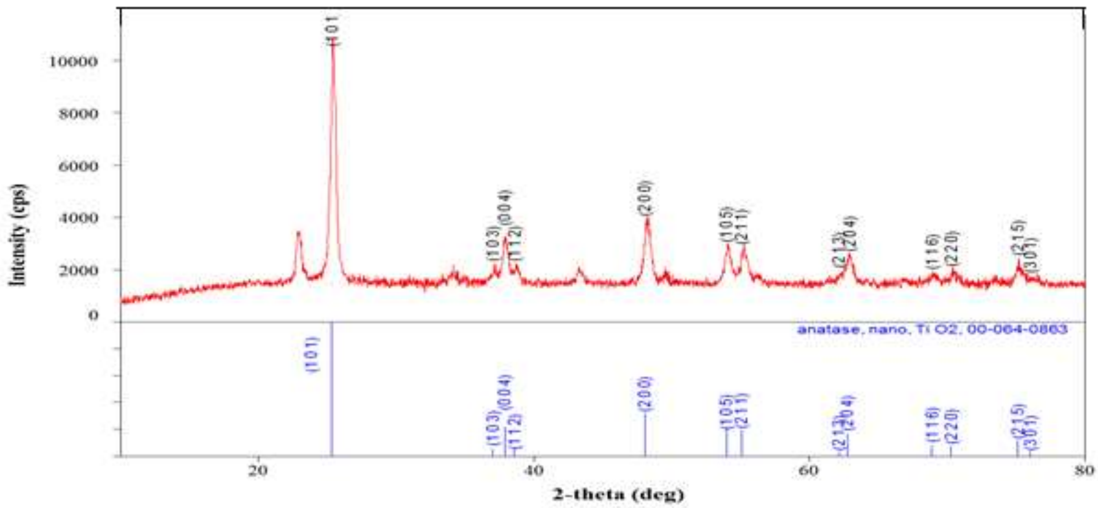

Figure 2 (a) XRD pattern of TiO_2 nanoparticles (30 ml of extract solution) at 500 °C

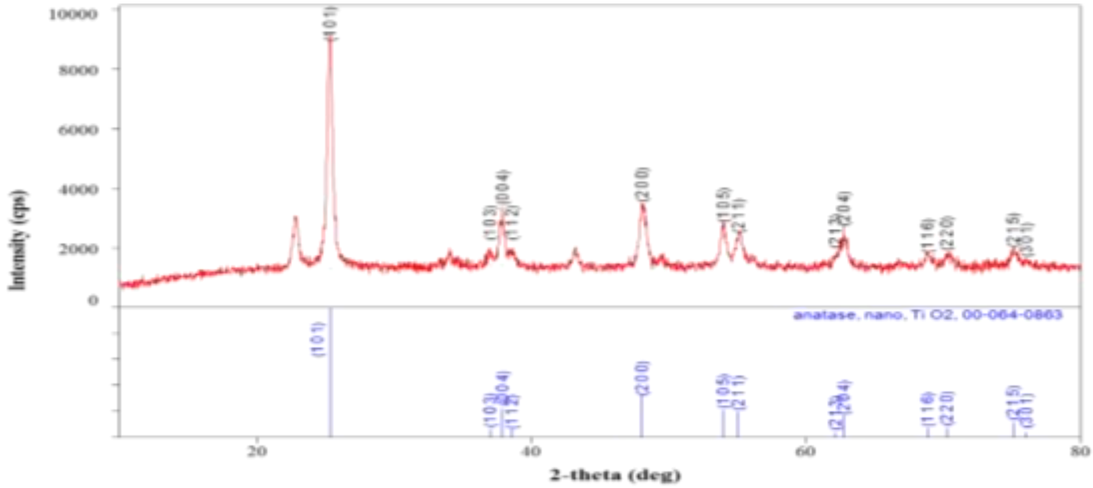

Figure 2 (b) XRD pattern of TiO_2 nanoparticles (60 ml of extract solution) at 500 °C

SEM Analysis of Titanium Oxide (TiO_2) Nanoparticles

The microstructural properties of TiO_2 nanoparticles by green synthesis were observed by using Scanning Electron Microscopy (SEM). Materials evolutions were obtained grain size, surface roughness and pore distribution. Figure 3 (a-b) showed the SEM photographs of TiO_2 crystalline powder with different extract solution at process temperature 500 °C. As detail analysis of SEM micrograph, it looked crack-free and little dense. Porosity and grain growth pattern were significantly observed on SEM images. These images were smooth and seemed to be front-oriented and the grain distribution was uniform but some of grain sizes were slightly larger. According to SEM results, these TiO_2 nanoparticles were composed of regular and sphere grains and TiO_2 nanoparticles were reported to be an average grain size of 35.53 nm for 30 ml of extract solution and 30.33 nm for 60 ml of extract solution with spherical shape by SEM images at process temperature 500 °C. These figures indicated that most of the grain size was regular structure and a few number of large grain size were found. It looks fairly dense and rough. These consisted of well-defined grain but marked difference. This fact indicated that structural properties were influenced by different extract concentration. From the images, it was clearly found that the little amount of pores and grain growth were examined and the orientation of grain was towards left for all images. TiO_2 nanoparticles were reported to be an average grain size of 35.53 nm for 30 ml of extract solution and 30.33 nm for 60 ml of extract solution with spherical shape by SEM images. In these results, the average grain size of the TiO_2 nanoparticles were depended varying different extract solution of *Centella asiatica* Leaf.

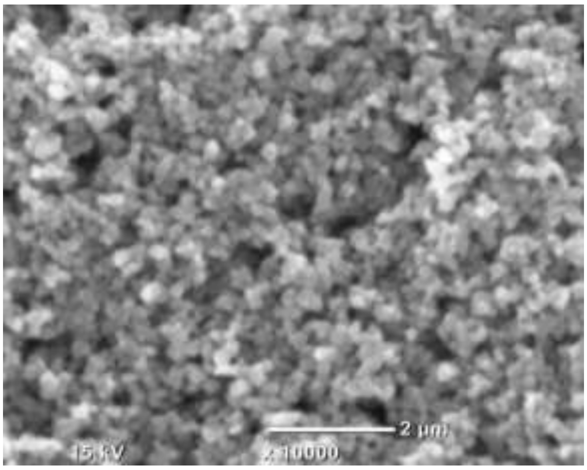

Figure 3 (a) SEM image of TiO_2 anoparticles (30 ml of extract solution) at 500 ºC

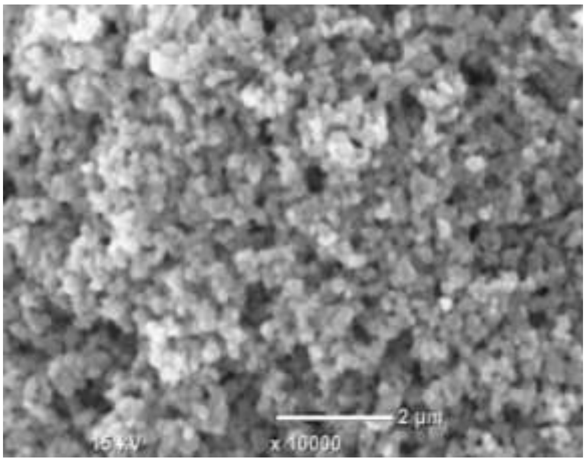

Figure 3 (b) SEM image of TiO_2 nanoparticles (60 ml of extract solution) at 500 ºC

UV-Vis Analysis of Titanium Oxide Nanoparticles

The absorbance spectrum of titanium oxide TiO_2 nanopowders were measured by using UV-Vis Spectrometer. The optical absorption spectra for a range of samples of titanium oxide (TiO_2) nanoparticles (TiO_2) with different Centella asiatica leaf extract concentration was obtained in UV/ VIS/ NIR region (up to1100 nm) using a SHIMADZU U-1800 UV/ VIS Double Beam Spectrophotometer. The wavelength range of spectrum lied between 190 nm and 400 nm. As the different leaf extracts were added to aqueous mixture solution, the colour of the solution changed from the whitish colour of the mixed solution changed into yellow and finally to colloidal pale yellow indicating titanium oxide nanoparticles (TiO_2) formation. The colour change is due to the Surface Plasmon Resonance phenomenon. From different literatures, it was found that the titanium oxide nanoparticles show SPR peak at around (370 nm – 431 nm). In this result, the dominant sharp band of titanium oxide nanoparticles (TiO_2) was observed around 385 nm in case of 30 ml extract solution whereas the band for 60 ml extract solution was found around 435 nm. The $(\alpha h\upsilon)^2$ and $h\upsilon$ characteristic curve of TiO_2 nanoparticles for 30 ml of extract solution and 60 ml of extract solution) at 500 ˚C were shown in figure 4 (a & b). On the characteristic curve, the extrapolating the straight line onto horizontal axis ($(\alpha h\upsilon)^2 = 0$), give the value of energy band gap and the obtained nanoparticles had a direct band gap 3.808 eV with 30 ml of extract solution and 3.295 eV with 60 ml of extract solution for the absorbance spectra. According these result, the intensity of absorption peak increases with increasing more extract solution. It was observed that the titanium oxide nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV- Vis absorption spectrum.

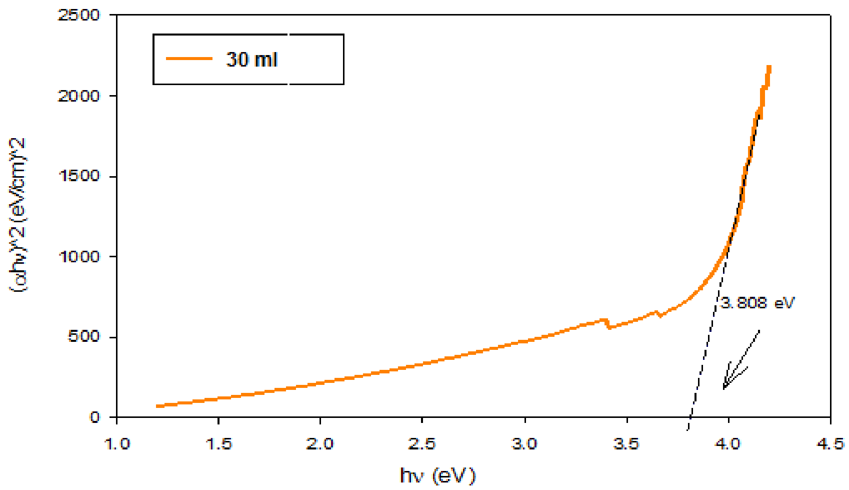

Figure 4 (a) Energy band gap calculation of TiO_2 Nanoparticles (30 ml extract solution)

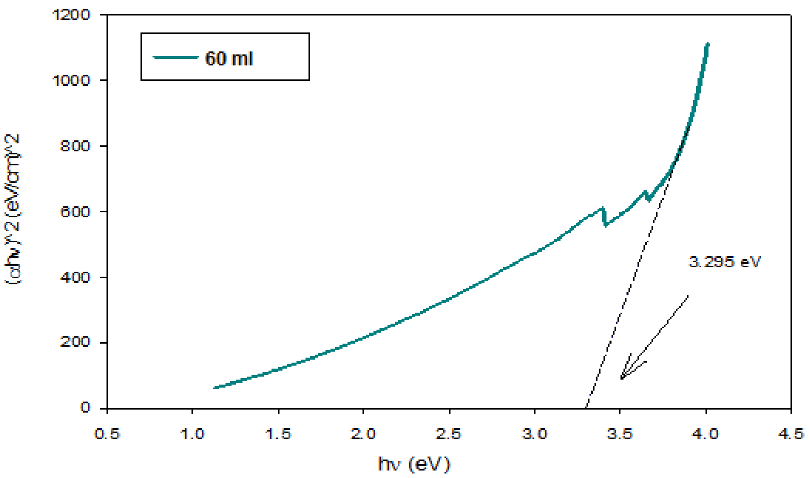

Figure 4 (b) Energy band gap calculation of TiO_2 Nanoparticles (60 ml extract solution)

Antimicrobial Activity of Titanium Oxide by paper disc diffusion assay

Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30 °C for 72 hours on a reciprocal shaker at 200 rpm. In this research, test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicons*, *Escherichia coli*, *Pseudomonas flurorescens*, *Klebsiella pneumonia.* 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30 °C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive compounds which inhibit the growth of test organisms. The diameters of inhibition zones that appeared were shown in Table 1 and figure 5 (a-c) According to experimental result, the inhibition zone of 60 ml extract solution was showed more against on microorganism than 30 ml of extract solution.

Table 1 Inhibitory zone of titanium oxide nanoparticles with different extract solution

No	Test organisms	Inhibition zones (mm)		Diseases
		30 ml of extract solution	60 ml extract solution	
1	*Aspergillus flavous*	9	10	Bronchitis
2	*Bacillus subtilis*	16	30	Pathogenic group
3	*Candida albicons*	12	18	Skin infection
4	*Escherichia coli*	12	20	Cholera, diarrhea and vomiting
5	*Pseudomonas flurorescens*	8	8	Bacteria for leaf blight
6	*Klebsiella pneumonia*	14	28	Infections in the urinary tract, liver abscess

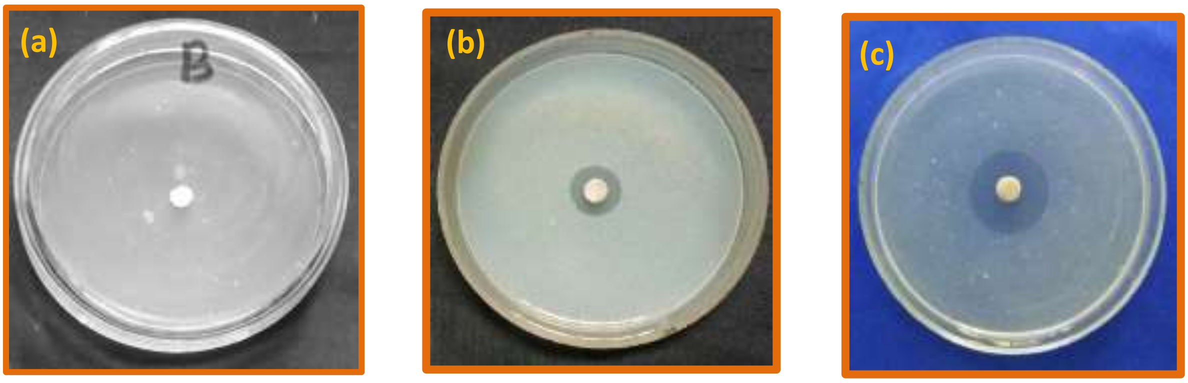

Figure 5(a) Control media without bacteria, (b) Zone of inhibition on *Bacillus subtilis* (30 ml extract solution) and (c) Zone of inhibition on *Bacillus subtilis* (60 ml extract solution)

Conclusion

In conclusion, the green synthesis of titanium oxide (TiO_2) nanoparticles has been demonstrated by the Centella asiatica leaf extract. Titanium oxide nanoparticles (TiO_2) have been appropriately characterized using XRD, SEM, and UV-Vis spectroscopy analysis. According to XRD analysis, all the peak heights and peak positions of different leave extract solution were in good agreement with library file of XRD machine. XRD analysis showed that nanoparticles were crystallized in the tetragonal structure and their average crystallite sizes were observed to be 42.39 nm for 30 ml of extract solution and 35.54 nm for 60 ml of extract solution. TiO_2 nanoparticles were reported to be an average grain size of 35.53 nm for 30 ml of extract solution and 30.33 nm for 60 ml of extract solution with spherical shape by SEM images. UV-Vis showed that the dominant sharp band of titanium oxide nanoparticles (TiO_2NPs) was observed the intensity of absorption peak increases with increasing more extract solution. It was observed that the titanium oxide nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV- Vis absorption spectrum. According to the antimicrobial experimental result, the inhibition zone of 60 ml extract solution was showed more against on microorganism than 30 ml of extract solution.

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