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Eclipta prostrata leaf aqueous extract mediated for the synthesis of titanium dioxide nanoparticles and its larvicidal activity against malaria vector

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Abstract- **Eco-friendly, nontoxic, inexpensive, abundantly available hitherto unreported *Eclipta prostrata* leaf extract is used for the biosynthesis of titanium dioxide nanoparticles (TiO_2 NPs). The TiO_2 NPs were characterized by FTIR, XRD, AFM and FESEM analysis. FTIR peak implicated the role of carboxyl group O-H stretching amine N-H stretch in the formation of TiO_2 NPs. XRD characterized in crystallographic plane of rutile phase. AFM showed uneven surface morphology which indicates the presence of both individual and agglomerated nanoparticles. FESEM analysis showed shape in spherical clusters, quite polydisperse and it ranges in size from 36-68 nm with calculated average size of 49.5 nm. In this paper, we have demonstrated a novel biological route for the synthesis of TiO_2 NPs and first report to assess the larvicidal activity against malaria vector.**

Keywords: Titanium dioxide nanoparticles, *Eclipta prostrata*, FTIR, Atomic force microscopy, Larvicidal activity XRD

1. INTRODUCTION

TiO_2 is technologically very important material especially as dielectrics. Various microbes are known to reduce metal ions to the metals. Minimum time, miniaturization and non-hazardous processes are key parameters for any kind of technology acceptance.

TiO_2 is used in cosmetic and skin care wareses, particularly in sun blocks, where especially nanosized particles (<100 nm) help to protect skin from UV rays; it is widely used to provide whiteness and opacity to products such as paints, plastics, papers, inks, food colorants and toothpastes [1].Materials with nano-sized dimensions have attracted considerable attention of the researchers due to their exponential promises in almost all walks of life [2]. For time immemorial, nature has made noble metal oxide part of our daily life. Metal oxide nanoparticles [3], exemplified by titanium dioxide (TiO_2) are of great technological grandness in the field of heterogeneous catalysis [4]. Preparation of nanoparticles using green technologies is advantageous over chemical agents due to their less environmental consequences. In the biosynthesis method, extracts from plant may act both as reducing and capping agents in synthesis of nanoparticles. TiO_2 nanoparticles are known to react with O_2 and -OH adsorbed on the surface to obtain oxygen free radical and hydroxyl free radical which are capable of directly attacking the cell wall and cell membrane.

Eclipta prostrata L. (Asteraceae) commonly known as False Daisy has been used as a traditional medicine [5]. In the present investigation, In the present investigation, TiO_2 NPs were synthesized using *E. prostrata* leaf by simple aqueous reduction method and thus obtained were characterized using FT-IR, XRD, FESEM, and AFM. Hence, This is the first report to assess the larvicidal activity of using *E. prostrata* leaves synthesized TiO_2 NPs.

II. EXPERIMENTAL PROCEDURE

The salubrious leaves of *E. prostrata* were collected from C.Abdul Hakeem College, Melvisharam, Vellore, India. $TiO(OH)_2$ was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Aqueous extract of *E. prostrata* was prepared using freshly amassed leaves (10 g). They were surface cleaned with running tap water, followed by distilled water and boiled with 100 mL of double distilled water at 60°C for 10 min. This extract was filtered through nylon mesh (Spectrum), followed by Millipore hydrophilic filter (0.22 µm) and used for further experiments. For synthesis of TiO_2 NPs, the Erlenmeyer flask containing 100 mL of $TiO(OH)_2$ (5mM) was stirred for 2 hr. 15 mL of the aqueous extract of *E. prostrata* was added in 85 mL of 5 mM TiO_2 at room temperature under stirred condition for 24 hrs. The control was maintained without *E. prostrata* extract.

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Characterization involved FTIR analysis of the dried powder of synthesized TiO_2 NPs by Perkin– Elmer Spectrum One instrument spectrometer in attenuated total reflection mode and using spectral range of 4000–400cm^{-1} with a resolution of 4 cm^{-1}. The morphology of the plant synthesized TiO_2 NPs were examined using field emission scanning electron microscopy (FESEM, FEI Novanano 600, Netherlands), and for the images it was operated at 15 kV on a 0° tilt position. XRD patterns of all samples were collected in the range of 20–80 °C (2θ) using Phillips PW 1830 instrument (CuKα radiation, λ=1.5406 Å), operated at 40 kV and 30 mA. AFM images have been processed using WSxM software ver. 4.0.

A. LARVICIDAL BIOASSAY

During preliminary screening with the laboratory trial, the larvae of *A. subpictus* were collected from the insect-rearing cage and identified in Zonal Entomological Research Centre, Vellore, India. One gram of aqueous leaf extract was first dissolved in 100 ml of distilled water (stock solution). From the stock solution, 100 mg/L was prepared with dechlorinated tap water for bioassay test of plant extract. The larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of Rahuman et al. (2000). The bioassay test was carried out as per the method of Rajakumar and Rahuman (2011) and Santhoshkumar et al. (2011). aqueous leaf extract of *E. prostrata,* $TiO(OH)_2$ and Synthesized TiO_2 NPs toxicity test was performed by placing 20 mosquito larvae into 200 mL of sterilized double distilled water with nanoparticles into a 250mL beaker (Borosil). The nanoparticle solutions were diluted using double distilled water as a solvent according to the desired concentrations (10, 8, 6, 4 and 2 mg/L) for aqueous leaf extract of *E. prostrata,* $TiO(OH)_2$ and Synthesized TiO_2 NPs. Each test included a set control groups (distilled water) with five replicates for each individual concentration. Mortality was assessed after 24 h to determine the acute toxicities on fourth instar larvae of *A. subpictus*.

III RESULTS AND DISCUSSION

TiO_2 NPs synthesized by a novel, biodegradable materials and simple green chemistry procedure using *E. prostrata* leaf extract. FTIR spectroscopy was used to determine different groups on *E. prostrata* leaf extract and predict their role in nanoparticle synthesis. After reaction of *E. prostrata* extract with $TiO(OH)_2$ the color was changed in to light green color. The FTIR spectrum of the $TiO(OH)_2$ showed characteristic bands at 3417 cm^{-1} and 1632 cm^{-1} correspond to the surface water and hydroxyl group (Fig. 1a). The band intensities in different regions of the spectrum for the powder *E. prostrata* and synthesized TiO_2 NPs test samples were analyzed. There was a shift in the following peaks: 3421 to 3427, 2922 to 2927, 2853 to 2856, 1631 to 1639 and 1103 to 1124 cm^{-1}(Fig 1 b and c). A comparison of these results with earlier reports [6] indicated that alcohols, phenols, alkanes, primary amines and aliphatic amines in *Eclipta* may be participating in the process of nanoparticle synthesis.

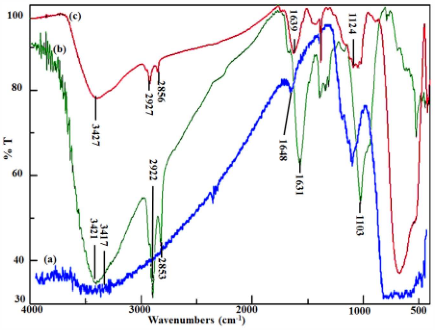

Fig. 1 FTIR spectra of (a) TiO_2NPs synthesized from aqueous leaf extract of *E. prostrata* with 5 mM TiO_2 (b) dried leaf extract (c) synthesized $TiO(OH)_2$

The *E. prostrata* leaves contains beta-amyrin, wedelolactone, triterpenoids, flavonoids, luteolin-7-o-glucoside, L-terthienyl methanol and stigmasterol [7]. Water soluble heterocyclic compounds such as flavones are the reducing and capping ligands of the nanoparticles [8]. Functional groups associated with these the cause for the bioreduction of $TiO(OH)_2$ to TiO_2 nanoparticles.

The nanoparticles were characterized by XRD in rutile phase. The positions of principal peaks in XRD were found to be in agreement with the literature [9]. The XRD sample shows dominant peak of 2θ=27.811 which matches the 110 crystallographic plane of the rutile structure indicating that the crystal structure is predominantly rutile dominant (Fig. 2). This pattern reflects the shape of the wave functions of the electronic eigenstates of the Ti-O-Ti-O chain on the TiO_2 (110)/H_2O interface [10].

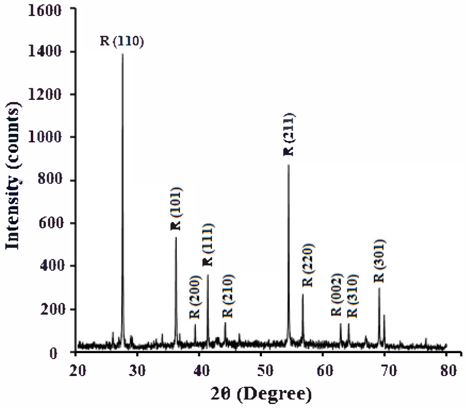

Fig. 2 XRD pattern of the TiO_2NPs synthesized from aqueous leaf extracts of *E. prostrata*

The particles size estimation was performed by the Scherrer's formula. d= 0.94 λ / β Cos θ where d is the mean diameter of the nanoparticles, λ is wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ and the data obtained was matched with the database of Joint Committee on Powder Diffraction

Standards (JCPDS) file No. 89-4202. The plant synthesized TiO_2 nanoparticles are quite polydisperse and ranges in size from 36-68 nm with calculated average size of 49.5 nm. Characterization of the synthesized nanoparticles using AFM offered a three-dimensional visualization. The uneven surface morphology explained by the presence of both individual and agglomerated nanoparticles. The strong crystalline nature can be seen in the form of diagonal formations with ridges(Fig. 3).

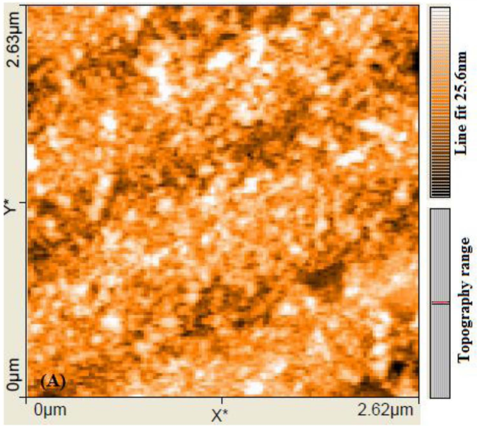

Fig. 3 Topographical view from Atomic force microscopic (AFM) image of the synthesized of TiO_2

The surface of synthesized nanoparticles was characterized using FESEM. The micrograph shows nano-scaled TiO_2 particles with the detailed surface morphology of nanoparticles (NPs) and microspheres. The nanoparticles are poorly dispersed with spherical clusters with agglomeration size up to 95 nm. Agglomeration makes it difficult to study individual nanoparticles (Fig. 4 A). The variation of shapes is due to unclear separation and explained by the high packing of individual particles. Further separation has not been feasible by magnetic stirring for even upto 24 hours. The morphological shapes and size of the aggregations were described in terms of the fractal dimensions, and a box-counting method was used to get the fractal dimensions of systems [11].

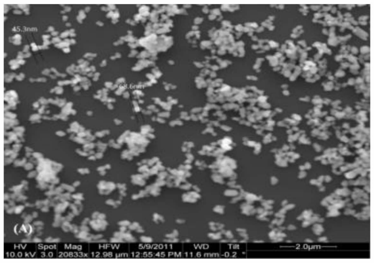

Fig. 4(A) FESEM images of the TiO_2 nanoparticles formed by the reaction of 5 mM TiO_2 and *E. prostrata* leaf broth 20833X

In the present study, the toxic effect on fourth instar larvae after 24 h of exposure; however the mortality of aqueous crude leaf extract of *E. prostrata,* TiO_2 NPs against the larvae of *A. subpictus* (LC_{50}=8.20, 4.13 and 0.89mg/L) respectively (Table 1). Earlier authors reported that the *Nelumbo nucifera* [12] *Mimosa pudica* [13], *Tinospora cordifolia* [14], *E. prostrata* [15] leaf synthesized AgNPs showed the highest mortality against larvae of *A. subpictus and C. quinquefasciatus.* The ecotoxicology of NPs, aiming to assess NPs' harmful effects to the ecosystem [16]. Various theories have been proposed to explain the phenomena of nanoparticles toxicity including: generation of reactive oxygen species (ROS) which can disrupt cell structures; binding with macromolecules making them dysfunctional [17].

TABLE I

Mortality of *A. subpictus* larvae at various concentrations of aqueous leaf extract of *E. prostrata,* $TiO(OH)_2$ and TiO_2 NPs

Extract	Concentrations (mg/L)	% Mortality[a] (mg/L) ± SD	LC_{50} LCL –UCL (mg/L)
Aqueous extract	10	63±0.415	8.20 (7.31 - 9.20)
	8	48±0.941	
	6	31±0.297	
	4	17±0.746	
	2	09±0.689	
TiO_2 NPs	10	100±0.000	4.13 (3.68 - 4.64)
	8	87±0.410	
	6	71±0.637	
	4	43±0.741	
	2	21±1.005	
$TiO(OH)_2$ 5mM (Positive control)	10	5±0.541	-
	8	4±0.214	
	6	3±0.871	
	4	2±0.586	
	2	-	

[a] = five replicates, [b] = the mortality were adjusted using Abbott formula,
- = Nil mortality, significant at $P<0.05$ level,
LC_{50} lethal concentration that kills 50% of the larvae,
UCL upper confidence limit, LCL lower confidence limit

Green synthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity and eco-friendly bio-organisms in plant extracts contain proteins, which act as both reducing and capping agents forming stable and shape-controlled TiO_2 NPs. This environmentally friendly method of biological TiO_2 NPs production provides rates of synthesis faster or comparable to those of chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods and sunscreen products applications. TiO_2 is stable in aqueous media and is tolerant of both acidic and alkaline solutions. It is inexpensive, recyclable, reusable and relatively simple to produce [12].

IV CONCLUSION

In conclusion, the present novel method is capable of producing TiO_2 nanoparticles with *E. prostrata* leaf extracted solution as a solvent instead of organic solvents. The advantages of this method include (i) use of cheap, nontoxic and environmentally benign precursors and (ii) simple procedures without time-consuming polymerization and problem with treatment of a highly viscous polymeric resin. This method can be used to prepare nanocrystalline oxides of other interesting materials for diverse fields of coating, cosmetics, food additive, etc. An attempt has been made to evaluate the role of aqueous leaf extract of *E. prostrata*, and synthesized TiO_2 NPs, larvicidal bioassay against *A. subpictus* activity.

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