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RESEARCH ARTICLE

Green Synthesis of Titanium Dioxide and its Application on Anti-Fungal Activity

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ABSTRACT:

In recent years, nanotechnology was an emerging field which has wide range of application in various science fields. Nanoparticles has significant approaches in medicinal field. The nanoparticles can be synthesized from various sources depending upon the applications. In this current study, the green synthesis of Titanium dioxide nanoparticles from *Calotropis gigantae* and its antifungal activity was studied. The precursor for titanium nanoparticles is titanium tetraisopropoxide. Usually the plant material assisted in reduction process. The green synthesis approach was eco-friendly, cost effective, non-toxic and renewable resources. Titanium dioxide nanoparticles were characterized by SEM, FTIR, XRD and EDAX. Scanning Electron Microscope analysis gives the details of TiO_2 nanoparticles spherical shape and other structural properties. X-Ray Diffraction analysis gives the crystalline nature of Titanium dioxide Nanoparticles. Fourier Transform Infrared spectroscopy results indicates the presence of various bioactive compounds. Energy Dispersive X-ray Analysis brings about the elemental composition of titanium nanoparticles. Titanium dioxide nanoparticles shows the antifungal activity against particular microorganism.

KEYWORDS: Green synthesis, Titanium dioxide nanoparticles, *Calotropis gigantae*, SEM, XRD, EDAX, FTIR, Antifungal activity.

INTRODUCTION:

Recent years, Nanoscience shows great development in various fields of science and research. Nanomaterials have high potential in environmental as well as in medicinal field. In the path of research, various nanomaterials with growing application of sustainability, green synthesis and biodegradability is produced. The particles, which ranges from 1 to 100nm in its size is generally regarded as Nanoparticles. Nanoparticles possess unique physical and chemical properties when compared to other macro scaled particles.

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The antimicrobial activity of nanoparticles are highly effective. Among different nanoparticles, metal oxide nanoparticles are used for effective antimicrobial studies. Generally, the metal oxides used are Ag, MgO, Au, Zn, CuO and TiO_2. These metal oxide nanoparticles are effective against various microorganisms. Mostly, it shows antimicrobial activity against bacterial, viral and fungal strains. Nanoparticles used for medicine is used widely, it depends on production of nanoparticles by industries. Nanoparticles are not specifically comprised of particular material; it also consists of organic and inorganic molecules[1]. The nanoparticles of various size and shape are synthesized by following different process [2]. Noble metals are used to synthesize metallic nanoparticles[3]. Titanium is one of the metal converted to metal oxide nanoparticle. It has sequence of application in photocatalysis, adsorbents, sensors and antimicrobial agent[4]. Titanium dioxide nanoparticles are synthesized by both biological and chemical method. Green synthesis of Titanium nanoparticles have greater

efficient than the other chemical method. Green synthesis is also defined as biogenic synthesis of nanoparticles, which utilizes plants, bacteria, fungi, algae etc. This green synthesis is biocompatible, cost effective, renewable and environment friendly. Nanoparticles produced by green synthesis has high catalytic activity and limits the usage of toxic chemicals. By hydrolysis technique, cyanide is removed from wastewater this is because of photocatalytic properties observed in Titanium nanoparticles[5]. Nowadays nanoparticles produced from green synthesis is increased. Titanium nanoparticles can produced using different plant extracts. This titanium nanoparticle has significant antimicrobial activity mainly against bacterial and fungal pathogens. It has high potential as antifungal agent. Titanium metal oxide nanoparticles usage are found recently. Hence, we investigate with green synthesis of Titanium nanoparticles and its antifungal activity. For the green synthesis of TiO2 nanoparticles plant extract from *Calotropis gigantae* is used.

The different parts of *C. gigantae* has various features of pharmaceutical properties like antibacterial[6], antifungal[7], antioxidant, anti-inflammatory[8], antinociceptive, anticonvulsant, hypolipidemic activities, diuretics and hypoglycemic[9,10]. *Calotropis gigantae* is also known as "Sweta Arka" in ancient ayurvedic medicine[11]. The parts of Calotropis has different pharmacological effects and possess phytochemicals. Mainly it was found that Usharin, gigantin, calcium oxalate, alpha and beta-calotropeol, beta-amyrin., fatty acids (both saturated and unsaturated), hydrocarbons, acetates and the benzoates, a mixture of tetracyclic triterpene compounds, terols, giganteol are present[12] Leaves of this plant is used as extract and antifungal activity was studies against *Aspergillus niger*. Here green synthesis of Titanium nanoparticles, its characterization and its antifungal activity was briefly discussed.



Figure - 1: ***Calotropis gigantae***

MATERIALS AND METHODS:

Materials:

The Leaves of *Calotropis gigantae* were collected from nearby area of Namakkal. For anti-microbial activity, fungal pathogen *Aspergillus niger* was taken. Titanium isopropoxide was used as metal precursor for Titanium nano particles. Potato dextrose agar is used as a medium for fungal pathogen. Ethanol is used for sterilization. All glass wares were sterilized using autoclave and hot air oven.

Preparation of plant extract:

The fresh leaves of *Calotropis gigantae* was washed thoroughly using distilled water and finely cut into pieces, which weighs nearly 10grams. These finely cutted leaves was boiled in 200ml of sterile distilled water. It was allowed to boil for one hour in water bath [13]. Then nearly 100ml of leaf extract was obtained. It was filtered using Watt man filter paper. This extract was stored at 4^0C for future use.

Green synthesis of titanium nanoparticles:

TiO_2 nanoparticles are synthesized using Titanium isopropoxide as precursor. The filtered leaf extract was taken in an Erlenmeyer flask and kept in a magnetic stirrer. The required amount of Titanium isopropoxide was added to the extract drop by drop. The amount of Titanium isopropoxide added was based on finding the normality of titanium isopropoxide. It was kept in magnetic stirrer for one hour. There was a change in colour from pale brown to yellowish colour after one hour of continuous stirring. This colour change preliminarily confirmed that TiO_2 nanoparticles formation. This is due to reduction of titanium metal ions[14]. This is because that leaf extract has the ability to stabilize and assists in nanoparticle production. Plant extract mainly influence the characteristics of nanoparticles[15]. Then, it was allowed for centrifugation at 5000rpm for 20 minutes. The synthesized nanoparticles was calcinated. This TiO2 nanoparticles was subjected to antifungal activity. It was characterized by using different analytical techniques.

Characterization of nanoparticles:

The synthesized nanoparticles was characterized using SEM, XRD, EDAX and FTIR. TiO_2 nanoparticles was characterized using SEM for determining the structural properties in order to know its size and shape. FTIR as a good analytical technique to illustrate the functional groups on nanoparticle surface and its synthesized solution. Nanoparticles have surface charge, which can be expressed in terms of zeta potential that usually gives its relative stability and colloidal stability[16]. In FTIR, the spectrum ranges from 4000 to 400 cm^{-1}. Energy dispersive analysis of X- ray was used for elemental analysis and for determination of chemical

characterization of the Titanium nanoparticles. X- ray diffraction was used to determine the crystalline structure of the Titanium nanoparticles.

Well diffusion assay:

The antimicrobial sensitive assay was discovered by Kirby boeyer. It involves two different methods. One is disc diffusion method and another one is well diffusion method. The antifungal activity of titanium dioxide nanoparticles were determined using well diffusion method. Malt extract agar plates were prepared. Malt extract agar was mainly used for fungal culturing. *Aspergillus niger* was taken from the inoculum using sterile cotton swab. It was streaked all over the surface of Malt extract agar media. Then three wells are made using cork borer in the plate. 10 mg/mL of Titanium nanoparticles aqueous solution was taken and loaded in one well. The other two wells contains positive and negative controls respectively. The plate is incubated at 37^0 C for 42 h. Distilled water and plant extract were used as controls. After incubation, the zone of inhibition was measured [17].

RESULT AND DISCUSSION:

In this present work, Titanium nanoparticles was synthesized using leaf extract of *Calotropis gigantae*. The nanoparticles were subjected to different analytical characterization like SEM, EDAX, XRD and FTIR. The antifungal activity against *Aspergillus niger* was found. Anti-fungal activity was found by using well diffusion method. The various phytochemicals in *C. gigantae* shows higher affinity to the fungal growth. The combined activity of Titanium nanoparticles and phytochemicals of *C. gigantae* has effective antimicrobial activity.

FTIR spectrum of titanium dioxide nanoparticle:

The synthesized TiO_2 nanoparticles using *C. gigantae* leaf extract was estimated using FTIR instrument Shimadzu, in which wave number ranges from 400 to 4000 cm^{-1}. The synthesized nanoparticles FTIR spectra was given in Figure 2. The wave number below 1200 cm^{-1} shows the characteristic absorption band of Titanium dioxide nanoparticles[18]. These peaks are resultants of Ti -O- Ti vibrations. The absorption band at 591.19 cm^{-1} and 951.89 cm^{-1} indicates the presence of bond between O -Ti- O. Generally the alkenes and aromatic peaks are higher than 3000 cm^{-1}. The extending vibration of OH appeared in high vitality area 3336.91 cm^{-1}, which has a broad crest. The extending vibration of C=C group was appeared in peak range of 1634.70 cm^{-1}, which was estimated as non-cyclic compounds. The crest at 1407.10 cm^{-1} shows the twisting vibration of O-H group that determines the carboxylic group. The peak at 2887.49 cm^{-1} represents the H-C=O stretch of aldehyde group. The crest at 622.05 cm^{-1} indicates the vinyl biomolecule with –CH extending group. Generally the organic compounds containing halogens[19,20] like C – Br ranges from 690 cm^{-1} to 515 cm^{-1}. Basically main functional properties of Titanium nanoparticles are estimated using FTIR spectra.

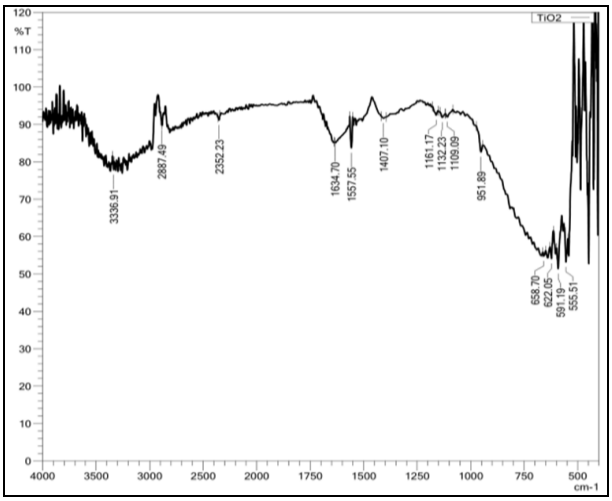


Figure - 2: FTIR Spectrum Analysis Of TiO_2 Nanoparticles

Scanning electron microscope (SEM):

Surface morphologies like shape, size and its structure was determined using Scanning Electron Microscope. The SEM image of Titanium oxide nanoparticles from *C. gigantae* were shown in figures 3. The SEM image concludes that TiO_2 nanoparticles are evenly distributed on the surface. It was found that synthesized nanoparticles are spherical in shape and its size ranges from 20nm to 90nm[21]. The poor dispersion of nanoparticles is due to the agglomeration. This made to analyze the individual nanoparticles [22].

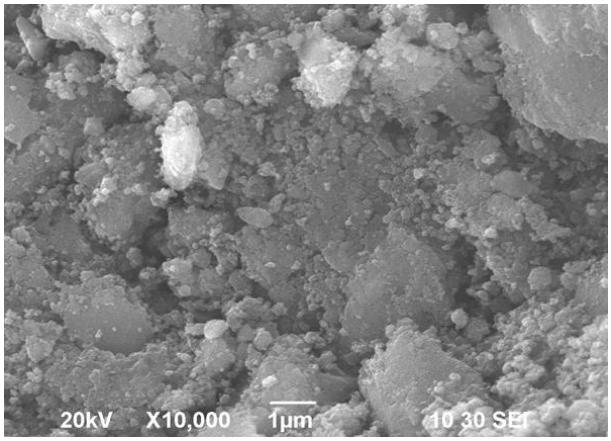
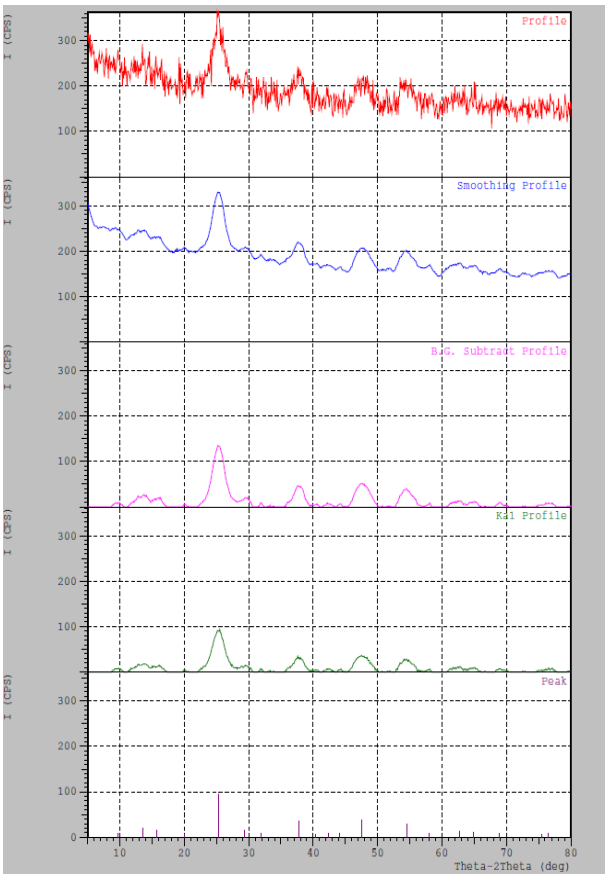


FIG- 3: SEM Image of TiO_2 Nano Particles

XRD characterization:

The XRD pattern of green synthesized TiO2 nanoparticles was given in figure 4. The anode material was copper. It represents the formation of amorphous titanium dioxide nanoparticles with diffraction angles of

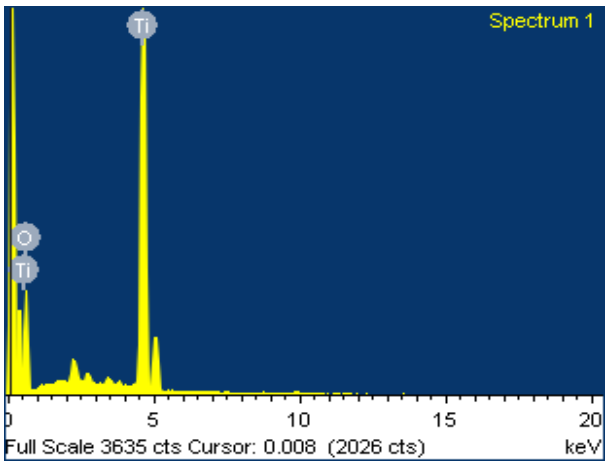
25.29^0(100), 37.81^0(038), 47.52^0(039), 54.52^0(030), 62.65^0(013), 64.82^0(011) and 76.55^0(009). The diffraction angle perceived at 25.2 is associated to (100) crystallographic anatase only. The absence of rutile phase is due to the increased oxygen concentration during the growth, results in delaying the conversion from anatase to rutile phase [23]. The size of the particle can be determined using Scherrer's equation [24]. During synthesis, the stoichiometry of the nanoparticles depends on its partial pressure [25]. Therefore this nanoparticles have stoichiometric properties. The XRD analysis of TiO_2 nanoparticles was carried out using X- ray diffractometer in the diffraction angles of 2θ scanning ranging from 10 to 80^0.



XRD Analysis of TiO_2 Nanoparticles:

EDX analysis:

The elemental composition of the sample was determined using the Energy dispersive X- ray spectroscopy. X- Ray spectrum from the entire scan gives the EDX spectrum. Every elements generates X-Ray based on their energy levels. Thus, the energy levels 0.452 Kev and 0.52 Kev was assigned to TiO_2 nanoparticles. The peaks represents the presence of oxygen and titanium particles in the sample. The presence of TiO_2 nanoparticles was further confirmed by EDX spectroscopy[26].



EDX Analysis of TiO_2 Nanoparticles

Antifungal activity:

Malt extract agar plate were prepared, after solidification of agar media, the fungal culture was stabbed on this plate. The well was made using well borer. In here the synthesized TiO_2 nanoparticles of 10 mg/mL was poured in one well and other two wells contains distilled water and plant extract of *C. gigantae* as controls. The plate is allowed for brooding for about 42 h. After that, on the following day zone of inhibition (mm) was observed. The zone of inhibition against *Aspergillus niger* was observed to be 10mm. The zone of inhibition for plant extract and distilled water is found to be 1mm and 5mm respectively. The antifungal mechanism was given in figure 5. The mechanism involves enzymatic and DNA/RNA damage to the cells.

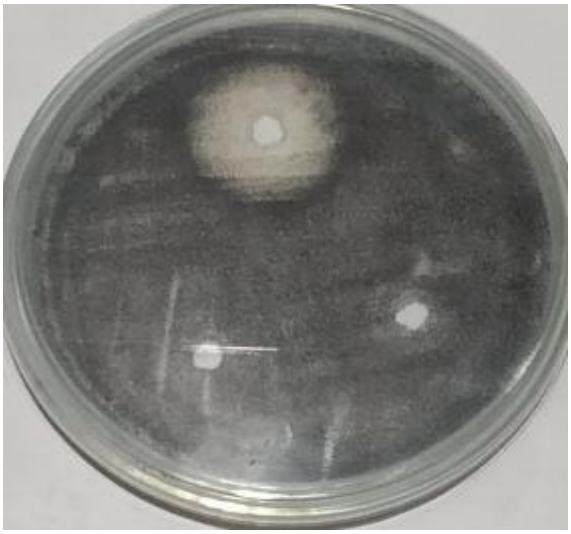


Figure -5: Antifungal Activity Against *Aspergillus niger* BY TiO_2 Nanoparticles.

CONCLUSION:

In current research, nanoparticles become the promising and novel antimicrobial agents. Nowadays, the development of nanotechnology led to the rapid

production of nanoparticles from laboratory scale to industrial scale. The bioactive compounds present in C. gigantae favors the green synthesis of Titanium dioxide nanoparticles. This type of biogenic synthesis of nanoparticles are very effective in pharmacological uses and environmental uses. TiO_2 Nanoparticles can be produced using different plant extracts [27]. These nanoparticles usually possess great stability, chemical inertness, high quantum efficiency and specific surface area[28]. This green synthesis of nanoparticles were eco-friendly by reducing the usage of hazardous, toxic compounds. There are lots of application of titanium nanoparticles in various fields. Anatase Titanium nanoparticles pigments has less abrasive power which provides a great progress in rubber, ceramics and paper manufacturing industries[29]. In addition, these nanoparticles shows toxic effects on humans due to the bioaccumulation, bio concentrate and bio magnify in mammal and vertebrae tissues[30]. In historical monuments, colloidal suspension of titanium nanoparticles are sprayed on travertine limestone to maintain the stone surface effectively[31]. Titanium nanocomposite has significant photocatalytic degradation properties, mainly it degrades beta napthol using X- ray diffraction and TEM[32,33]. Titanium nanoparticles are also used in aircrafts for its less weight and greater stability[34]. Medical applications like these nanoparticles are used to treat hyper pigmented skin and several dermatological and non-dermatological diseases[35]. It is used in Nano therapeutics, prosthetic implants and photodynamic therapy[36,37]. The green synthesized Titanium dioxide nanoparticles shows massive antifungal activity. This nanoparticle are very effective against bacterial and fungal strains[38]. The characterisation of green synthesized nanoparticles has provided the essential morphological details, presence of different functional groups and its stoichiometric properties. Various analytical techniques like SEM, FTIR, EDX and XRD were used for confirmation of titanium dioxide nanoparticles and to study the different physical and chemical properties of titanium dioxide nanoparticles. The FTIR analysis brings out the various functional groups present in TiO_2 nanoparticles. SEM analysis gives the knowledge of structural properties. XRD analysis made to know the crystalline structure of TiO_2 nanoparticles. EDX results in elemental analysis of TiO_2 nanoparticles. Thus the green synthesis approach, various Characterizational studies and antifungal activity provides an effective results of Titanium dioxide nanoparticles. The continuous dependents on antibacterial, antiviral, anticancer, antifungal, diagnostic purposes, usage of nontoxic chemicals, solvents acquiescent to environment and targeted drug delivery considerably led to the biosynthesis of nanoparticles[39]. These nanoparticles have anti-proliferative activity an effective against tumors[40]. Textiles, food storage containers and mainly in medical devices possess nanoparticles for its antimicrobial activity[41]. Revolutionary changes are occurring due to development of nanoscience.

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