Section A-Research paper
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Green Synthesis of Titanium Dioxide Nanoparticles Using *Pandanus odorifer* leaves extract and their anti-microbial activity

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ABSTRACT

The present study deals with the synthesis of titanium dioxide (TiO_2) nanoparticles using *Pandanus odorifer (Forssk.) Kuntze* leaf extract and explores the antimicrobial potential of synthesized nanoparticles. The biosynthesized TiO_2 nanoparticles were characterized using different spectroscopic and microscopic techniques. The absorption spectrum of synthesized TiO_2 nanoparticles was primarily characterized by Ultraviolet-visible (UV–vis) spectrophotometer. The functional groups associated with the TiO_2 nanoparticles and the *Pandanus odorifer* leaf extract were examined by Fourier Transform Infrared (FTIR) spectroscopy. The crystalline structure of nanoparticles was analyzed by X-ray diffraction (XRD) examination. Morphological characters were examined by Scanning Electron Microscopy (SEM). Presence of elemental composition of synthesized TiO_2 nanoparticles was characterized by Energy Dispersive X-ray (EDX). The antimicrobial properties of the TiO_2 nanoparticles were observed to be highly toxic against bacterial strains are *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) , and the fungal strains are *Aspergillus niger (A. niger), Candida albicans (C. albicans).* The zone of inhibition was estimated by disc diffusion assay and moreover, minimum inhibitory concentration was evaluated by the Resazurin Microtitre Assay. It can be concluded that titanium dioxide nanoparticles manifest a strong antimicrobial action and thus can be developed as a novel type of a antimicrobial material for the cure of microbial infections.

Keywords: *Pandanus odorifer*, titanium dioxide, SEM, antimicrobial.

INTRODUCTION

In the physical, chemical, biological, medicinal, optical, mechanical, and engineering sciences, where cutting-edge methods are being developed to probe and control single atoms and molecules, the creation of metal and metal oxide nanoparticles has garnered substantial attention. Due to their large surface area and high atom content, metal, and metal oxide nanoparticles exhibit intriguing features such as antibacterial, magnetic, electrical, and catalytic activity. In general, the size, shape, composition, morphology, and crystalline phase of nanoparticles determine their characteristics. Due to their potential oxidation strength, photo stabilityility, and lack of toxicity, titanium dioxide nanoparticles stand out among the different metal oxide nanoparticles in terms of applications in air and water purification, DSSC.

Additionally, titanium nanoparticles exhibit intriguing optical, dielectric, antibacterial, antimicrobial, chemically stable, and catalytic features that lead to commercial applications such as pigment, fillers, catalyst supports, and picture catalyst. The majority of metal and metal oxide nanoparticles were traditionally produced frequently using a variety of physical and chemical techniques. Non-sputtering, solvothermal, reduction, sol-gel technology, and electrochemical technique are a few of the frequently utilized synthetic processes. These techniques, however, are costly, poisonous, energy-intensive, high-pressure, difficult to separate, and possibly dangerous. Because of the environmentally friendly products, biocompatibility, and long-term economic sustainability of trustworthy biosynthetic, an environmentally friendly approach has gained significant relevance. It also helps to prevent negative consequences during their use, especially in the medical industry.

The major reaction that takes place during the biosynthesis of nanoparticles is reduction/oxidation. Metal and metal oxide nanoparticles are often produced using microbial enzymes or plant phytochemicals with antioxidant or reducing capabilities. The three environmentally friendly and green chemistry approaches for the synthesis of nanoparticles are the selection of the solvent medium, reducing agent, and non-toxic substance for the stabilisation of nanoparticles, respectively.

Recently, it was possible to synthesise nanoparticles using bacteria, fungi, actinomycetes, and plant extracts from neem, camellia sinensis, coriandrum, nelumbo lucifera, ocimum sanctum, and other plants that are in line with the principles of green chemistry. The utilization of plant extracts offers benefits over other biosynthetic methods, including being readily available, safe to use, and having a wide range of metabolite viability. Terpenoids, flavones, ketones, aldehydes amides, and other phytochemicals play major roles in the creation of nanoparticles.

TiO_2 nanoparticles are used successfully because of their increased surface area, which raises surface energy and improves microbial and bacterial suppression. TiO_2 NPs have been discovered to be effective in treating microbial and bacterial infection disorders; as a result, production of these nanoparticles may aid in the treatment of bacterial infections, which have become a serious threat since they are becoming increasingly resistant to existing treatments. In the manufacture of TiO_2 NPs and other metal and metal oxide NPs, both chemical and physical processes have been used, with the former being the approach more frequently used in industry. These techniques do, however, have certain drawbacks, including the need for high temperatures, potential danger, lack of environmental safety, use of harmful chemicals as reducing and capping agents throughout the synthesis process, and high cost (Eneyew et al., 2020).

One of the most urgent challenges of recent years is antibiotic resistance, and it will only get worse. The fast development of the bacterial genome has resulted in bacteria developing resistance to antimicrobial substances. Biogenic NPs have therefore demonstrated positive results in the treatment of multidrug-resistant bacteria and may represent a viable option in the struggle against such resistant pathophysiology in the quest for a novel medicine. NPs and other conjugates have been mixed with various organic and inorganic chemicals to enhance the antibacterial response.

Pandanus odorifer is an aromatic monocot species of plant in the family Pandanaceae, native to Polynesia, Australia, South Asia (Andaman Islands), and the Philippines, and is also found wild in southern India and Burma. It is commonly known as a fragrant screw-pine.The Fragrant Screw Pine, also known as the *pandanus odorifer* , is a plant native to Southeast Asia and the Pacific islands.

It is a tropical evergreen plant that can grow up to 6 meters tall and has long, spiky leaves with a pineapple-like scent when crushed.The plant is used in a number of countries. The leaves are used for coloring rice dishes in the Philippines. In Indonesia, the plant’s fruit is used to make traditional medicine. mats, baskets, and hats can be made from the leaves of plants.

The essential oil of *Pandanus odorifer* is used in a variety of fragrances. The leaves and fruit of the plants have compounds that can be used for a variety of purposes. More research is needed to fully understand the properties.The scent of the *Pandanus odorifer* makes it a unique addition to any tropical garden or landscape.

The aim of the present study is the synthesis of Titanium dioxide nanoparticles, characterization of UV, FTIR, XRD and SEM/EDAX analysis, and analyse its anti-microbial property using aqueous extract of *Pandanus odorifer* leaves.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The *Pandanus odorifer* leaves are collected from Andaman and Nicobar island.

COLD EXTRACTION

10 gm of sample was weighed and soaked in 100 ml of sterile distilled water. The extract was allowed to stand 24h and filtered using sterile filter paper. The filtrate was collected and used for synthesis of titanium dioxide nanoparticles.

Synthesis of Titanium dioxide nanoparticles (Thakur et al., 2019; Mohammad Zaki Ahmad et al 2022)

10 ml of extract was added to 90 ml of 5 mM aqueous TiO_2 solution and stirred on a magnetic stirrer at 500 rpm for 2 h at room temperature. This solution was incubated at room temperature for 6 h. After 6 hr centrifuged at 8000 rpm for 10 min. Collect the pellet and washed with distilled water. This content was dried at 60^0 C for 1 h. This Titanium dioxide nanoparticles was stored for further analysis.

Characterization of Nanoparticles

UV–Visible Spectrophotometer:

UV–visible spectrophotometer was used to obtained spectral response of ZnONPs. The sample was monitored by absorbance measurements carried out on UV–visible spectrophotometer in the wavelength range of 200-800nm (Thermo Scientific—Evolution 201). It refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

Fourier Transforms Infrared Spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) analysis was used for the identifying the functional group of the nanoparticles. It was used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. It was analyzed by SHIMADZU spectrometer in the range of 500–4000 cm−1.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) is used to determine the atomic and molecular structure of nanoparticles. This method was carried out for irradiating of the material with incident X-rays and to measure the intensities and scattering angles of the X-rays which was scattered by the nano material. The X-ray beam is diffracted by the sample and detected at various angles. The XRD (RIGAKU miniflex-600, Japan) was performed using an X-ray diffractometer–Cu, Kα radiation λ 1.54 nm in the 2θ range of 30-80 operated data voltage of 40kV and a current of 30 mA. The graph is detected between 2θ in x-axis and intensity on y-axis with different peaks corresponding to different planes of the crystal.

SEM/EDAX Analysis

Scanning electron microscopy (SEM) analyzed for the size and shape of the nanoparticles were examined. SEM provides detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or backscattered electron signal. The images of titanium dioxide nanoparticles were examined using scanning electron microscopy (SEM; TESCAN VEGA3 SBU).

ANTIBACTERIAL ACTIVITY

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay:

Preparation Of Resazurin Solution

The resazurin solution was prepared by dissolving 270 mg in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

ANTIFUNGAL ACTIVITY

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay:

Preparation Of Resazurin Solution

The resazurin solution was prepared by dissolving 270 mg in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of potato dextrose broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10µl of fungal suspension was added to each

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well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

RESULTS AND DISCUSSION

This current investigation was to examine titanium dioxide nanoparticles were efficiently green synthesized by using ***Pandanus odorifer*** leaves was extracted with aqueous solution (Figure:-1, 2, 3).

Figure 1. *Pandanus odorifer* leaves

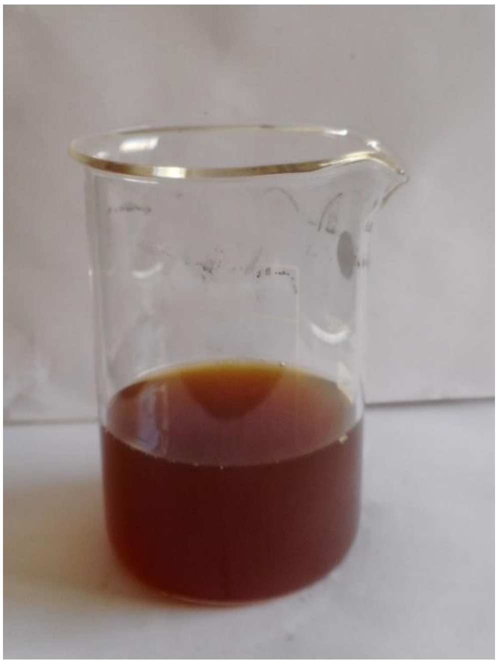

Figure 2. *Pandanus odorifer* leaves extract

Figure 3. *Pandanus odorifer* filtrate

The colour change from white precipitate to pale yellow indicates the synthesis of titanium dioxide nanoparticles (TiO_2NPs) (Figure 2).

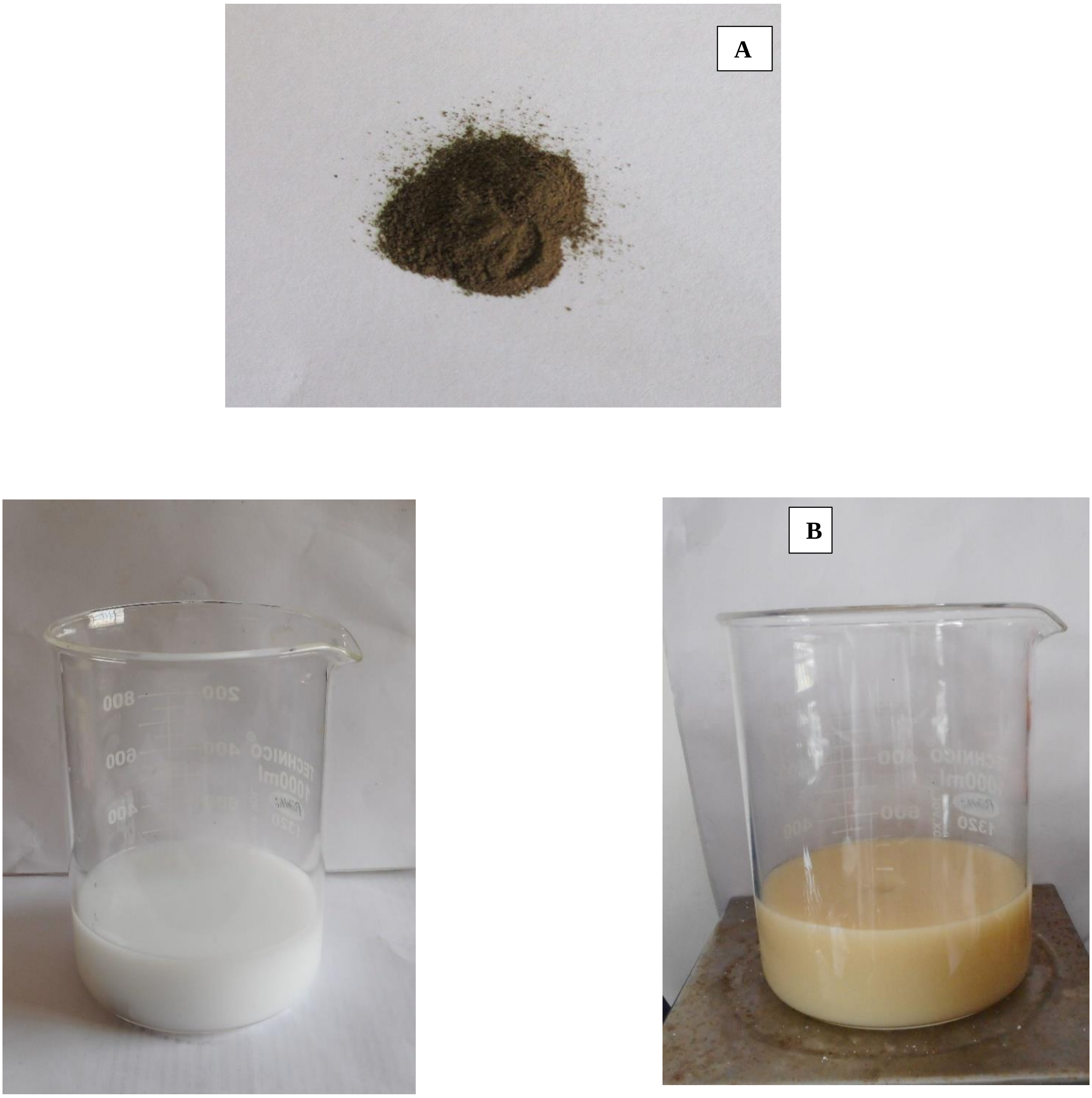

Figure 4. Synthesis Of Titanium Dioxide Nanoparticles Using Aqueous Extract Of *Pandanus Odorifer* Leaves A. TiO_2NPS Powder B. Titanium Solution C. After Synthesis

VISIBLE APPEARANCE

The copper nanoparticles were synthesized by using titanium dioxide as a precursor and plant extract as a capping agent during the chemical process. There is no color change in the extract, although the chemical reaction takes place where the phytochemicals present in the plant extract react with titanium dioxide. After centrifugation, pellets formed were washed with ethanol to dissolve the impurities and sent for characterization.

UV-VISIBLE SPECTROSCOPIC ANALYSIS OF TITANIUM DIOXIDE NANOPARTICLES

UV-Visible absorption spectra of the green synthesized titanium dioxide nanoparticles were logged at a different wavelength from 200-1200nm as shown in **figure 5**. The titanium dioxide nanoparticles formed using titanium dioxide and ***P. odorifer*** as the capping agent displays an absorption peak of 274.09 nm. The surface plasmon absorbance chiefly depends on the size of NPs and every researcher reports different peak values for the NPs synthesized using different plant extracts.

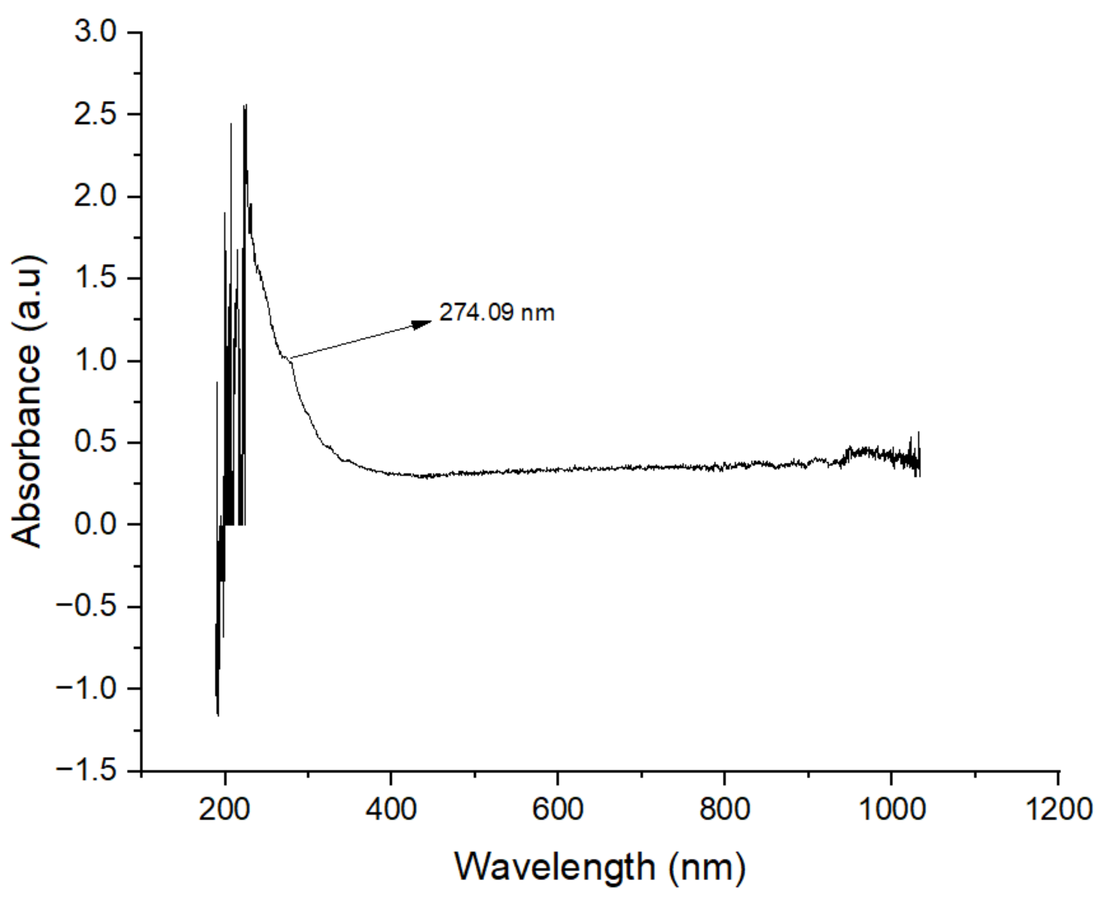

Figure 5. UV analysis of Titanium Dioxide nanoparticles

FT-IR SPECTRAL ANALYSIS

FT-IR analysis is done to determine the biological compounds present in ***Pandanus odorifer*** extract and the functional group of organic compounds which are responsible for the synthesis of titanium dioxide nanoparticles. The FT-IR analysis is done at a scale between 4500 to 500nm. The peaks can be seen at 1033 cm^{-1}, 1074 cm^{-1}, 1411 cm^{-1}, 1565 cm^{-1}, 2853 cm^{-1}, 2923 cm^{-1} . The peak at 1033 cm^{-1} and 1074 cm^{-}1 indicates C-N stretching (aliphatic amines), 1411 cm^{-1} indicates C-C stretching(in-ring) (aromatics), 2853 cm^{-1} and 2923 cm^{-1} indicates C-H stretching (alkanes).

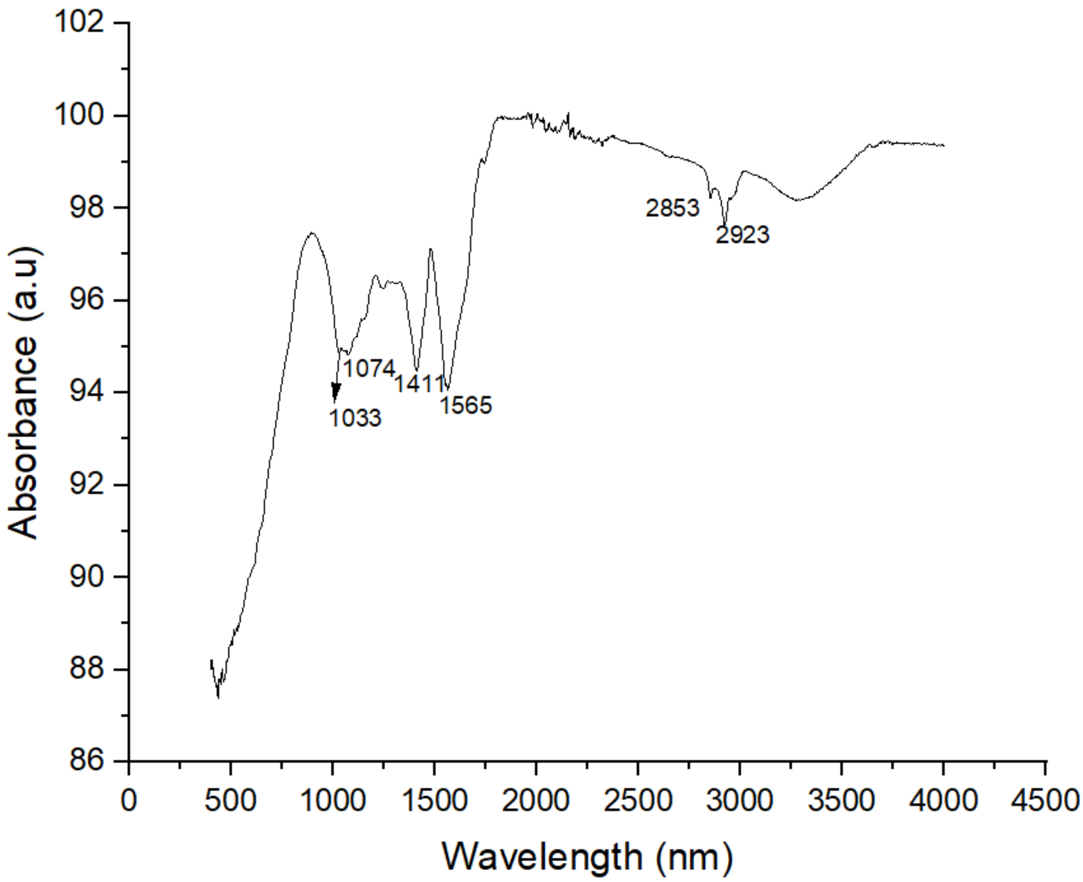

Figure 6. FTIR analysis of Titanium Dioxide nanoparticles

XRD SPECTRUM OF GREEN MEDIATED COPPER NANOPARTICLES:

The crystalline phase of titanium dioxide nanoparticles was figured by XRD analysis. XRD pattern of synthesized titanium dioxide nanoparticles is shown in **figure 7**. The diffraction was recorded. The highest peak shows the presence of titanium dioxide.

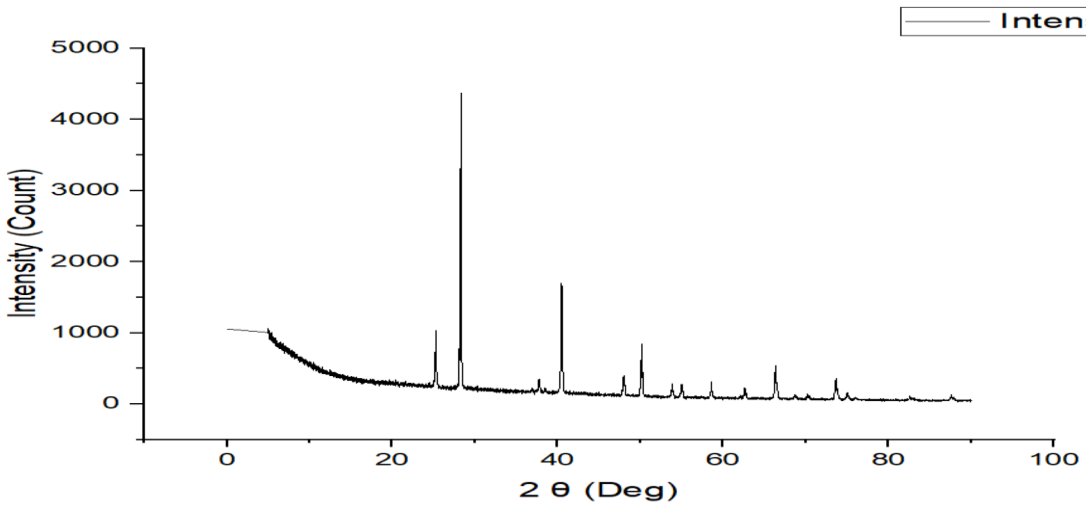

Figure 7. XRD Analysis Of Titanium Dioxide Nanoparticles

No.	2θ, °	d, Å	Height, counts	FWHM, °	Int. I., counts °	Int. W., °	Asymmetry	Decay(ηL/mL)	Decay(ηH/mH)	Size, Å
1	25.263(3)	3.5224(4)	877(21)	0.103(5)	105.2(14)	0.165(7)	1.26(19)	1.22(10)	0.72(11)	822(43)
2	28.1340(13)	3.16920(14)	600(13)	0.060(3)	29.8(14)	0.079(7)	1.71(8)	0.55(4)	0.66(4)	1431(73)
3	28.3057(7)	3.15037(8)	3944(56)	0.0718(8)	352(2)	0.094(2)	1.71(8)	0.55(4)	0.66(4)	1191(12)
4	36.91(4)	2.433(2)	202(3)	0.16(3)	6.8(10)	0.18(4)	1.3(11)	0.4(7)	0.0(9)	558(117)
5	37.763(9)	2.3803(5)	308(8)	0.128(15)	32.3(11)	0.22(2)	1.9(8)	1.2(2)	1.2(3)	686(81)
6	38.487(9)	2.3372(5)	201(3)	0.14(2)	8.5(9)	0.20(3)	0.5(4)	1.4(3)	0.0(6)	611(103)
7	40.478(2)	2.22670(13)	1499(33)	0.133(3)	240(2)	0.178(6)	1.80(15)	0.62(4)	0.63(7)	663(14)
8	48.002(6)	1.8938(2)	347(11)	0.157(7)	49.0(10)	0.213(15)	2.2(4)	0.57(10)	0.89(18)	580(25)
9	50.157(3)	1.81732(11)	753(23)	0.104(4)	103.8(15)	0.161(8)	1.21(18)	1.22(10)	0.55(10)	881(31)
10	53.872(8)	1.7005(2)	235(9)	0.164(9)	31.3(9)	0.22(2)	2.7(7)	0.62(13)	0.8(3)	568(31)
11	55.010(6)	1.66794(16)	243(9)	0.136(8)	31.6(10)	0.209(19)	0.69(15)	1.54(15)	0.22(17)	690(39)
12	58.589(4)	1.57429(10)	275(11)	0.104(6)	30.6(9)	0.161(14)	0.76(15)	1.46(15)	0.36(18)	917(57)
13	62.073(8)	1.49402(18)	109(3)	0.107(16)	4.0(4)	0.14(3)	2.2(6)	0.50(17)	0.6(3)	906(139)
14	62.667(8)	1.48129(16)	194(8)	0.165(8)	24.4(8)	0.21(2)	2.2(6)	0.50(17)	0.6(3)	589(29)
15	66.358(4)	1.40755(7)	471(17)	0.182(4)	91.8(11)	0.231(13)	2.3(3)	0.32(5)	0.84(11)	545(11)
16	68.726(16)	1.3647(3)	117(4)	0.20(2)	11.6(8)	0.25(4)	1.5(5)	0.6(3)	0.0(5)	501(50)
17	70.266(16)	1.3385(3)	125(5)	0.169(15)	11.8(8)	0.20(3)	2.8(14)	0.0(3)	1.0(6)	599(54)
18	73.677(5)	1.28476(8)	308(14)	0.201(5)	62.4(10)	0.254(18)	2.2(3)	0.32(7)	0.79(14)	515(13)
19	74.987(11)	1.26553(16)	146(7)	0.177(12)	19.8(8)	0.23(3)	0.6(2)	1.1(3)	0.0(2)	590(39)
20	76.01(3)	1.2510(4)	80(2)	0.21(3)	5.1(7)	0.24(6)	1.8(12)	0.3(7)	0.0(11)	491(77)
21	82.137(16)	1.17251(18)	64.6(17)	0.14(4)	2.0(5)	0.16(6)	2.4(9)	0.0(4)	0.6(6)	804(206)
22	82.667(15)	1.16634(17)	98(5)	0.202(15)	10.7(8)	0.23(4)	2.4(9)	0.0(4)	0.6(6)	547(40)
23	83.132(19)	1.1610(2)	69.0(18)	0.20(4)	4.2(6)	0.23(6)	2.4(9)	0.0(4)	0.6(6)	548(100)
24	87.602(12)	1.11289(12)	117(6)	0.205(13)	19.4(7)	0.28(3)	0.8(2)	1.0(2)	0.3(2)	560(34)

Table 1. XRD Analysis Of Titanium Dioxide Nanoparticles

SEM/EDAX Analysis

SEM analysis was carried out to understand the topology and the size of the TiO_2-NPs, which showed the synthesis of higher density polydispersed spherical TiO_2-NPs of various sizes. The SEM image showing the high density titanium dioxide nanoparticles synthesized by the ***Pandanus odorifer*** extract further confirmed the development of titanium dioxide nanostructures. Most of the nanoparticles aggregated and 38 only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 40-80nm as well the cubic, face-centred cubic structure of the nanoparticles.

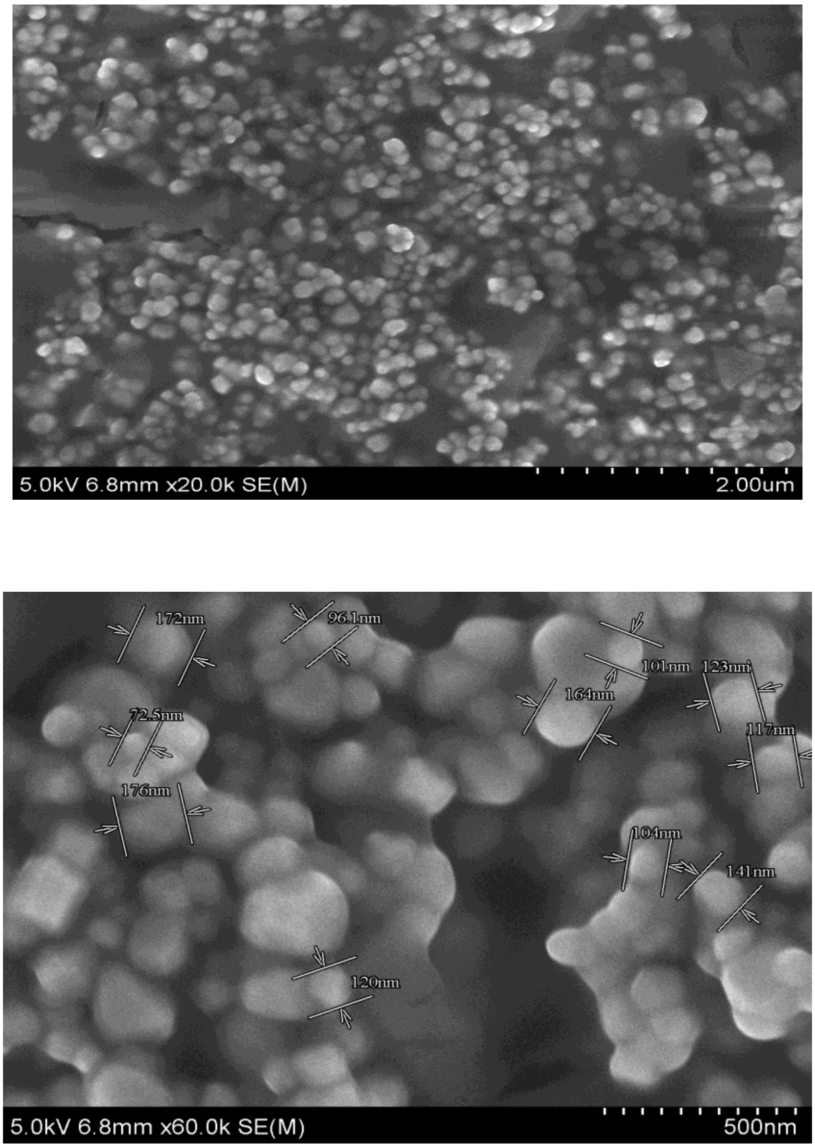

Figure 8. SEM Analysis Of Titanium Dioxide Nanoparticles

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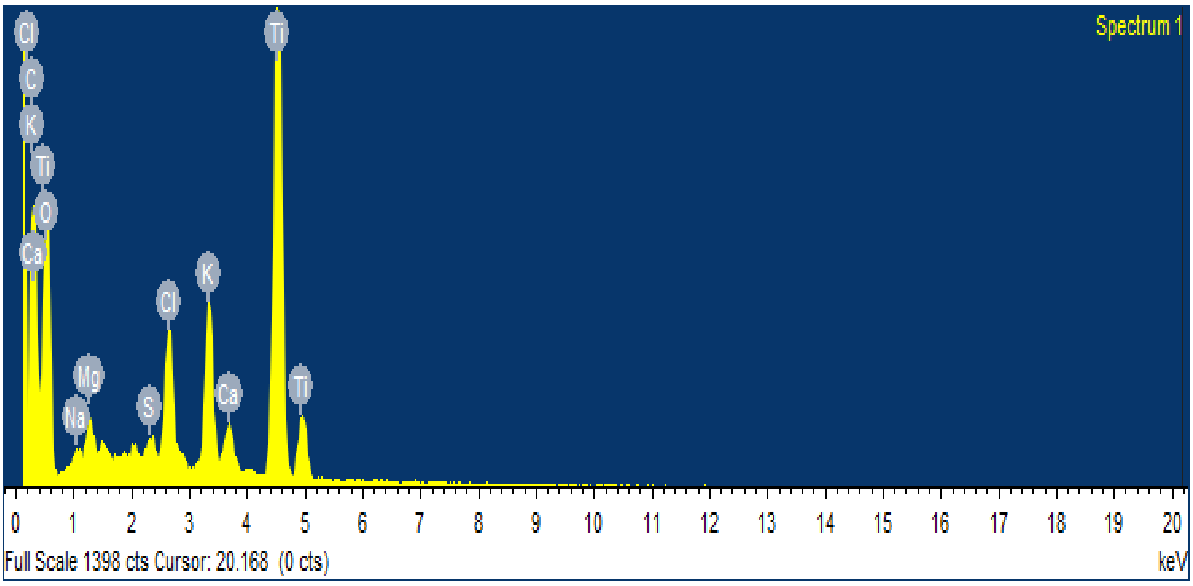

Figure 9. EDAX analysis of Titanium oxide nanoparticles

Element	App Conc.	Intensity Corrn.	Weight%	Weight % Sigma	Atomic %
C K	9.23	0.8484	46.11	7.07	59.05
O K	2.99	0.3547	35.69	4.74	34.31
Na K	0.06	0.6858	0.35	0.10	0.23
Mg K	0.10	0.6657	0.67	0.11	0.42
S K	0.06	0.9672	0.28	0.06	0.13
Cl K	0.36	0.8440	1.80	0.25	0.78
K K	0.65	1.0816	2.54	0.34	1.00
Ca K	0.14	1.0126	0.60	0.10	0.23
Ti K	2.30	0.8157	11.97	1.58	3.84
Totals			100.00		

Table 2. EDAX Analysis Of Titanium Oxide Nanoparticles

ANTIMICROBIAL ACTIVITY:

Resazurin is an oxidation-reduction indicator used for the evaluation of cell viability, especially in different cytotoxicity assays . It is a blue, non-fluorescent, and non-toxic dye that turns pink and fluorescent when reduced to resorufin by oxidoreductases within the viable cells. Resorufin is further reduced to hydroresorufin. Bluish purple indicates that the sample can kill the microbes. The pink color indicates that the sample has low to no capacity to kill the microbes. Minimum inhibitory concentration (MIC) values for both bacteria and fungi is shown in **figure 10.**

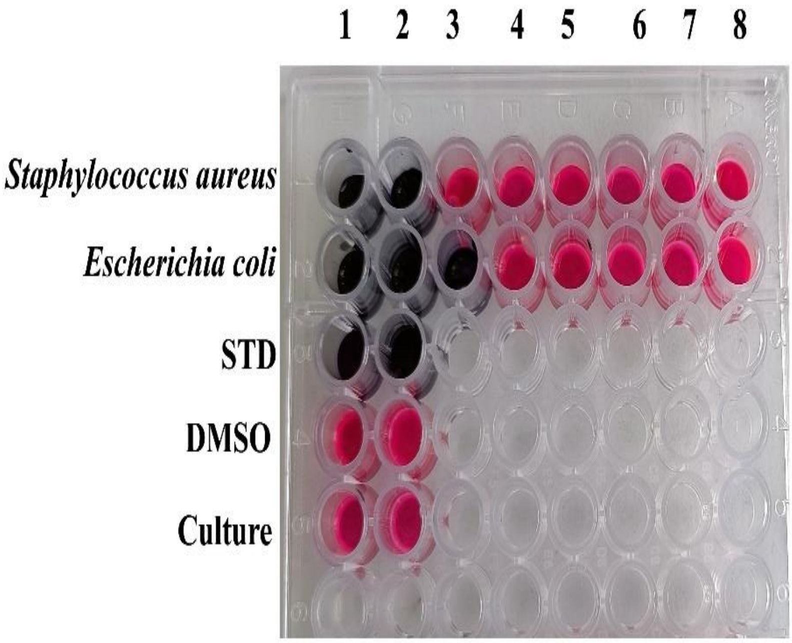

Figure 10. Resazurin Assay For Bacteria

S. No	**Microorganisms/sample**	**Growth of inhibition**										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	STD Steptomycin 10µg	DMSO	Culture
1	*Escherichia coli*	-	-	-	+	+	+	+	+	-	+	+
2	*Staphylococcus aureus*	-	-	+	+	+	+	+	+			

Table 3. Positive And Negative Activity Of The Viable Bacterial Cells

Microorganisms/sample	**MIC Value (µg)**
Escherichia coli	250
Staphylococcus aureus	500

Table 4. MIC values for the bacterial cells

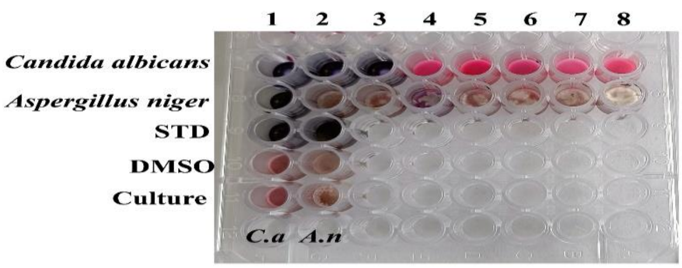

Figure 11. Resazurin Assay For Fungi

S.No	**Microorganisms/sample**	**Growth of inhibition**										
		1000 µl	500 µl	250 µl	125 µl	62.5 µl	31.2 µl	15.6 µl	7.8 µl	STD Steptomycin 10µg	DMSO	Culture
1	*Candida albicans*	-	-	-	+	+	+	+	+	-	+	+
2	*Aspergillus niger*	-	+	+	+	+	+	+	+	-	+	+

Table 5 . The Positive And Negative Activity Of The Fungi Cells

Microorganisms/sample	**MIC Value (µg)**
Candida albicans	250
Aspergillus niger	1000

Table 6. MIC values for the fungi cells

CONCLUSION

From the above results, we conclude that *Pandanus odorifer* can synthesize titanium dioxide nanoparticles in a simple, harmless, environment-friendly, and low-cost method. In this study, we have used a smaller amount of chemicals for the synthesis of titanium dioxide nanoparticles hence it is called green synthesis. The characterization of titanium dioxide nanoparticles was done by UV visible spectrometer, FT-IR analysis, XRD, and SEM/EDAX analysis. Further studies will be done using higher advanced technologies. The synthesized titanium dioxide nanoparticles will be evaluated for the bio-actives they have.

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