Green Synthesis of Titanium Dioxide Nanoparticles Using Stevia Leaves Extracts.

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ABSTRACT

In this study, the biogenic synthesis of economically viable $Ti0_2$ nanoparticles using stevia leaf broth was assessed for its in vitro antioxidant, antibacterial, and antifungal properties. In this study, we have created a simple and environmentally safe process that uses stevia leaf extract to create titanium dioxide nanoparticles from titanium dioxide solution. Fourier transform infrared spectroscopy (FTIR), UV absorption spectroscopy, scanning electron microscopy (SEM), and x-ray diffraction (XRD) were used to analyze the produced nanoparticles. The XRD pattern's strong peaks demonstrate the titanium dioxide nanoparticles' purity and crystallinity. The nanoparticles, which range in size from 48 to 94 nm, are spherical, according to scanning electron microscopy.

Keywords: **stevia broth; biogenic preparation; $Ti0_2$ nanoparticles; anti-oxidant; antimicrobial; antifungal activity.**

Introduction

Nanoparticles are incredibly tiny particles, with sizes ranging from 1 to 100 nm. Compared to their larger, visually unseen counterparts, nanoparticles can have radically different chemical and physical properties [1, 2].

Studies on nanotechnology and nanoscience have expanded dramatically in recent years across a wide range of product sectors. In situations where conventional technologies may have reached their limits, it presents potential for the production of novel materials, particularly those for therapeutic purpose. It is incorrect to view nanotechnology as a singular, narrowly focused strategy. The single "tiny science," commonly referred to as nanotechnology, encompasses more than merely minuscule buildings and goods. A subset of nanotechnology employed in the medical and health fields is called nanomedicine [3, 4].

White pigment titanium dioxide has good diffraction properties and is widely used in paints, plastics, textiles, toothpaste, cosmetics, and pharmaceuticals. In sunscreens, it is utilized in nanoscale form to provide UV protection and a smooth application on the skin.The nanoparticles encourage the destruction of cancer cells by using light energy [5, 6].Research suggest that, in comparison to bigger particles, titanium dioxide nanoparticles may be more harmful in cell culture due to their smaller size. Because they can infiltrate biological structures, nanoparticles may be harmful to the skin [7, 8, 9].

Stevia Rebaudiana (Bertoni), Botanical Name: Stevia Rebaudiana (Bertoni), Common Name: Sweet Leaf, Sugar Leaf, Candy Leaf, Family: Asteraceae, Species: S. Rebaudiana [10, 11].

China grows 32,000 hectares of stevia, which makes about 75% of the worldwide stevia yield. In recent years, stevia has been successfully grown in a number of Indian states, including Hyderabad, Kerala, Rajasthan, and Maharashtra. One plant that is utilized as a natural sweetener is stevia. The stevia plant's leaves contain glycoside. Stevia also contains a high concentration of antioxidant, flavonoid, and phenolic activity. Research from the WHO suggests stevia improves blood pressure, inhibits cavities, stimulates the pancreas to release more insulin, and has antibacterial properties. [12]

Previously, investigated the safety of titanium dioxide nanoparticles in cosmetics and concluded that nano TiO2 is a non-sensitizer and mild- or non-irritant to the skin. [13], Reported the stevia-synthesized NiO-NPs were more efficient against Gram-negative bacteria, Escherichia coli, than Gram-positive bacteria, Bacillus, Streptococcus Pneumonia, after producing NiO Nanoparticles Using Stevia Leaf Extract via phytogenic synthesis. [14], A green synthesis method to manufacture TiO2nanoparticles using Moringa Oleifera Leaf Extract and concluded that biosynthesis of TiO2 nanoparticles using Moringa Oleifera Leaf Extract is a new methodology that uses inexpensive precursors. [15]

The goals of this research were to investigate the in-vitro antioxidant, antimicrobial, and antifungal activities of stevia leaf extract-derived titanium dioxide nanoparticles produced using phytogenic synthesis.

Materials and Methods

Materials: Titanium Dioxide (Research lab fine chem industries), Ethanol (Research lab fine chem industries Mumbai), Methanol (Research lab fine chem industries Mumbai). DMSO (Research lab fine chem industries Mumbai), Acetone (Research lab fine chem industries Mumbai). DPPH (Sisco research laboratories Pvt. Ltd. Mumbai).

METHODS:

Collection and Authentication of Plant:

The plant was collected from wakad (pimpari chinchawad), Pune, and authenticated by the Botanical Survey of India (BSI), Koregaon Park, Pune.

Preparation of stevia leaf broth:

For preparation of stevia leaf broth, stevia leaves were collect and washed with distilled water and dried under shade. 5g of crushed leaves were mixed with 100 ml distilled water in 200 ml conical flask, which was then boil for 2 min. The boiled solution was filtered by using muslin cloth and formed filtrate was called broth. This broth was used for preparation of Titanium dioxide nanoparticles. [16]

Preparation of Titanium Dioxide Nanoparticles:

For preparation of titanium dioxide nanoparticles, 1gm of titanium dioxide mixed with 200 ml distilled water. In that solution 25 ml stevia leaf broth was added and stirred a solution for 2 hr. by using magnetic stirrer to attain a homogenous mixture. The prepared solution was heated until water was evaporated. [17]

Preliminary evaluation tests for nanoparticles: [18]

I. Solubility test:

The purpose of the solubility analysis was to check a suitable solvent system for dissolving the Prepared Titanium Dioxide and Stevia Nanoparticles as well as to assess its solubility in the dissolution medium. A Nanoparticle solubility test was conducted by dissolving an excess amount of the medication in various solvents such as water, ethanol, methanol, acetone, DMSO, etc.

II. pH Determination:

A pH determination study was used to determine the acidity or alkalinity of the solution. pH is the concentration of hydrogen ion in the solution. A solution containing more H^+ ions remains acidic while the solution containing more OH^- ion remain alkaline. pH of prepared nanoparticles was determined by used pH paper.

Analytical techniques for the analysis of nanoparticles:

1) Scanning Electron Microscopy (SEM):

The scanning electron microscopy used to determine the morphological features of prepared nanoparticles**.**

2) UV-Vis Spectroscopy:

The UV-Vis Spectroscopy is used to determine the optical property of prepared nanoparticles. UV-Vis Spectroscopy was used to determine band gap of Prepared Titanium Dioxide and Stevia Nanoparticles. The band gap of prepared $TiO2$ nanoparticles is determined using UV-Vis spectroscopy.

The following equation was used to calculate the energy band gap of the prepared nanoparticle:

$$Eg = hc/ \lambda$$

Where,
h is a planks constant (4.135×10-15)
c is velocity of light (3×108)
λ is the wavelength in (nm).

3) Fourier Transform Infrared Spectroscopy (FTIR):

By comparing the infrared spectrum of an "unknown" with previously recorded reference spectra, the infrared spectrum can be utilized as a fingerprint for identification. This first principles method is predicated on the observation that a molecule's structural characteristics, whether they be the molecule's backbone or the functional groups connected to it, result in distinct and repeatable absorptions in the spectrum. The presence of unsaturation and/or aromatic rings in the structure can then be ascertained. Finally, it is possible to deduce whether specific functional groups are present of the group and its local environment and/or location in the structure. The FTIR study used to determine the functional group present in extract, which play an active role in preparation of nanoparticles.

4) X-ray Diffraction (XRD):

X-ray Diffraction Spectroscopy was used to investigating the structure-property relationship and discovering new properties, in order to achieve relevant improvements in current state-of-the materials. XRD technique is based on elastic scattering of X-ray from structure that have long range order. The X-ray get diffracted by crystal because the wave length of X-ray similar to inter atomic spacing in the crystal.

❖ Antioxidant Study: [21, 22]

The antioxidant activity (AA %) of prepared titanium dioxide nanoparticles was determined by DPPH free radical scavenging capacity. The reaction mixture was comprised of 0.5 ml of titanium dioxide nanoparticles (10-200 µg/ml) of absolute ethanol and 0.3 ml of DPPH solution. The reaction between DPPH and the hydrogen donating antioxidant result in the reduction of DPPH, and lead to the colour change from deep violet to light yellow. The absorbance of the resultant product was read at 517 nm after 30 min 0f dark incubation. Ethanol used as a blank.

The scavenging activity percentage (AA %) was calculated using the following equation:

$$\text{Scavenging activity} = \frac{(A\ (blank)\,517 - A\ (sample)\,517) \times 100}{(A\ (blank)\,517)}$$

❖ Antimicrobial and Antifungal Study: [26]

Antimicrobial and Antifungal activity of the prepared Titanium dioxide nanoparticles will determined using Gram-negative bacteria (Pseudomonas aeuroginosa), Gram-positive bacteria (Staphylococcus aureus) and fungi (Candida albicance). Ciprofloxacin (antibiotic) as a standard. The disc diffusion method was used to study the microbial in activation potential of prepared titanium dioxide nanoparticles. The inhibition zone diameter (mm) will measured using verniar caliper after incubating the bacteria for 24h at 37℃.

1) Disc diffusion method:

Disc susceptibility testing of Pseudomonas aeruginosa NCIM 5029 (ATCC 27853), Staphylococcus aureus NCIM 5021 (ATCC 25923) and Candida albicans NCIM 3100 was carried out against Sample (Stevia-TiO2 nanoparticles) and ciprofloxacin (antibiotic) as recommended by Clinical and Laboratory Standards Institute (CLSI, USA, 2017) on Mueller-Hinton agar (HiMedia, India) plates. O.D. of the overnight grown culture was adjusted to 1x105 CFU/mL. 100 µL of the culture was spread on MHA plates with spreader. Sterile discs were placed onto the plates and loaded with 20µL (200 µg/disc) of Sample 1. Plates were incubated at 37˚C for 24 h and inhibition zone diameter was measured.

2) Minimum inhibitory concentration (MIC):

MIC of Titanium Dioxide and Stevia Nanoparticles against Pseudomonas aeruginosa NCIM 5029 (ATCC 27853), Staphylococcus aureus NCIM 5021 (ATCC 25923) and Candida albicans NCIM 3100 was determined using broth micro dilution method as per the CLSI guidelines (CLSI, USA, 2018). MIC was tested at concentrations ranging from 2-1024 µg/mL. The microplates were incubated at 37˚C for 24 hr. and growth was measured by reading the absorbance at 540 nm on a microplate reader.

Results and Discussion:

- **Preliminary Evaluation Test**
  - ➢ **Solubility test:**

The nanoparticles have an organic component on their surface and are resistant to irreversible aggregation. The solubility of nanoparticles colloidal suspension is reversible and temperature dependent. The precipitate is formed when nanoparticles aggregate and settle out due to gravity.

- ➢ **pH Determination:**

The pH of prepared nanoparticles was ranges from 6-7.

- **Evaluation of Nanoparticles by Instrumetal Techmiques:**

1) Scanning Electron Microscopy (SEM)

SEM was employed to evaluate the morphological features of TiO2 nanoparticles, as seen in the image. The synthesized TiO2 was found to be spherical in size, ranging from 48 to 94 nm. Sharpe edges, which have a high surface-to-volume ratio and less cohesive energy than bulk material, make nanoparticles highly reactive.

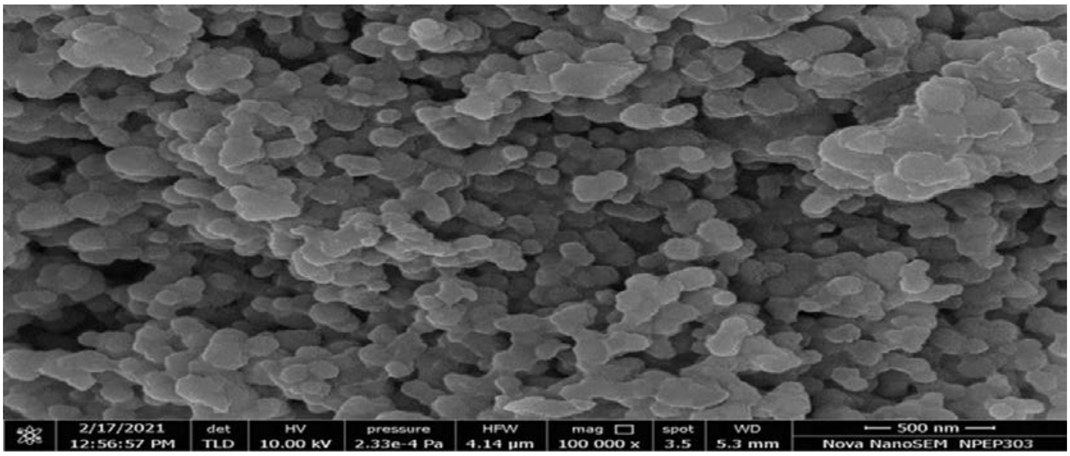


Fig 1: Scanning Electron Microscopy (SEM) image of Titanium Dioxide a Stevia nanoparticles.

2) UV-Vis Spectroscopy.

At a wavelength of 283.5 nm, the band gap was calculated to be 4.3 eV, which corresponds to the absorption peak.

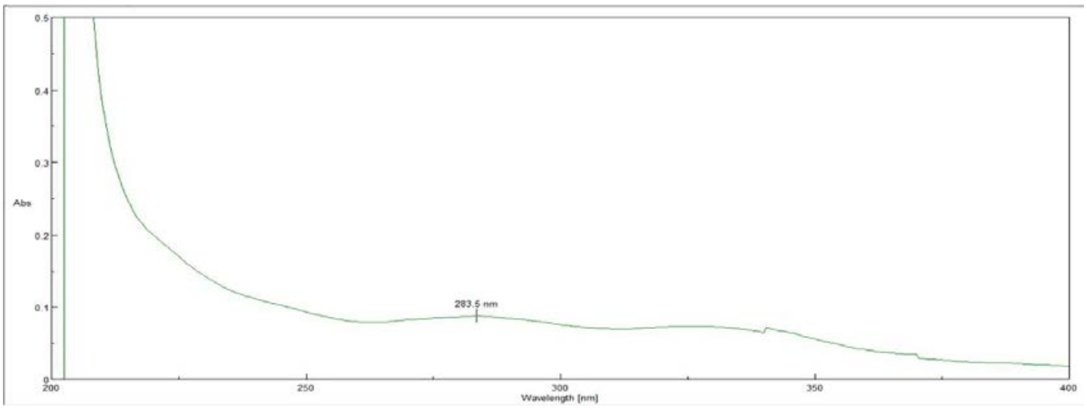


Fig 2. UV-Vis absorption graph of Titanium Dioxide and Stevia nanoparticles

3) Fourier Transform Infrared Spectroscopy (FTIR) Study:

The functional group of produced nanoparticles was established using Fourier Transform Infrared Spectroscopy (FTIR) and the results are shown in the table below.

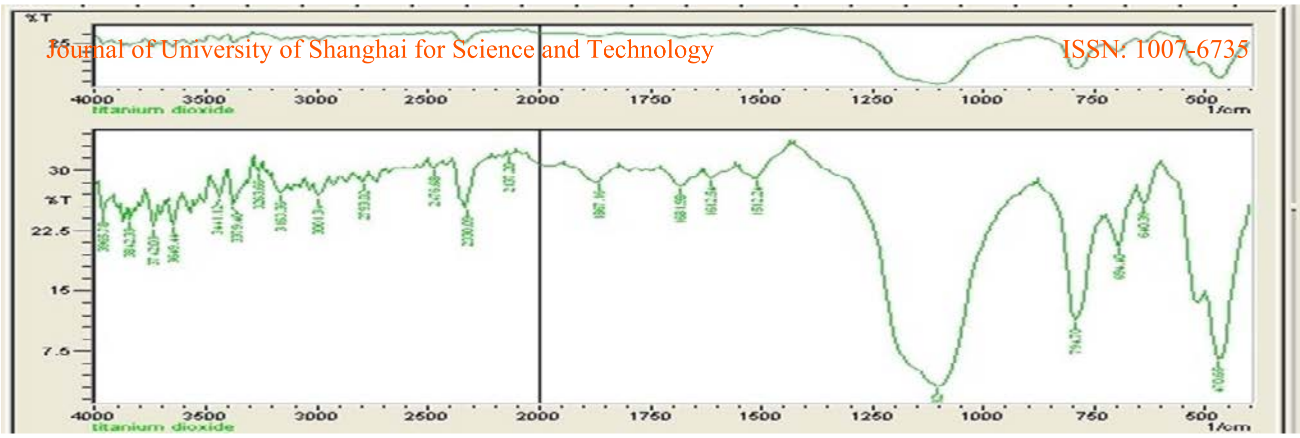
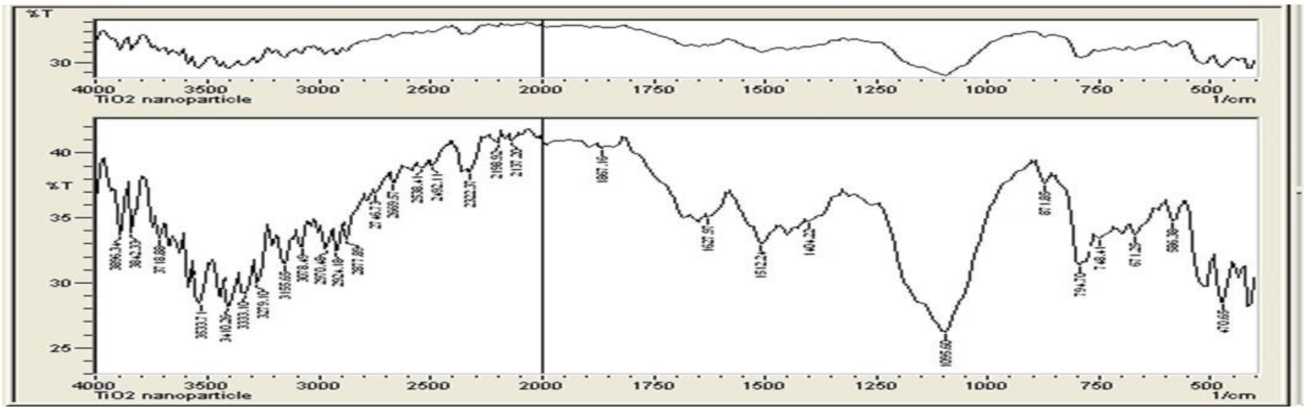


Fig 3:



FTIR Spectra of stevia powder

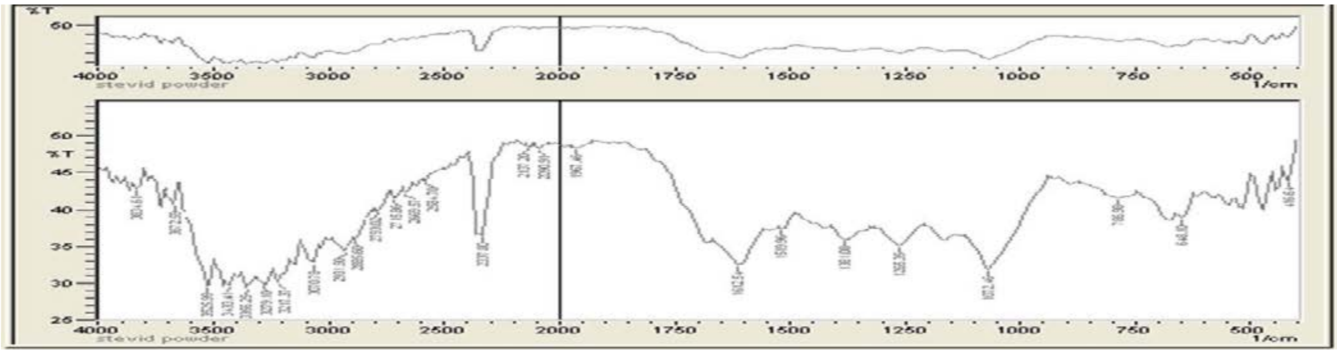


Fig 4: FTIR Spectra of titanium dioxide

Fig 5: FTIR Spectra of stevia-TiO2 nanoparticles

The principle peak of the spectra obtained for the formulation was not altered in the FTIR study, indicating that there was no significant interaction between the stevia and titanium dioxide used in formulation.

Sr.no	Functional group and its standard value	Observed Titanium dioxide Spectra value in FTIR.	Observed Titanium Dioxide value of FTIR spectra in formulation of Titanium Dioxide and Stevia nanoparticles

Table 1: FTIR ranges of Titanium Dioxide and Titanium Dioxide and Stevia Nanoparticles

1	Titanium dioxide (900-1200 cm^{-1})	1095.60 cm^{-1}	1103.32 cm^{-1}

Table 2: FTIR ranges of Stevia and Titanium Dioxide and Stevia Nanoparticles.

Sr.no.	Functional group & its standard value.	Observed Stevia Spectra value in FTIR.	Observed Stevia Spectra value of FTIR in formulation of Titanium Dioxide and Stevia Nanoparticles
1	Phenolic OH stretching (3200-3550 cm-1)	3217.37, 3525.99 cm-1	3219.10,3533.71 cm-1
2	Phenyl ring (sharp ring) (1400-1620 cm-1)	1612.54, 1519.96 cm-1	1404.22,1512.24,1627.97cm-1
3	Phenyl ring (intense bend ring) (700-1000 cm-1)	786.98 cm-1	748.41, 794.70, 871.85 cm-1
4	c-o-c (1070-1150 cm-1)	1072.45 cm-1	1095.60 cm-1
5	Carbonyl group (1600-1900 cm-1)	1612.54 cm-1	1867.15 cm-
6	c=o (1650-2000 cm-1)	1967.46 cm-1	1867.16 cm-1
7	OH stretching (3200-3550 cm-1)	3253.66, 3549.44 cm-1	3219.10, 3533.71 cm-1

X-ray Diffraction Analysis:

The produced nanoparticles are crystalline in nature, as determined by X-ray Diffraction (XRD) measurement data.

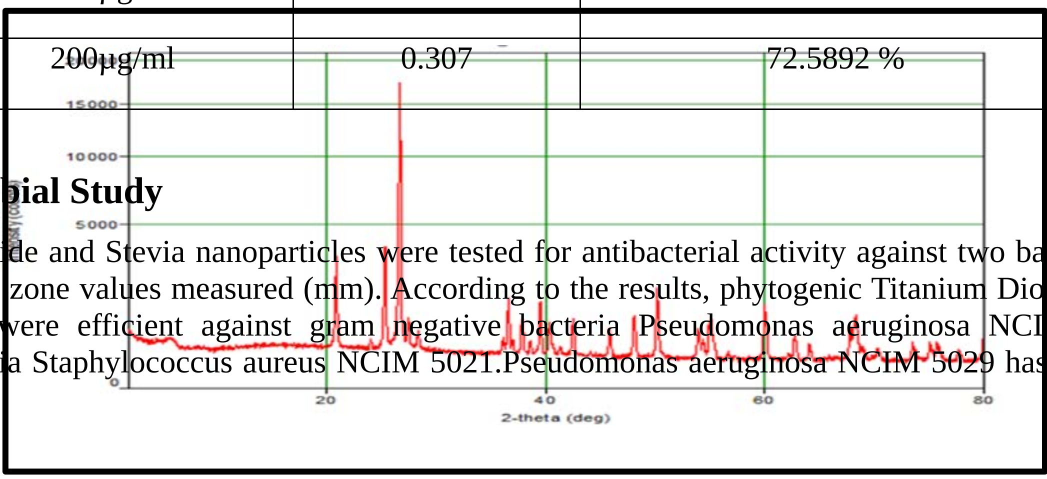


Fig 5: XRD graph of Titanium Dioxide and Stevia Nanoparticles.

Antioxidant Study, Antimicrobial Study & Antifungal Study.

1 Antioxidant Study

The DPPH reduction the results of the test indicated that titanium dioxide-Stevia nanoparticles have high antioxidant activity. The scavenging capacity of titanium dioxide-Stevia was dose-dependent, with the highest value of 72 percent found at 200g/ml. The antioxidant property of the Titanium dioxide-Stevia nanoparticles because of DPPH free radical quenching in a time-dependent manner, which occurs through the exchange of electrons between the reactant and derived product.

Table 3: Antioxidant Study ranges of Titanium Dioxide and Stevia nanoparticles

Concentration	Absorbance	% Scavenging Activity
Ethanol (blank)	1.12	-
10µg/ml	0.972	13.2142 %
50µg/ml	0.812	27.5 %
100µg/ml	0.463	58.6607 %
150µg/ml	0.397	65.44 %
200µg/ml	0.307	72.5892 %

2 Antimicrobial Study

Titanium Dioxide and Stevia nanoparticles were tested for antibacterial activity against two bacterium strains, with inhibition zone values measured (mm). According to the results, phytogenic Titanium Dioxide and Stevia nanoparticles were efficient against gram negative bacteria Pseudomonas aeruginosa NCIM 5029, gram positive bacteria Staphylococcus aureus NCIM 5021.Pseudomonas aeruginosa NCIM 5029 has a 23-mm zone of inhibition, Staphylococcus aureus NCIM 5021 has a 21-mm zone of inhibition. The MIC for TiO2- Stevia nanoparticle against Pseudomonas aeruginosa NCIM 5029 and Staphylococcus aureus NCIM 5021 was >1024 g/mL, whereas the MIC for ciprofloxacin was less than 2 g/mL for both isolates tested.

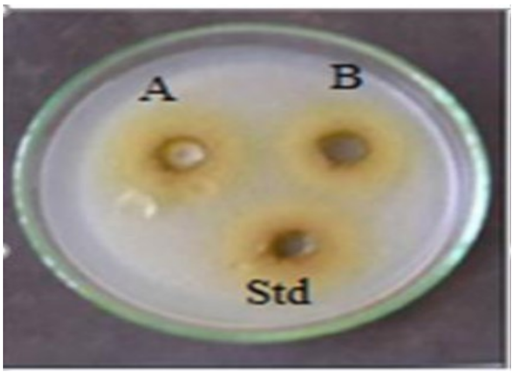
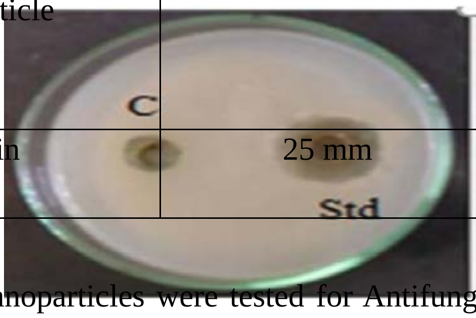


Fig 6: Zone of inhibition was observed for ciprofloxacin tested against Pseudomonas aeruginosa NCIM 5029 and Staphylococcus aureus NCIM 5021 Shown in the plate.

Table 4: Zone of inhibition of ciprofloxacin against gram positive and gram negative bacteria.

Compound	Pseudomonas aeruginosa	Staphylococcus aureus
Titanium Dioxide – Stevia nanoparticle	23 mm	21 mm
Ciprofloxacin	25 mm	25 mm



3 Antifungal Study:

Titanium Dioxide and Stevia nanoparticles were tested for Antifungal activity against one fungal strain, with inhibition zone values measured (mm). According to the results, phytogenic Titanium Dioxide and Stevia nanoparticles were efficient against fungal strain Candida albicans NCIM 3100Candida albicans NCIM 3100 has a 10-mm zone of inhibition.

Fig 7: Zone of inhibition was observed for ciprofloxacin tested against Candida albicans NCIM 3100 Shown in the plate.

Table 5: Zone of inhibition of ciprofloxacin against fungal strain.

Compound	Candida albicans
Titanium Dioxide-Stevia nanoparticle.	10 nm
Ciprofloxacin	19 nm

Conclusion:

The present work proves that phytogenic generation of nanoparticles is useful method using cheap precursors like titanium dioxide and Stevia. This is a simple, cost-effective, time saving and environmentally friendly synthetic approach that creates opportunities for a wide variety of applications. The eco-friendly phytogenic generation approach by the use of these leaf extracts for the synthesis of nanoparticles will increase their economic viability and sustainable management. In SEM synthesized TiO_2 was found to be spherical in size, ranging from 48 to 94 nm. Sharpe edges, which have a high surface-to volume ratio and less cohesive energy than bulk material, make nanoparticles highly reactive. UV-Vis Spectroscopy was performed at a wavelength of 283.5 nm, and the absorption peak-corresponding band gap was found to be 4.3 eV. FTIR study used to detect the functional group present in extract, which play an active role in preparation of nanoparticles. Phenolic OH stretching at 3219.10, 3533.71 cm-1,Phenyl ring (sharp ring) at 1404.22,1512.24,1627.97 cm-1, Phenyl ring (intense bend ring) at 748.41, 794.70, 871.85 cm-1, ether group at 1095.60 cm-1, Carbonyl group at 1867.15 cm-1, and c=o group at 1867. The produced nanoparticles are crystalline in nature, as determined by XRD measurement data. Antioxidant Study scavenging efficiency of titanium dioxide-Stevia was dose-dependent, with the highest value of 72 % observed for 200g/ml. Antimicrobial and Antifungal Studies is shows that , phytogenic Titanium Dioxide and Stevia nanoparticles were effective against gram negative bacteria Pseudomonas aeruginosa NCIM 5029, gram positive bacteria Staphylococcus aureus NCIM 5021, and fungal strain Candida albicans NCIM 3100.

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