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Green Synthesis Of Akarkara (Anacylus Pyrethrum) Root Extract With Titanium Oxide Nano Particles And Its Antibacterial Activity

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Abstract:
Titanium oxide nanoparticles (TiO_2 NPs) are extensively used in nanotechnology for their potent antibacterial activity, chemical stability, and biocompatibility. This study deals with an environment friendly and biosynthesis process for pharmacological applications of titanium oxide nanoparticles, derived from Akarkara (Anacyclus pyrethrum) root aqueous extract. The formation and characterisation of TiO_2NPs were confirmed by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscope (SEM) with energy-dispersive spectroscopy (EDX).The antibacterial activities were carried out cells with different concentrations respectively. The TiO_2 nanoparticles inhibited the growth of the bacterial pathogens significantly, in a dose and duration dependent manner.

Keywords: Anacyclus pyrethrum, Titanium dioxide nanoparticles, Antibacterial Activity.

Introduction:
In recent years, there has been a growing interest in the use of herbal medicines for disease control due to their effectiveness, affordability, and limited side effects [1]. Natural products, particularly those derived from plants, have been a valuable source of raw materials for the discovery of bioactive compounds used in various industries, such as nutrition, cosmetics, and pharmaceuticals [2]. Anacyclus pyrethrum (L.) is one of wild species of flowering plant in the Asteraceae family and the Anacyclus genus, commonly known as pellitory. A.pyrethrum tends to flourish in well-drained soil and prefers open, unshaded areas with sparse tree and shrub cover within Mediterranean vegetation zones [4]. Extensive phytochemical screening of A. pyrethrum has unveiled a plethora of secondary metabolites, including alkaloids, tannins, coumarins, and flavonoids [5]. The root of A. pyrethrum is particularly rich in N-isobutyldienediylamide and polysaccharides [6].

Additionally, numerous experimental studies have demonstrated the wide range of biological effects associated with A. pyrethrum, including antioxidant and anti-inflammatory properties [7]. Apart from its medicinal properties, A. pyrethrum has been traditionally used as a stimulant, cordial, and rubefacient [8]. It is recommended for gargling to alleviate symptoms of conditions such as rhinitis, neuralgia, rheumatic discomfort, and musculoskeletal pain. In Ayurvedic medicine, root of pellitory is combined with Withania somnifera and Vitis vinifera roots to treat epilepsy [9]. Furthermore, it has shown promising results in the remedy of conditions such as sciatica, paralysis, hemiplegia, and amenorrhea [10]. Anacycline, isobutylamide, inulin, and trace amounts of essential oil have been identified in the pellitory root [11]. Remarkably, there have been observations indicating that A. pyrethrum may lead to a reduction in insulin needs for those with insulin-dependent diabetes mellitus, along with the potential to decrease plasma glucose and serum cholesterol levels following oral administration for a duration of 3-6 weeks [12]. These attributes arise from a diverse array of phytochemical compounds, with over a hundred distinct compounds identified thus far. These compounds include phenolic compounds, flavonoids, and alkaloids [3,5,8].Molecular studies on A. pyrethrum have provided valuable insights into its genetic makeup and the underlying mechanisms of its pharmacological properties. It has been documented to exhibit a range of biological effects, including antimicrobial, antiviral, diuretic, antioxidant, and analgesic properties [15,13]. These studies have also revealed the biosynthetic pathways and regulatory mechanisms that contribute to the plant medicinal properties [3]. Furthermore, molecular studies have also facilitated the identification and selection of high-yielding and disease-resistant cultivars of A. pyrethrum [14], In this context, this study aims to deals with an environment friendly and biosynthesis process for pharmacological applications of titanium oxide nanoparticles, derived from Akarkara (Anacyclus pyrethrum) root aqueous extract. Our objective is to uncover novel titanium oxide nano particles and its anti-bacterial activity and chemical profile.

Materials and methods:

Sample collection:

Akarkara (Anacylus pyrethrum) is used for the synthesis of titanium oxide nano particles and were collected from the Farm Quartee 100% natural root powder. It is padded, marketed& exported by Authorised seller Prem enterprises, Jaipur, Rajasthan. The collected root powder was stored and the chemicals received from the vendor is directly used without any modifications.

Preparation of the Akarkara (Anacyclus pyrethrum) root extract:

Biosynthesis of Akarkara (Anacyclus pyrethrum) root extract with Ethanolic solution TiO_2 NPs:

The 40 grams root powder is taken in round bottom flask and is dissolved in ethyl alcohol was macerated in 300 mL of ethanol for 20 days for solvent extraction. Then extract was taken out and excess ethanol was removed by using simple distillation method. The ethanolic plant extract of Akarkara (Anacyclus pyrethrum) was stored at 5ºC in refrigerator and used for further analysis. The obtained extract colour was golden brown. Taken 2 ml of ethanolic plant extract Akarkara (Anacylus pyrethrum) bio synthesis by titanium oxide 50mM and 1% KOH alcoholic solution diluted 200ml added to the plant extract it gives orange colour solution biosynthesized is product formed and further analysis.

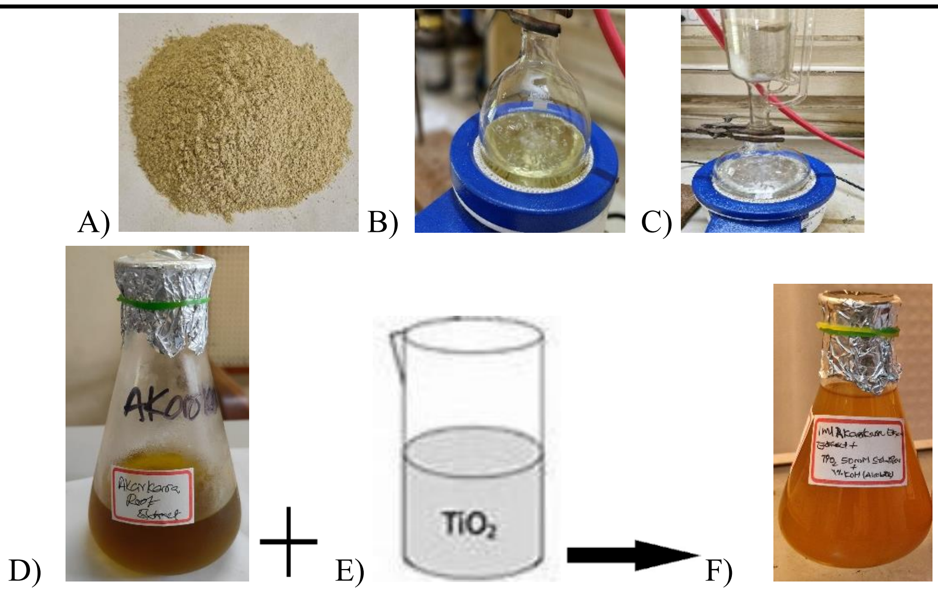


Fig. A) Akarkara (Anacylus pyrethrum) root powder, B &C) Soxlate extraction with ethyl alcohol , D) Akarkara (Anacylus pyrethrum) root extract, E)Titanium oxide alcoholic solution, F)Biosynthesized Titanium oxide NPs.

Results and discussion:

UV–Visible Spectroscopic Analysis:

Usually, TiO2 nanoparticles absorb most of their light between 250 and 400 nm. Depending on the precise synthesis conditions and particle size, this common phase can have specific peaks reported anywhere from 290 nm to 376 nm, with an absorption edge typically seen around 380-388 nm with a slightly narrower band gap (about 3.0 eV) and a slightly longer wavelength of absorption, this phase extends slightly into the near-visible range. Its absorption edge and peaks are located around 396-410 nm. For different synthesis techniques, additional distinct absorption peaks have been reported in the literature at 280 nm, 303 nm, 317.6 nm, 352 nm, and 356 nm.

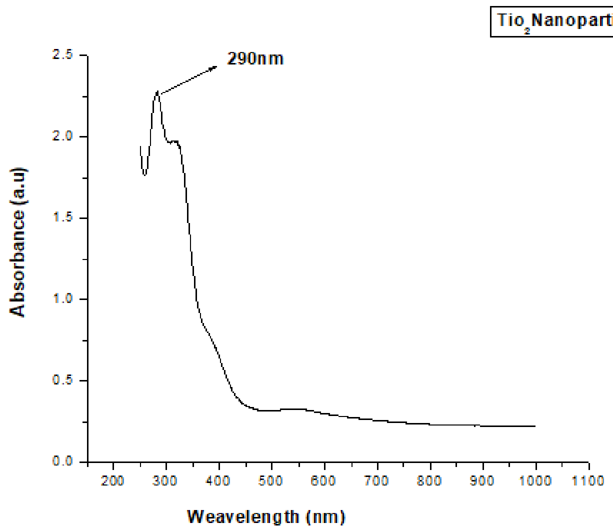


Fig. 1- UV spectra of TiO2 NPs

Fourier Transform Infrared Spectroscopic Analysis (FTIR):

The broad absorption band appear at 3381 cm^{-1} O-H stretching vibration corresponds to alcohol and phenol hydroxyl groups indicates plant phytochemical compounds and surface hydroxyl groups on TiO2 & peaks at 2976 cm^{-1},2888 cm^{-1} shows the presence of the alkane C-H stretching from bioactive compounds like alkaloids, terpenoids, flavonoids and glycosides. The sharp peak at 1650cm-1, corresponds to the carbonyl stretching vibrations present in aldehydes, ketones, carboxylic acids and amides, group of quinone compounds (commonly found in alkaloids, terpenoids, flavonoids, phenolic compounds) The peak at 1045cm-1 indicates the C-O stretching vibrations of alcohols, esters, ethers, polysaccharides and other phytochemicals acting as capping/stabilizing agents and the peak at 880 cm^{-1} and indicates strong peak in the lower wavenumber region correspond to Ti–O–Ti stretching vibrations, confirming the formation of TiO_2 nanoparticles.

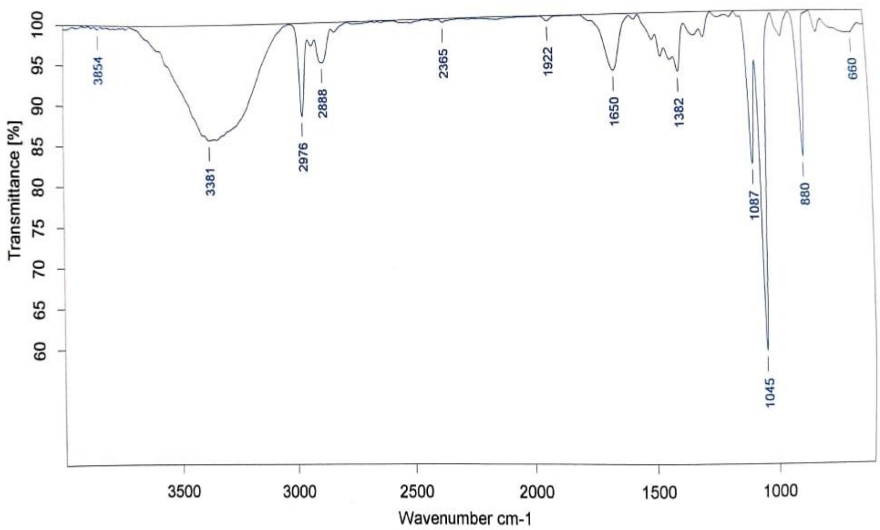


Fig. 2- FTIR spectra of TiO2 NPs

X-ray Diffraction (XRD):

The crystalline nature of TiO2 nanoparticles were identified from the X-ray diffraction analysis and this is shown in Fig.3. From the figure a relative broad and low intensity peaks were observed for the 2 theta (degree) values 25.3,37.8,48.0,54.0,62.7,68.8 for miller indices (101) (004) (200) (105) (204) (116) which confirms the crystalline nature of akarkara root extract bio synthesized titanium oxide nano particles. From the XRD data the phase anatase (tetragonal) was identified. for green synthesized and the diffraction pattern revealed the anatase phase of the developed TiO_2 root extract of Akarkara (Anacylus pyrethrum) with TiO2 nanoparticles by the Scherrer equation.

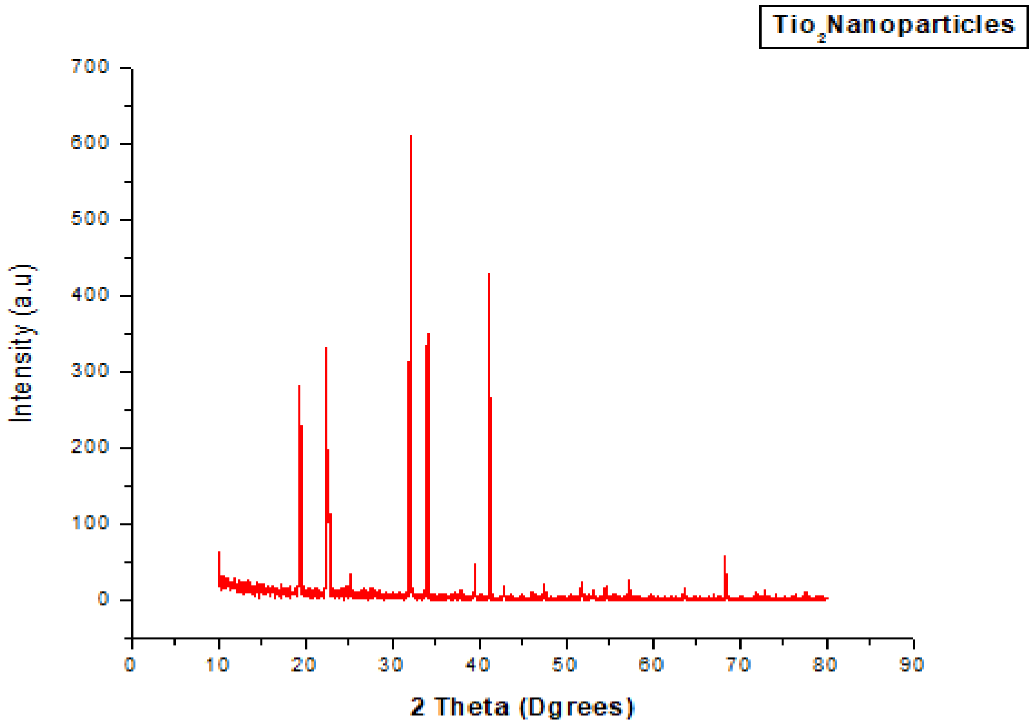


Fig. 3 – XRD diffractogram of TiO_2 NPs

Scanning Electron Microscope (SEM):

Fig. 4 shows the scanning electron microscope (SEM) of the prepared TiO2 nanoparticles. The nano particles formed here with uniform morphology. The figure shows the range of about 35.5 nm to 110 nm nano particles and agglomerated which would be found in the biological preparation of the nano particles.

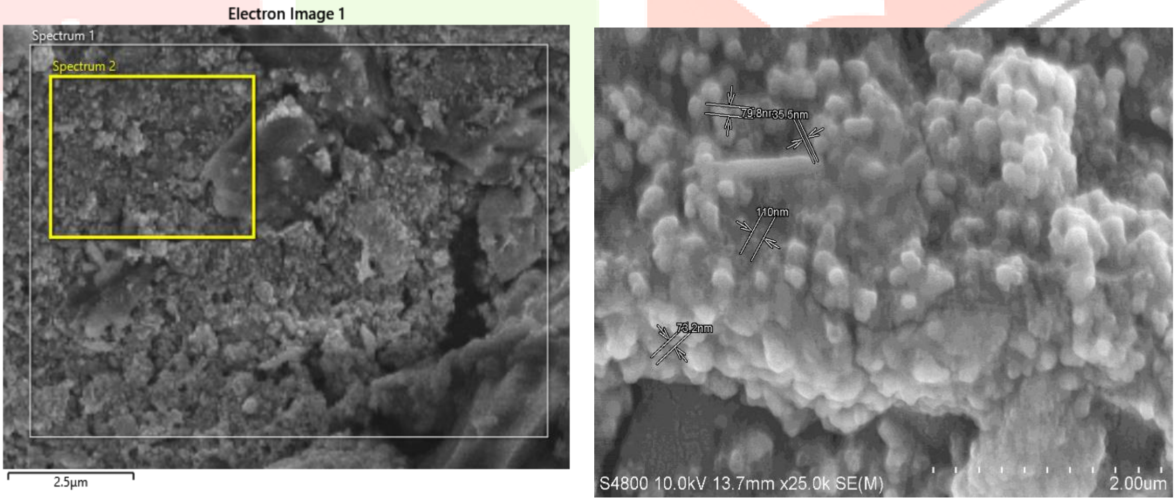


Fig. 4 SEM images of root extract of Akarkara (Anacyclus pyrethrum) TiO2 nanoparticles

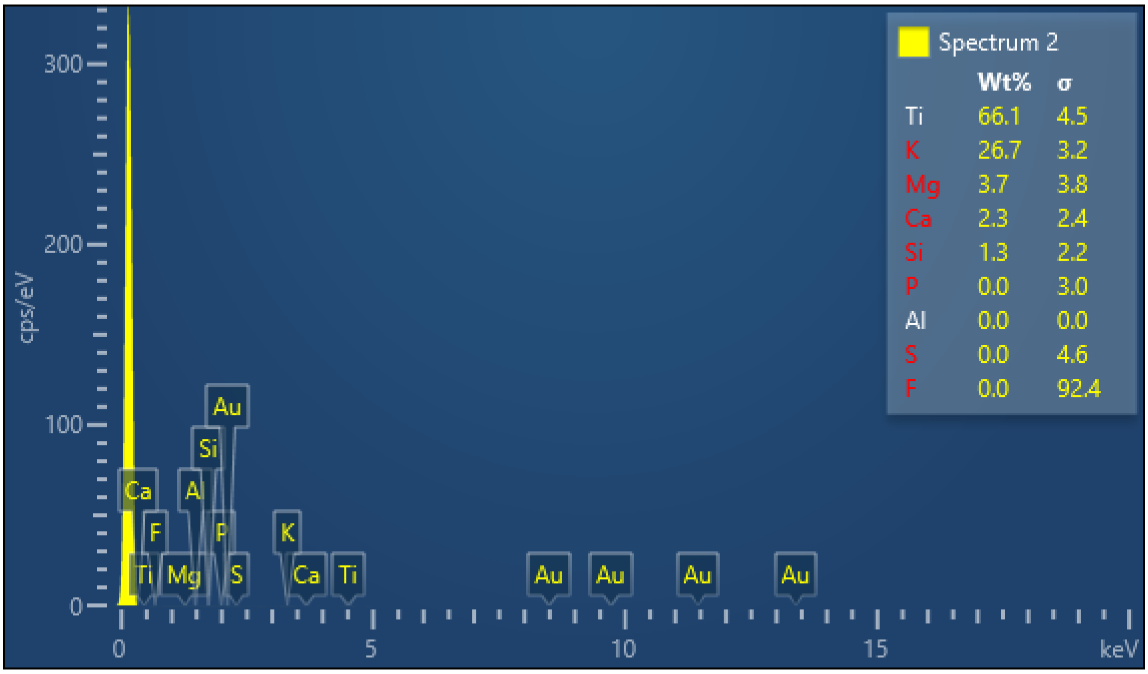


Fig. 5 EDS (Energy Dispersive X-ray Spectroscopy)

Biological Application:

Anti-Bacterial Activity:

The antibacterial potential of **Titanium Oxide**, and the **standard antibiotic ampicillin** was determined using the **agar well diffusion method**. Four bacterial strains—*Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 3160), and *Bacillus subtilis* (MTCC 121)—were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Each bacterial strain was cultured in **nutrient broth** and incubated at **37 °C for 24 hours** to achieve active growth. The bacterial suspension was adjusted to a turbidity equivalent to **0.5 McFarland standard** (approximately 1×10^8 CFU/mL). Sterile **nutrient agar plates** were prepared, and 100 µL of each standardized inoculum was spread evenly across the surface using sterile cotton swabs to ensure uniform lawn formation. Two wells of **6 mm diameter** were made aseptically in each plate using a sterile cork borer. The wells were filled with 50 µL of each test sample: well 1 Titanium Oxide, and well 2 served as the **positive control containing ampicillin (20 µg/mL)**. The plates were left at room temperature for 30 minutes to allow proper diffusion of the samples into the agar medium and then incubated at **37 °C for 24 hours**. After incubation, the **zones of inhibition (in mm)** around each well were measured using a digital Vernier caliper, and the results were recorded to compare the antibacterial efficacy of the test substances against each bacterial strain.

Results

The antibacterial activity of Titanium Oxide, and the standard antibiotic ampicillin was evaluated against *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 3160), and *Bacillus subtilis* (MTCC 121) using the agar well diffusion method. All test organisms exhibited measurable inhibition zones, confirming the antimicrobial efficacy of the tested agents. Among the two, ampicillin demonstrated the highest inhibitory effect against all pathogens, indicating its strong bactericidal action. The Titanium Oxide showed moderate inhibition. *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed pronounced sensitivity toward Titanium Oxide, suggesting its potential as an antibacterial compound. Comparatively, *E. coli* and *Bacillus subtilis* displayed moderate inhibition zones with Titanium Oxide, reflecting strain-specific variations in susceptibility. The quantitative measurements of inhibition zones are presented in Table 1.

Table 1. Zone of inhibition (mm) of different test samples against selected bacterial pathogens

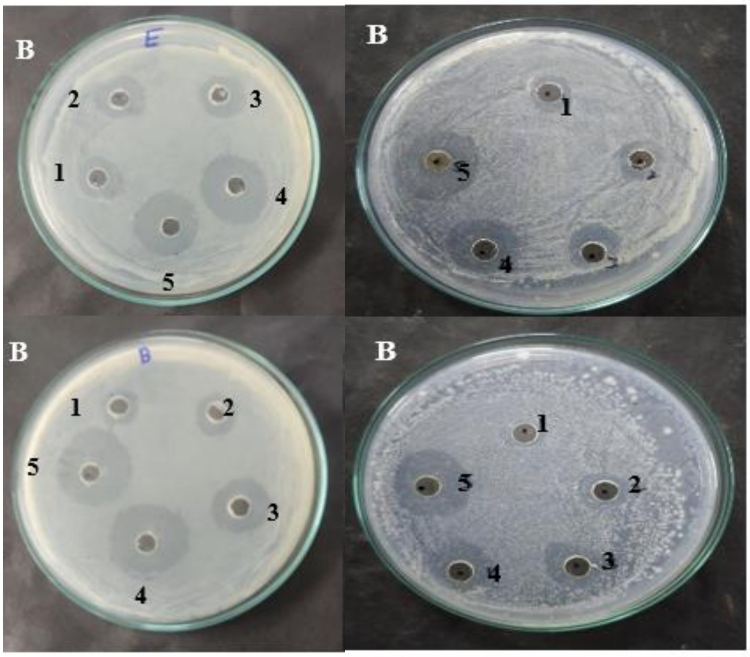
Test Organism	Titanium oxide	Ampicillin
Escherichia coli MTCC 443	12 ± 0.4	18 ± 0.5
Pseudomonas aeruginosa MTCC 424	13 ± 0.3	17 ± 0.6
Staphylococcus aureus MTCC 3160	15 ± 0.4	19 ± 0.5
Bacillus subtilis MTCC 121	11 ± 0.3	17 ± 0.4

Methodology for minimum inhibitory concentration (MIC) well diffusion method

The minimum inhibitory concentration (MIC) of **Titanium Oxide** is determined by the **agar well diffusion assay** against selected bacterial pathogens to evaluate their concentration-dependent antibacterial efficiency. The bacterial strains used in the study were *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 3160), and *Bacillus subtilis* (MTCC 121), obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Each bacterial culture was freshly inoculated in nutrient broth and incubated at **37 °C for 24 h** to obtain an active log-phase culture. The optical density was adjusted to **0.5 McFarland standard** (≈ 1×10^8 CFU/mL). Sterile **nutrient agar plates** were prepared and uniformly seeded with 100 µL of each standardized bacterial suspension using a sterile cotton swab to ensure uniform growth. Five wells of **6 mm diameter** were aseptically made in each plate using a sterile cork borer. Different concentrations of the test samples (62.5, 125, 250, 500, and 1000 µg/mL) were prepared in sterile distilled water. Each well was filled with **50 µL** of the corresponding concentration, with **B plates representing Titanium Oxide**. The plates were allowed to stand for **30 minutes** at room temperature to facilitate diffusion of the test materials into the medium, followed by incubation at **37 °C for 24 hours**. After incubation, the plates were examined for the presence of **zones of inhibition** around the wells. The diameter of each inhibition zone (in mm) was measured using a digital Vernier caliper, and the MIC value was determined as the **lowest concentration that produced a visible inhibition zone.**

Results

The antibacterial efficacy of Titanium Oxide was assessed at graded concentrations ranging from 62.5 to 1000 µg/mL using the agar well diffusion method. Test materials exhibited a concentration-dependent inhibition of bacterial growth. Among the tested concentrations, 1000 µg/mL of sample demonstrated the maximum inhibitory effect, while 62.5 µg/mL showed minimal or no inhibition in most organisms. Titanium Oxide showed superior antibacterial activity at all concentrations, indicating the higher potency of the bioactive metabolite[16,18]. The maximum inhibition was observed against *Staphylococcus aureus* (MTCC 3160) and *Pseudomonas aeruginosa* (MTCC 424), while moderate inhibition was recorded for *Escherichia coli* (MTCC 443) and *Bacillus subtilis* (MTCC 121). The observed data clearly indicate that the inhibitory zone diameters increased proportionally with the concentration of the test agents, suggesting a dose-dependent response pattern[17,18]. The quantitative data for the inhibition zones are presented in Table 2.



Escherichia coli (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 121), and *Staphylococcus aureus* (MTCC 3160).

Table 2. Zone of inhibition (mm) at different concentrations of Titanium Oxide against test bacteria

Test Organism (MTCC No.)	Sample	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
Escherichia coli (443)	Titanium Oxide	08 ± 0.4	10 ± 0.3	13 ± 0.4	15 ± 0.5	18 ± 0.5
Pseudomonas aeruginosa (424)	Titanium Oxide	09 ± 0.3	12 ± 0.3	15 ± 0.4	17 ± 0.4	19 ± 0.3
Bacillus subtilis (121)	Titanium Oxide	10 ± 0.4	13 ± 0.4	16 ± 0.3	18 ± 0.4	21 ± 0.5
Staphylococcus aureus (3160)	Titanium Oxide	08 ± 0.4	11 ± 0.3	13 ± 0.4	16 ± 0.5	18 ± 0.4

Conclusions:

TiO_2 nanoparticles were successfully synthesized using Anacyclus pyrethrum plant extract without adding any chemical agent. The XRD result proves the crystalline nature of

the material and the crystallite size was calculated to be 35.5nm to 110 nm. The functional moieties were identified from the FTIR studies. Morphological studies of TiO2 nanoparticles confirmed the formation of fine, less agglomerated particles. The anti-bacterial activity of the bio synthesized titanium oxide nano particles at different concentrations are exhibited a concentration-dependent inhibition of bacterial growth.

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