

GREEN SYNTHESIS OF TiO₂ NANOPARTICLES USING *OPUNTIA FICUS INDICA* FRUIT PEEL EXTRACT AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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The green synthesis pathway is cost-effective, environmentally benign (using non-toxic materials), and frequently produces better results than traditional methods. In the current study, an extract from a by-product such as the peel of *Opuntia ficus indica* (OFI) fruits was used in an improved phyto-synthesis process to create TiO₂ nanoparticles (NPs). With the aid of stabilizing molecules found in the extract, titanium isopropoxide (TiTP) functions as a precursor of titanium to create TiO₂ NPs. To characterize the appearance and structure of the synthesized nanoparticles, a range of analytical methods was utilized. The techniques employed encompassed X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and ultraviolet-visible spectroscopy (UV-Vis), in addition to scanning electron microscopy paired with energy dispersive X-ray spectroscopy (SEM with EDX) and zeta potential analysis. Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* bacteria were effectively inhibited by the as-prepared TiO₂. Nanoparticles prepared by this phyto-synthesis method may be thus suitable for a wide range of biological and medicinal applications.

Keywords: TiO₂ nanoparticles, *Opuntia ficus indica*, green synthesis, antimicrobial

INTRODUCTION

Various methods of synthesizing nanoparticles, based on chemical reduction, such as the sol-gel process, hydrothermal, solvothermal, sonochemical, electrochemical, and others,¹⁻⁵ while being simple and affordable, become hazardous because of the toxic reducing and stabilizing agents used. Therefore, researchers have shown interest in novel synthesis techniques aligned with the principles of “green chemistry”. Thus, alternative routes for the reduction of precursors may involve biosynthesis, based on bacteria, fungi, actinomycetes, marine sources, and yeasts; or phyto-synthesis using extracts from various parts of plants (leaves, roots, flowers, and fruits). Such methods are simple, affordable, quick and environmentally friendly, yielding safe products for a variety of uses.⁶

A family of nanoparticles known as metal oxide nanomaterials has garnered attention due to its intriguing properties and extensive practical applications, particularly in environmental remediation, for eliminating hazardous gases, heavy metals, organic pollutants, and biological contaminants.⁷ Nanoparticles can also be used for producing functional materials by adding them to textiles. For example, zinc oxide (ZnO) nanoparticles can impart significant antibacterial activity, provide robust UV protection, and induce hydrophobicity, which is responsible for self-cleaning.⁸ Utilizing biogenic copper oxide (CuO) nanoparticles prepared using *Mimosa pudica* mucilage is a practical way for wastewater remediation. Under solar radiation, these nanoparticles act as potent photocatalysts that aid in the breakdown of organic contaminants.⁹

Metal oxide-based nanomaterials have also been studied for their potential medical uses, especially as antibacterial, antifungal, and anticancer agents. Their biocompatibility, the controlled release kinetics of the active principle, and their capacity to target particular cells or tissues make them more effective than conventional medications, when utilized in clinical medicine for drug administration and biomedical imaging.¹⁰ Green synthesis of ZnO nanoparticles using an extract of *Fraxinus*

rhynchophylla led to nanoparticles alleviated pain in several nociceptive models, including pain caused by chemical and thermal stress, with fewer side effects than morphine.¹¹ The greater specific surface area and increased reactivity of these exceptional oxide metals in comparison to bulk materials are responsible for their outstanding features.

Due to their chemical stability, unique characteristics, and affordability, titanium dioxide (TiO₂) nanoparticles (NPs) are among the most widely used and produced metal oxide nanoparticles.^{12–13} Recently, various uses have been developed for TiO₂ nanoparticles, such as in sunscreens, UV-blocking fabrics, self-cleaning, anti-static and anti-fogging surfaces, and water and air purification technologies. Therefore, environmentally friendly, inexpensive, and quick synthesis processes are of high importance. As a result, in recent years, green chemistry techniques for obtaining TiO₂ NPs using plant extracts from leaves, flowers or peels have attracted much research interest.^{14–19}

One of the few plants that can withstand harsh weather, *Opuntia ficus indica* (OFI) is utilized in a wide range of industries, including the food, pharmaceutical, and cosmetics sectors, although its primary use is as fodder. It thrives in tropical and subtropical regions, including Mexico, Sicily, Chile, Brazil, Spain, in portions of northern Africa, including Algeria, Tunisia, Egypt, and Morocco, and even in the Middle East, as in Saudi Arabia, Iran, Pakistan *etc.*^{20–21} Despite studies on cladodes, fruits, stems, seeds, and even peel extracts of *Opuntia ficus indica*, only a few plant parts have been utilized in green chemistry, particularly in the synthesis of nanoparticles.

Silver nanoparticles (AgNPs) were biosynthesised using *Opuntia* extract, and their antibacterial activity was examined by Gad *et al.*²³ Also, Bocarando-Chacon *et al.*²⁴ and L. P. Ramírez-Rodríguez *et al.*²⁵ have demonstrated the adaptability of *Opuntia ficus indica* in nanoparticle synthesis by developing lead nanoparticles (Pb NPs) using the extract. Further, Rab Alvarez Andal *et al.*²⁶ synthesized gold nanoparticles (Au NPs) and other inorganic nanoparticles, such as zinc oxide, graphene *etc.*, using the same extract.^{27–28} Also, the green production of nanoparticles has been reported using the aqueous extract from the peels of prickly fruits.²⁹

In this work, we concentrate on the synthesis of TiO₂ NPS using an extract from the fruit peels of *Opuntia ficus indica*, which are typically seen as agricultural waste. By offering a green alternative to conventional chemical synthesis methods, which rely on hazardous reducing agents and energy-intensive procedures that harm the environment, this strategy supports the principles of sustainable and environmentally friendly nanotechnology. Furthermore, this approach promotes the valorization of agricultural waste, by avoiding waste to valuable plant components – fruits, cladodes, and seeds. For applications where environmental impact is a significant challenge, the green synthesis of TiO₂ NPs is essential. The resultant nanoparticles will be investigated in terms of their antibacterial properties, in order to evaluate their potential for specific targeted applications.

EXPERIMENTAL

Materials

Every reagent utilized in the production of TiO₂ nanoparticles was purchased directly from Sigma Aldrich. The fruits of *Opuntia ficus indica* (OFI) were gathered in September 2019, at Thenières El Had Tissemsilt, in northern Algeria. After being separated, the fruit peels were repeatedly cleaned with tap water and distilled water before being left to air dry. The peels were then prepared for investigation using two distinct methods:

¹ To achieve a fine consistency, the peels were mashed in a mechanical blender;

² The peels were cut into homogeneous cubes, each with a side length of 1 to 1.5 cm.

To prepare the aqueous OFI extract, 200 mL of distilled water was mixed with 40 g of peels from each procedure (crushed or sliced), and the mixture was kept in a water bath at 60 °C for two hours under constant stirring. The prepared OFI aqueous extract was filtered and considered ready for use.

To obtain TiO₂ nanoparticles, 5 milliliters of titanium isopropoxide (TiTP) were mixed with 100 milliliters of the orange-colored OFI extract. The mixture produced an orange-yellow hue. Overnight, the entire mixture was stirred magnetically. The liquid was then centrifuged for 10 minutes at 4000 rpm. The remaining plant extract was removed by repeatedly washing the solid phase with distilled water after precipitation. Then, the precipitate was dried for 24 hours at 70 °C in an oven, and powder was obtained. As seen in Figure 1, the latter was calcined for three hours at 400 °C with an increase of 2 °C every minute.

Characterization

Several techniques were used for characterizing the TiO₂ NPS. The sample's crystalline structure was identified using a Bruker D2 Phaser powder diffractometer, utilizing CuK α radiation with a wavelength of 1.5406 Å. For Fourier transform infrared (FTIR) analysis, a Jasco FTIR 4100 single-beam spectrophotometer

was used in the transmission mode, for detecting the functional groups present in the aqueous extract. The particle size distribution and zeta potential (ZP) were determined using the light scattering technique (SZ-100, Horiba Scientific) on dilution in distilled water at 25 °C. Morphological and elemental characterization analysis was performed using Scanning Electron Microscopy (SEM) by a Quanta 250 FEI SEM system, coupled with EDX analysis.

Antimicrobial test

The antimicrobial activity testing was based on the disk inhibition zone method. The as-prepared TiO₂ NPS were tested in terms of their antimicrobial resistance to gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and gram-positive *Bacillus subtilis* bacteria, respectively. To prepare the culture plates, 20 mL of Muller-Hinton agar was poured onto the plates and spread evenly. The agar surfaces were then buffered with *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* isolates to achieve confluent growth, ensuring accurate results. Sterile disks (6 mm) were utilised to load TiO₂ NPs suspensions at three different concentrations (200, 600 and 1000 µg/mL), dissolved in dimethyl sulfoxide (DMSO). A blank contrast sample was designated as the first filter paper. These disks were then placed onto appropriate disks to determine the minimum inhibitory concentration (MIC).

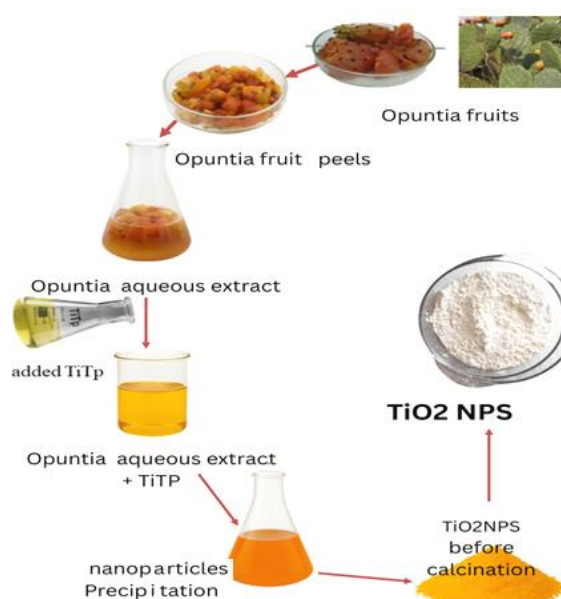


Figure 1: Green synthesis of TiO₂ nanoparticles using a peel extract of *Opuntia ficus indica*

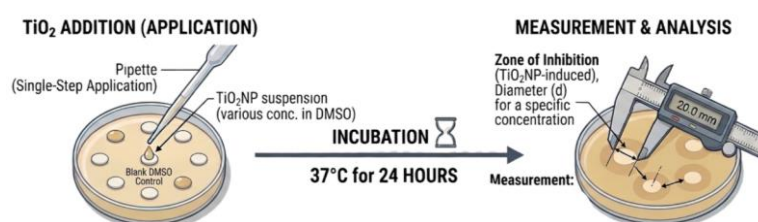


Figure 2: Protocol for the antimicrobial activity testing of TiO₂ NPs

After drying, the disks were positioned evenly spaced on the agar surface to prevent overlapping zones of inhibition. Each disk was gently pressed onto the middle surface and positioned at least 25 mm from the edge. The plates were then incubated at 37 °C for 24 hours in an incubator, where the activity was observed through the presence of inhibition zones surrounding the disks. The diameters of the inhibition zones (measured in millimeters) were recorded upon completion of the incubation period to assess the antibacterial efficacy of the as-prepared NPs (Fig. 2).

RESULTS AND DISCUSSION

The XRD pattern of *Opuntia* peel extract nanoparticles PEO-NPs is shown in Figure 3. The XRD pattern displayed strong diffraction peaks at 25°, 36° and 55° for both preparation procedures, confirming the anatase phase of TiO₂, compared with JCPDS (N°21-1272).³⁰ We noted the absence of

other phases of TiO₂ like rutile or brookite. Comparing the peak intensity observed in the X-ray diffraction (XRD) patterns, it is evident that the TiO₂ synthesized from the extract of cut peels exhibits significantly higher peak intensities, compared to the TiO₂ synthesized from the extract of the crushed peels. The results indicate superior crystallinity in the TiO₂ produced from the cut peels extract. For this reason, all subsequent experiments were conducted using the cut peel extract.

The nanoparticle size was determined using the Scherrer's formula:³¹

$$D = \frac{0.9\lambda}{\beta \cos(\theta)} \quad (1)$$

where D is particle size (nm), λ is wavelength (1.54 Å), β – line broadening at half the maximum intensity in radians, θ is Bragg's angle.

The resulting mesh parameters are $a = b = 3.73 \text{ Å}$, $c = 9.37 \text{ Å}$, $\alpha = \beta = \gamma = 90^\circ$. Volume of cell (10^6 pm^3): 130, 36, and calculated density (g/cm^3): 4.07. Based on the Scherrer formula, the average size of PEO-NPs was found to be 13 nm.

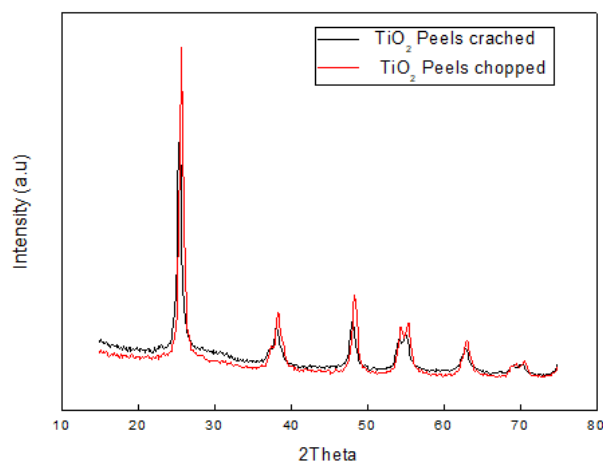


Figure 3: XRD patterns of TiO₂ nanoparticles obtained with extracts of crushed or chopped *Opuntia ficus indica* peels

FTIR analysis

The FTIR spectrum of the aqueous extract from OFI peels was used to discern the functional groups inherent in the extract, which may be responsible for the reduction of TiTp into TiO₂. Notably, the FTIR spectrum, as depicted in Figure 4, revealed distinctive peaks indicative of specific functional groups.

A sharp peak was observed at approximately 3400 cm^{-1} that is characteristic of hydroxyl (–OH) groups, characteristic of lignocellulosic source materials.³² Additionally, a distinct peak at 2073 cm^{-1} was identified, signifying the presence of alkenes, supporting the presence of phenolic compounds in the extract. Moreover, the presence of a peak at 1700 cm^{-1} is indicative of carbonyl (C=O) groups, whereas the peak at 1370 cm^{-1} confirms the presence of aromatic groups.³³ These functional groups are inherent components supposed to act as both reducing and capping agents during the synthesis of nanoparticles. These findings underscore the chemical complexity of the OFI peel extract, highlighting the presence of a variety of functional groups. These functional groups may play an important role in the mechanism behind TiTp reduction to TiO₂ nanoparticles.

UV-Vis analysis

The UV-Vis spectra of the *Opuntia ficus indica* (OFI) aqueous extract, displayed in Figure 5 (a), shows distinctive absorption peaks at roughly 270–290 nm and 320–400 nm. These peaks indicate the presence of flavonoids and polyphenols.²³ The aqueous extract of the fruit peels has been reported to contain more flavonoids, polyphenols, and betacyanin than the pulp, according to earlier research.³⁴⁻³⁵ During the synthesis of TiO₂ NPs, these molecules may have an essential role in reducing Ti ions.³⁶⁻³⁷

The UV-Vis spectrum of the colloidal nanoparticle suspension recorded after the addition of the TiTP precursor, prior to drying, (Fig. 5b) showed a noticeable change in both color and spectral

profile. Following synthesis, the TiO₂ nanoparticle samples exhibited a decrease in the intensity of the aromatic/flavonoid absorption bands and an increase in the optical background extending into the visible region, consistent with the formation of a colloidal dispersion of nanostructures.

The reduction of these bands is explained by the complexation of reducing agents (phenols, flavonoids), which participate in the reduction of Ti⁴⁺ ions, leading to the formation of TiO₂ nanoparticles. Such a mechanism is commonly observed in green synthesis routes. These findings align with the results reported by Ansari and Daneshjou, who used spinach leaf extracts in TiO₂ green synthesis.³⁷

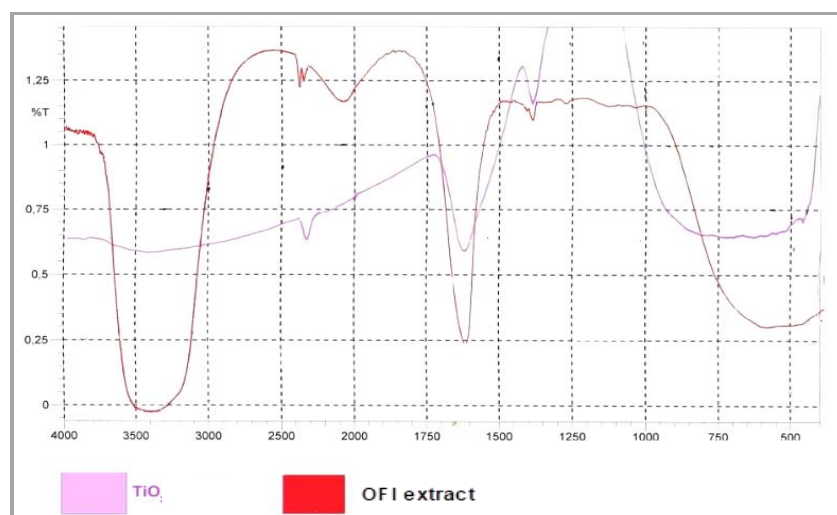


Figure 4: FT-IR spectra of the peel extract of *Opuntia ficus indica* and of TiO₂ NPs synthesized using the extract

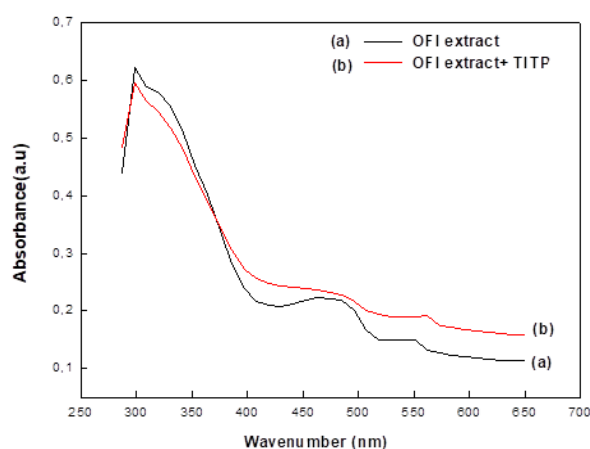


Figure 5: UV-Vis spectra of the peel extract of *Opuntia ficus indica* and of TiO₂ NPs synthesized using the extract

Furthermore, the UV-Vis spectrum presented in Figure 5 illustrates the optical properties of the TiO₂ nanoparticles synthesized in this study. As shown, the as-prepared nanoparticles exhibit an absorption peak at approximately 300 nm. When compared with the UV-Vis spectrum of bulk TiO₂, it becomes evident that the absorption band of the synthesized nanoparticles is shifted toward shorter wavelengths – a blue shift – which is characteristic of nano-sized TiO₂. As particle size decreases, quantum confinement effects arise, leading to the modification of their electronic structure and resulting in a blue shift in the absorption spectrum.³⁸

Particle size distribution and zeta potential analysis

To analyze the particle size distribution, green-synthesized TiO₂ NPs were subjected to dynamic light scattering (DLS) analysis. As shown in Figure 6, the size distribution frequency curve reveals a primary population of nanoparticles centered at approximately of 25 nm, and the calculated Z-average

diameter was found to be 147 nm, with a polydispersity index (PDI) of 0.42. This difference between the size distribution of 25 nm and the Z-average of 147 nm is explained by the mechanism of green synthesis mediated by plant extracts. There occurs a complex capping process by diverse biomolecules (such as polyphenols and proteins) present in the plant aqueous extract, coating the TiO₂ surface in layers of different thicknesses.³⁹ The value of PDI indicates a polydisperse nature, suggesting that while the core particles are fine, they form aggregates in the aqueous medium.

A monodisperse distribution and good stability were confirmed by a PI value of 0.42±0.02. In the literature, a PI<1 with particle sizes <200 nm suggests minimal aggregation and precipitation in colloidal solutions.⁴⁰

The zeta potential analysis shows a value of +46 mV, indicating excellent colloidal stability. This high positive surface charge ensures robust electrostatic repulsion between particles, preventing rapid sedimentation. Typically, a stable colloidal system is confirmed when zeta potentials are above +30 mV or below −30 mV, due to electrostatic repulsion avoiding particle aggregation.⁴¹ The electrophoretic mobility (0.000314 cm²/Vs) confirms the global cationic nature and the colloidal integrity of the system, consistent with stabilized green-synthesized metal oxides.

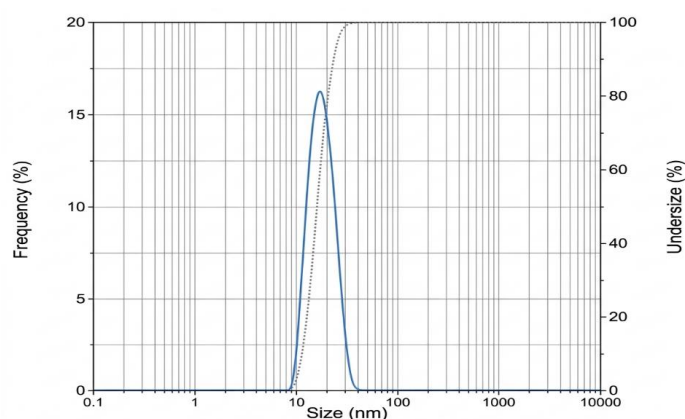


Figure 6: Particle size distribution of OFI green-synthesized TiO₂ NPs

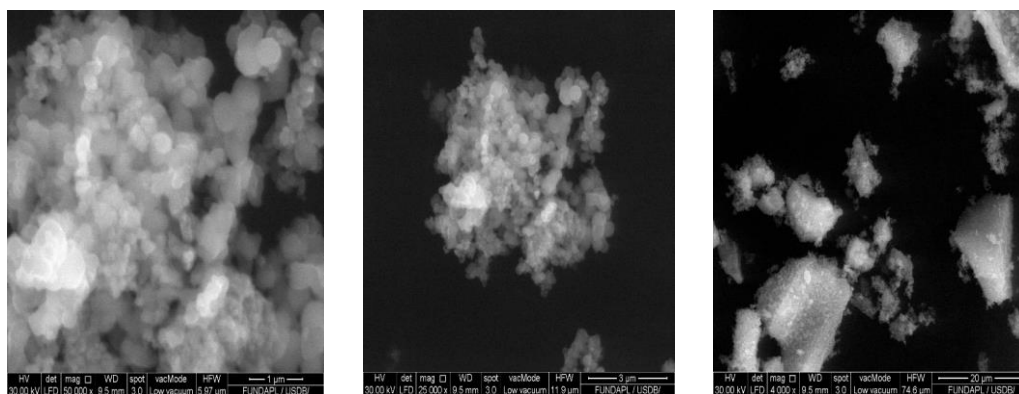


Figure 7: SEM micrographs of TiO₂ NPs obtained using the peel extract of *Opuntia ficus indica*

Scanning electronic microscopy

Spherically shaped nanoparticles with homogeneity, clear borders, and no agglomeration behavior are shown in the SEM images presented in Figure 7. These results are consistent with those of G. Rajakumar *et al.*⁴² for the fabrication of TiO₂ nanoparticles utilizing the leaf extract of *Eclipta prostrata*. The TiO₂ nanoparticles have an average diameter of 120 nm, according to quantitative examination of SEM images. This assessment highlights the nanoparticles' size homogeneity and monodispersity, which are essential for their performance in a variety of applications.

The EDAX spectrum of TiO₂ is represented in Figure 8, which reveals the presence of characteristic element peaks, such as titanium (Ti) peaks at around 4.5 keV and 5 keV, and an oxygen (O) peak at approximately 0.5 keV, confirming the presence of TiO₂.⁴³

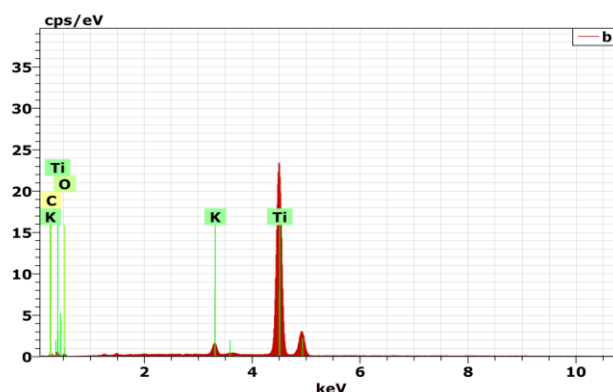


Figure 8: EDX spectrum of TiO₂ nanoparticles obtained using the peel extract of *Opuntia ficus indica*



Figure 9: Antimicrobial activity of TiO₂ nanoparticles against different microorganisms (*Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*)

Antimicrobial activity

The as-prepared TiO₂ nanoparticles demonstrated a pronounced inhibitory effect against bacterial growth. The zone of inhibition test revealed significant disparities in the antibacterial efficacy of TiO₂ NPs across various bacterial strains, indicating their differential susceptibility to nanoparticle-induced inhibition, as shown in Figure 9. Interestingly, the bacterial strains exhibited varying responses to TiO₂ NPs, even among strains belonging to the same gram classification. This variability underscores the complex interplay between nanoparticle properties and bacterial susceptibility mechanisms.⁴⁴

Notably, the zone of inhibition was most extensive for the highest nanoparticle concentration tested (1000 µg/mL), indicating a concentration-dependent effect on bacterial growth inhibition. This observation suggests that higher nanoparticle concentrations confer greater antimicrobial efficacy against the tested bacterial strains.

The enhanced antimicrobial efficacy at higher concentrations (compared to lower concentrations (e.g., 200 µg/mL) also highlights the importance of the incorporation of *Opuntia ficus indica* (OFI) extract in the synthesis of TiO₂ nanoparticles, and potential synergistic effects between TiO₂ nanoparticles and the bioactive compounds present in the OFI extract.

CONCLUSION

Fruit peel extracts represent an economical and sustainable resource for producing nanoparticles with strong antibacterial activity against a wide range of bacterial species. In particular, the *Opuntia ficus indica* (OFI) extract contains a rich variety of bioactive compounds that can be harnessed for nanoparticle synthesis. The use of OFI extract enhances the antibacterial efficacy of TiO₂ nanoparticles, highlighting a synergistic effect between the phytochemicals and the nanomaterial. This synergy underscores the potential of natural extracts in nanotechnology.

The antimicrobial performance of these nanoparticles offers a promising strategy for controlling microbial infections and may find a wide range of applications in environmental remediation, food safety, and healthcare. Moreover, the accessibility and low cost of fruit peels make them an attractive option for large-scale production, paving the way for the commercialization and widespread adoption of this green nanotechnology approach.

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