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Synthesis of Titanium Dioxide Nanoparticles (TiO_2) and Application for Reduction of Bacterial Growth

Mena Akram Ali *, Saba A. Mahdy, Nehia Neama Hussein

Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq

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ABSTRACT

The present study has been dedicated to analyzing the photocatalytic activity and chemical stability of TiO_2 nanoparticles, which in turn, could be employed in environmental and medical applications, such as antimicrobial coatings and cancer therapy. The work was focused on the synthesis of titanium dioxide nanoparticles (TiO_2) using titanium tetraisopropoxide $Ti[OCH(CH_3)_2]_4$ dissolved in isopropyl alcohol, sonication, and hydrothermal method at 140°C. The nanoparticles were afterwards cleaned, centrifuged, and dried by oven at 50°C for 3h, and stored at refrigerator until use. In the characterization of the fabricated nanoparticles, an anatase tetragonal phase with a particle size of around 74 nm was established. XRD analyses (X-ray diffraction) were exploited to learn more about the nanostructure, crystallite dimensions, and crystallographic planes. Spectroscopy of TiO_2 was done using UV-Vis absorbance, and the results were in line with TiO_2 photocatalytic activity which is particularly due to its interaction with light at 385 nm. The antibacterial properties of the nanoparticles were demonstrated in biological evaluations, and they were found to be effective against Proteus *mirabilis*. This may lead to finding out ways of using them for microbial targeting. Besides, the nanoparticles demonstrated concentration-dependent antioxidant properties, which were responsible for the suppression of oxidative stress. Moreover, TiO_2 showed the anti-cancer effects against Human Skin Squamous Cell Carcinoma (HSSCC) concentrating and time-dependent cell death processes. The research, further, studied the role of TiO_2 nanoparticles in the suppression of biofilms, especially against staphylococcal biofilms, but had a limited effect in the species like Pseudomonas *aeruginosa*, suggesting that the strategies should be specific to applications. In general, the TiO_2 nanoparticles synthesized demonstrate a wide range of applications in medicine and environment with their antibacterial, anti-oxidant and anti-cancer properties.

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INTRODUCTION

Titanium dioxide nanoparticles (TiO_2 NPs) have been acknowledged as a useful nanomaterial in environmental applications due to their unique photocatalytic properties and chemical stability [1]. The TiO_2 ability to decompose organic pollutants and inactivate microbial pathogens when irradiated with UV light has been broadly documented, which appears to be an effective path to water and air purification [2]. The efficiency of TiO_2 nanoparticles is often an issue due to the recombination of the photo-generated

* *Corresponding Author Email: as.21.55@grad.uotechnology.edu.iq*

electron-hole pairs and their limited absorption in the visible light spectrum [3]. The most recent achievements in nanotechnology, including TiO_2 nanoparticles, have proved to be effective against these shortcomings. The doping of TiO_2 with non-metals such as nitrogen and carbon has shown to shift the photo catalytic activity into the visible light spectrum, which increases the overall efficiency of the process [4]. Besides, the synthesis of nanostructured titanium dioxide in different morphologies (such as nanotubes and nanowires) has resulted in increased surface area and improved electron transport. This has again resulted in the enhancement of photocatalytic performance [5]. Even though these technologies make progress, the problems with the practical use of nanostructured TiO_2 are still here, and they are especially about the cost-effectiveness, the scalability of synthesis methods, and the long-term impact on the environment [6]. Facing these issues is a must for the full-scale implementation of TiO_2-related technologies in environmental decontamination.

Furthermore, TiO_2 nanoparticles have a wide range of applications in the medical field in addition to their environmental benefits. Accordingly, this type of materials inherently has antimicrobial properties that could be employed in the coating of medical devices and implants to decrease the amount of hospital-acquired infections [7,8]. TiO_2, the photocatalyst, is not only able to produce self-sterilized surfaces, which are favourable for utilization in bleached areas such as the operating rooms, but also TiO_2 is able to produce self-sterilized surfaces [9].

Also, TiO_2 nanoparticle's antioxidant properties have been identified, a research which led to the promotion of oxidative stress related diseases especially cancerous diseases such as [10]. The care by the number of football players is great. We have to treat the minor injuries and the bigger injuries too [11,12]. However, despite the fact that the safety and biocompatibility of ns- TiO_2 nanoparticles remain a challenge for nanoparticle interaction with biological systems, which may impact their toxicological effects [13]. The study aims at examining the crystal structure, antibacterial, antioxidant and anticancer properties of chemically synthesized titanium dioxide (TiO_2) nanoparticles for possible future medical and environmental uses.

MATERIALS AND METHODS

Materials

Titanium Tetra Isopropoxide (TTIP, $C_{12}H_{28}O_4Ti$, 97%), Ethanol (CH_3CH_2OH, 96%) isopropyl alcohol 50 ml, and distilled water was purchased from Merck India. All chemicals and reagents are of analytic grade and used without further purification. Bacterial pathogens, such as *Staph. epidermidies, Staph. haemolyticus* (gram-positive bacteria), *P. mirabilis*, *P. aeruginosa* (gram-negative bacteria) were used to study biological activities.

Synthesis of TiO_2 by hydrothermal method

The production of TiO_2 NPs was somewhat altered from the previously published literature [14]. Initially, 50 milliliters of isopropyl alcohol are mixed continuously for 30 minutes to dissolve 1.6 milliliters of titanium tetra isopropoxide. Finally, to create the dispersion medium, add a few drops of distilled water. The product spent twenty minutes in the ultrasonic bath. The solution was sonicated and then placed in an autoclave set at 140 °C for three hours.After allowing the solution to reach room temperature, the contaminants were eliminated by centrifuging and washing it with deionized water. Whatman No. 1 Filter paper is then used to filter it. The filtered sample was dried three hours at 50°C. The resulting TiO_2 nanoparticles were collected and subjected to further analysis.

Characterization of TiO_2 nanoparticles

The PANanalytical XPERT PRO Diffractometer was used to record the titanium dioxide nanoparticles under investigation's X-ray diffraction pattern. Using a Perkin Elmer spectrophotometer, the FTIR spectra was acquired between 400 and 4000 cm−1. SEM was used to visualize the surface morphology of TiO_2 nanoparticles. The homogeneity and elemental distribution of the chemical under investigation are ascertained using the EDS spectra. Quanta FEG-250 assisted in recording the SEM with the EDS spectrum. A Shimadzu 2700 spectrophotometer was used to record the UV-Visible Diffuse Reflectance Spectrophotometer (DRS) spectrum. The 200–800 nm range was used to record the reflectance spectra. Using the disk diffusion technique, the antibacterial activity of TiO_2 nanoparticles against gram-positive and gram-negative bacteria was investigated.

Antibacterial activity

Mueller-Hinton agar medium was created to examine the impact of titanium dioxide nanoparticles on the development of bacterial isolates. The McFarland tube was used to create and compare the bacterial solution used in this investigation. The plates containing the few selected strains were cultured and left to dry at the open air at room temperature. After properly sterilizing the wells, wells were then punched into agar. For the purpose of this assay, 80µL of different concentrations of three type of TiO_2NPs (20, 40, 60, 80, and 100)µgmL^{-1} for each bacterial plate. The plates were incubated for twenty-four hours at 37 **°C**, and diameter of the cleared zone against the microorganism was measured in millimeters [15,16].

Anticancer Activity

Preparation of Cancer Cell Lines

The cytotoxic effects of TiO_2-NPs, The efficacies of test sub-stances at specified concentrations (Control, 1. 95, 3. 9, 7. 8, 15. 62, 31. 25, 62. 5, 125, 250, 500, 1000 µg/mL) were evaluated on a HSSCC line in passage. The cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Calf Serum (FCS). The cytotoxic impact of the test substance was examined by culturing cells in tissue culture plates (96-Microtiter plates) with a flat bottom. The experiment consisted of three stages: Cells Seeding: After activating and proliferating cancer cell lines for 24 hr, the monolayer growth was treated with Trypsin-Versen solution. Subsequently, 25 mL of RPMI-1640 medium, prepared with serum, was added to each well, adjusting the cell count to 1x104 using a counting chamber. A volume of 100 µl of the cell suspension was distributed into the tissue culture wells, which were then incubated at 37 °C for 24 hr to allow cell attachment to the glass [17].

Preparation of TiO_2-NPs concentrations

Different concentrations of the test substance were prepared using a serum-free tissue culture medium. These concentrations were added to the wells containing adherent cancer cells, and solutions were prepared just before use. Six replicates were used for each treatment. The culture medium in the plates was poured out, designating column 1 as the negative control, to which 200 µl of serum-free culture medium was added. Columns 2 to 12 received increasing concentrations (200 µl per well) of the test substance. The plates were covered, incubated at 37°C, and exposed for different durations (24, 48, 72 hrs).

Minimum Inhibitory Concentration

Microtiter Plate Method

The antibacterial activity of was assessed using minimum inhibitory concentration MIC assays against Gram-negative S.*haemolyticus*, *P.mirabilis*, and *P.aeruginosa* [18] Stated that the MIC was determined on a 96-well microtiter plate using the resazurin assisted microdilution technique in Mueller-Hinton broth (MHB) as follows: Test material, which was: Plant extract *Q.infectoria* , TiO_2 nanoparticle, Preparation of test materials with the final required concentration. 100 µl of broth medium in each well from 1 to 10 were made.

100 µl of diluted test material was transferred to the first well. by transferring 100 µl from the first to the 10th well (the concentrations were 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19 µg/ml) in addition to the controls.

Each well was inoculated with 100 µl of bacterial suspension equivalent to McFarland standard no 0. 5 (1.5 ×10 8 CFU\ml).

The microtiter plate was incubated at 37 C for 24 h. 8- 30 µl of resazurin dye was added to each well (30 µl/well), and incubated for 2 to 4 hrs. for the observation of color change. After completion of the incubation, rows with no color change (blue resazurin color remained unchanged) were scored as above the MIC whereas the last blue well in the row recorded as MIC.

Anti-biofilm

The microtiter plate (MTP) assay is a qualitative technique that uses a microplate reader to determine an agent's effectiveness against biofilm formation. The minimum inhibition concentrations MIC obtained from the previous experiment were used to study the effect of the test materials on the formation or inhibition of biofilm of the studied S.*haemolyticus*, *P.mirabilis*,and *P.aeruginosa* isolates that produce strong biofilm, the test materials were: Plant extraction (*Q.infectoria* , TiO_2NPs.

The same previously mentioned protocol (MTP) was used for the biofilm formation assay. However, 100 µl of test compounds was added. the plate was incubated at 37 °C for 24h. After

that, all wells were washed, stained, and read at 600 nm wavelength using a micro-plate reader. percent of biofilm inhibition was calculated by the equation [19]:

$$\% \text{ Biofilm inhibition} = [(\text{OD Control- OD Sample})/\text{OD Control}] \times 100$$

Antioxidant

The method mentioned in [20] was followed in conducting the antioxidant antioxidant test, using the method (2, 2-diphenyl-1-picryl-hydrazylhydrate DPPH), by adding 0.024 grams of DPPH to 50 milliliters of absolute ethyl alcohol. It is dissolved well by mixing it on a magnetic stirrer without heat, then the volume is supplemented to 100 milliliters with absolute ethyl alcohol to give a final concentration equal to 0.024 mg/ml. Then, half a milliliter of serial concentrations of the test substance (0.0, 25, 50, 100, 150, 200) µg/mL were taken and added to a mixture of DPPH (0.5.) mM and mL (3.3) of absolute ethanol. The amount of color change was measured using The spectrophotometer was at a wavelength of 515 nm during 100 minutes of reaction at room temperature. The plank tube contained (3.3) mL of absolute ethanol and (0.5) mL of the sample, and the control tube contained (3.3) mL of absolute ethanol. And (0.5 mL) of DPPH. The removal percentage was calculated according to the equation shown below.

$$\text{Antioxidant Activity} = 100 - \frac{1 - \text{sample absorbency}}{\text{control absorbency}} \times 100$$

Ascorbic acid or vitamin C at a concentration of 1/1 (1 mg/100 ml of distilled water) is used as a positive control due to its high effectiveness as an antioxidant and is considered a standard material for comparison.

Statistical Analysis

One-way analysis of variance (ANOVA) and subsequent post-hoc tests were conducted using IBM SPSS Statistics for Windows, version 26 (SPSS Inc., Chicago, Illinois, United States). Variables were expressed as mean ± standard deviation (SD). The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

X-Ray Diffraction

Fig. 1, shown, plots intensity against 2Theta angles, revealing crucial information about the crystalline structure of TiO_2. Peaks labeled with Miller indices (such as 110, 101, and 200) correspond to specific crystallographic planes within the material. The X-ray diffraction (XRD) analysis revealed the presence of nano-sized particles in the tetragonal anatase phase of TiO_2. The XRD pattern confirmed a particle size of approximately 74 nm, and the specific surface area was determined to be 19.16 m^2/g. Importantly, the morphology index (MI) derived from the full width at half maximum (FWHM) of the XRD data provided insights into the interrelationship between particle size and specific surface area. The XRD data obtained during chemical synthesis of TiO_2 nanoparticles. Noteworthy observations include specific 2θ angles corresponding to

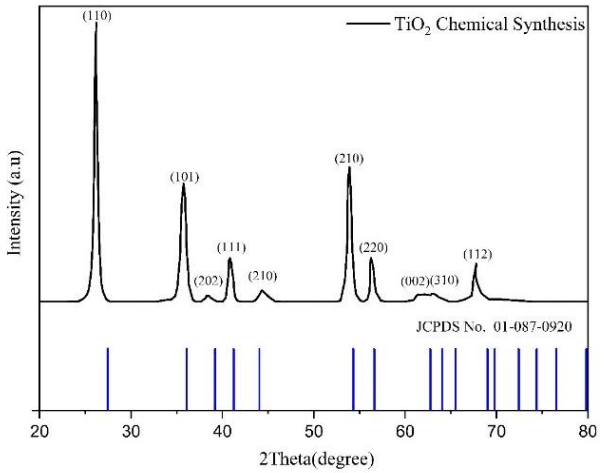

Fig. 1. Crystallographic Insights: X-ray Diffraction Pattern of Chemically Synthesized TiO_2.

crystallographic planes within the TiO_2 lattice. The d-spacing values provide information about interplanar distances, while the grain sizes indicate the dimensions of individual crystallites.

UV-Vis absorbance spectrum of TiO_2

The UV-Vis absorbance spectrum of TiO_2 shown in (Fig. 2) offers critical insights into its optical behavior. Notably, a strong absorbance peak occurs around 385 nm, indicating the material's interaction with light.

Minimum Inhibitory Concentration (MIC)

The MIC values of chemically synthesized titanium dioxide (TiO_2) nanoparticles against four different bacterial species are shown in the. Table 1.

two bacterial isolates i.e. *Staph. epidermidis and Staph. haemolyticus* from the data have the higher MIC value of 25 mg/mL implying that the concentration of TiO_2 nanoparticles must be high enough to inhibit the microbes. To intrinsic resistance of staphylococcal species to the antibacterial nature of these TiO_2 nanoparticles the means that these nanoparticles may become less resistant over time. Also, *P. aeruginosa*, a common and possibly resistant bacterium, also has an CIM value of 25 mg/ml; this, then, confirms the requirement of high TiO_2 levels to ensure repression. Unlike its relative *P. mirabilis* which denotes a MIC value which is higher by an order of 10 animalia just as the animals. At one hundred and fifty-nine DDL/ml, the bacterial cells do not appear to be hampered by TiO_2 nanoparticles as strongly as in the other experiments. Such a low MIC value means that just a small amount of TiO_2 nanoparticles is necessary to control the growth of *P. mirabilis*, which may make TiO_2 nanoparticles an efficient and inexpensive treatment against infections caused by this microorganism.

Antioxidant activity

The study involves testing various concentrations of TiO_2 NPs (0.0 µg/mL, 25 µg/

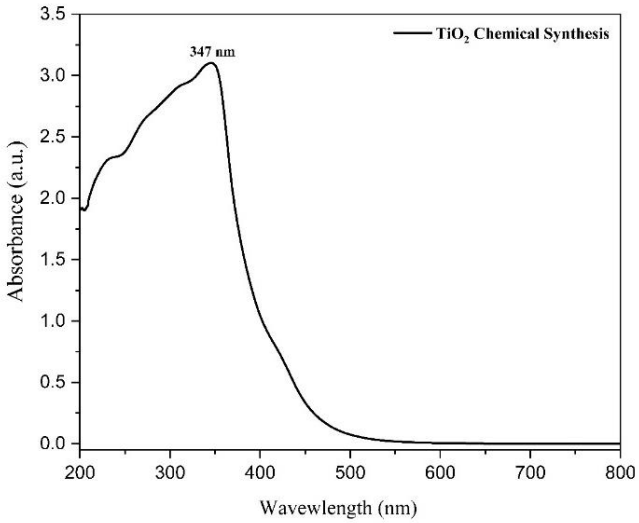

Fig. 2. UV-Vis Absorbance Spectrum of Chemically Synthesized TiO_2.

Table 1. MIC of TiO_2 NPs against some pathogenic bacteria

Microbes name	TiO_2 chemical synthesis mg/ml
S.epidermidis	25
S.haemolyticus	25
P.mirabilis	1.56
P.aeruginosa	25

mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, and 200 µg/mL) to determine their potential antioxidative properties. Table 2 From the data, it is evident that the base absorbance without any TiO_2 (0.0 µg/mL) is 0.822 with 0.0% antioxidant activity, serving as the control. As the concentration of TiO_2 NPs increases, there is a gradual decrease in absorbance and a corresponding increase in antioxidant activity. For instance, at 25 µg/mL, the absorbance decreases slightly to 0.810, yielding a modest antioxidant activity of 1.46%. This trend continues, with the antioxidant activity mildly increasing to 1.82% at 50 µg/mL.

A notable shift in both absorbance and antioxidant activity is observed at higher concentrations. At 100 µg/mL, the absorbance further decreases to 0.799, and the antioxidant activity increases to 2.79%. The antioxidant activity sees a significant increase at 150 µg/mL and 200 µg/mL, where it reaches 9.37% and 10.82%, respectively, accompanied by a more pronounced drop in absorbance to 0.745 and 0.733.

Anticancer activity

The effects of various concentrations of chemically synthesized titanium dioxide nanoparticles (TiO_2 NPs) on Human Skin Squamous Cell Carcinoma (HSSCC) over a period of 24, 48, and 72 hours. The results are displayed as mean values with corresponding standard deviations, and each experiment was conducted with two replicates (N=2). Concentrations of TiO_2 NPs ranged from 0 µg/mL (control) to 1000 µg/mL. Table 3 As observed from the table, the control group (0

Table 2. Impact of TiO_2 NPs Conc. on antioxidant activity

	Conc. µg/mL	Abs	Antioxidant activity %
TiO_2 chemical synthesis	0.0	0.822	0.0
	25	0.810	1.46
	50	0.807	1.82
	100	0.799	2.79
	150	0.745	9.37
	200	0.733	10.82

Table 3. Effects of different concentrations of Chemical synthesis TiO_2 on Human skin Squamous Cell Carcinoma (HSSCC), after 24, 48, and 72 hours of exposure.

Concentration (µg/mL)	Chemical synthesis TiO_2 nps			P value
	24 h (N=2)	48 h (N=2)	72 h (N=2)	
0	1.84± 0.01 a	1.87± 0.02 a	1.88± 0.01 a	0.12
1.95	1.77± 0.01 a	1.72± 0.02 ab	1.70± 0.01 b	0.04
3.9	1.62± 0.01 a	1.58± 0.04 a	1.55± 0.05 a	0.26
7.8	1.51± 0.01 a	1.42± 0.01 b	1.37± 0.01 c	0.01
15.62	1.46± 0.02 a	1.41± 0.01 a	1.31± 0.01 b	0.00
31.25	1.36± 0.01 a	1.32± 0.02 a	1.27± 0.03 a	0.06
62.5	1.31± 0.01 a	1.28± 0.02 a	1.21± 0.01 b	0.02
125	1.14± 0.01 a	1.12± 0.01 ab	1.08± 0.01 b	0.04
250	0.89± 0.01 a	0.82± 0.01 b	0.76± 0.01 c	0.00
500	0.74± 0.02 a	0.67± 0.04 ab	0.61± 0.01 b	0.04
1000	0.00± 0.00	0.00± 0.00	0.00± 0.00	—

Different small letters denote significant differences.
Similar small letters denote non-significant differences.

Table 4. Effectiveness of chemically synthesized TiO_2 NPs on biofilm inhibition in all bacterial isolates.

Isolates	control	Chemical synthesis TiO_2 Nps
s.haemolyticus	100%	100%
s.epidermidis	100%	100%
p.mirabilis	100%	28.9%
p.aeruginosa	100%	61.9%

µg/mL) maintained relatively stable cell viability across all time points, with negligible changes noted. However, increasing concentrations of TiO_2 NPs led to a decrease in cell viability, more prominently as the exposure time increased from 24 to 72 hours. Notably, the viability sharply decreased at higher concentrations, with the most substantial reductions observed at 250 µg/

Table 5. Antibacterial Activity of Chemically Synthesized TiO_2 Nanoparticles at Varying Concentrations Against Different Microorganisms

microorganism	TiO_2 (chemical synthesis) 100%	TiO_2 (chemical synthesis) 80%	TiO_2 (chemical synthesis) 60%	TiO_2 (chemical synthesis) 40%	TiO_2 (chemical synthesis) 20%
Staph.haemolyticus	28.33±1.52	23.66±1.15	20±2	15±2	14.66±1.15
Staph.epidermidis	24±1	22.33±1.52	18.33±1.15	14±2	13±2
P. mirabilis	20±2	18.66±2.08	17.66±1.15	16±2	15.33±1.52
P. aeruginosa	17.66±2.51	17±2	15.33±2.51	10.66±2.51	9.33±1.52

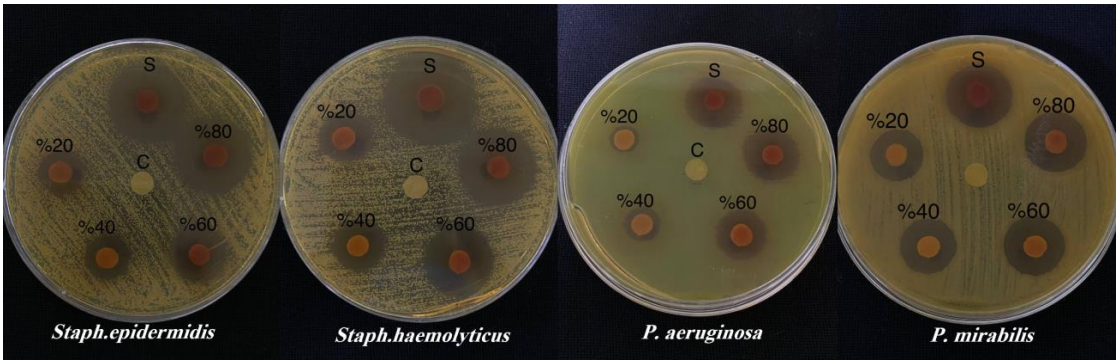

Fig. 3. Antibacterial activity of TiO_2 nanoparticles chemical method.

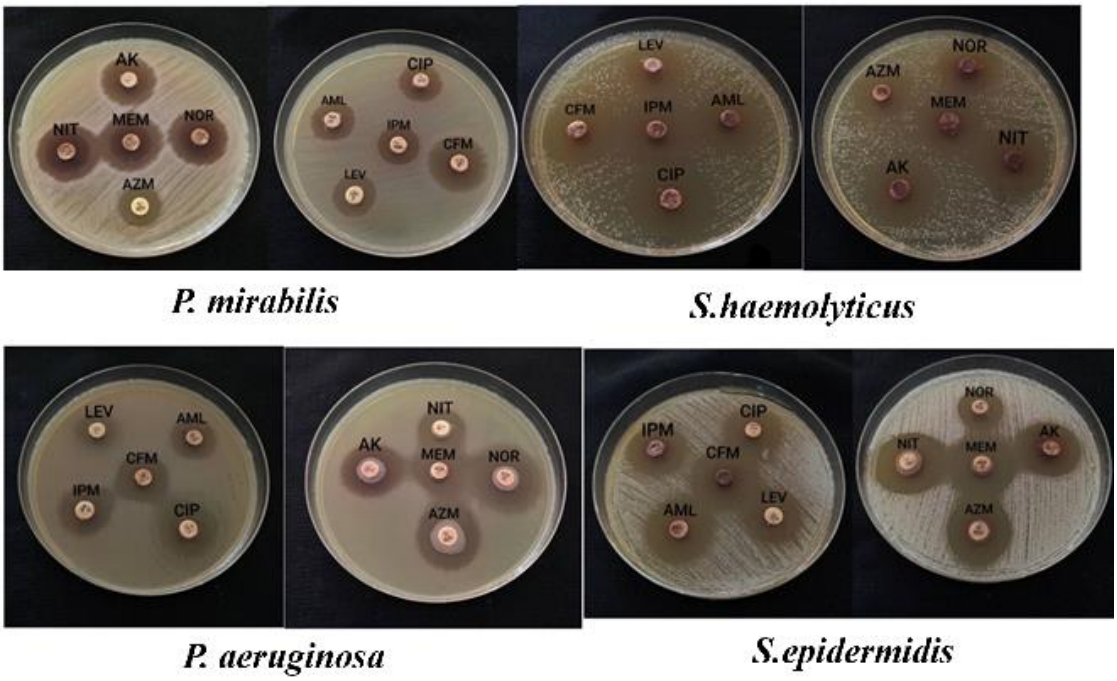

Fig. 4 .Comparison of Antibacterial Efficacy between Titanium Dioxide Nanoparticles with Antibiotics Across Different Bacterial Strains

mL and 500 µg/mL, where cell viability dropped to 0.76±0.01 and 0.61±0.01 at 72 hours, respectively. Complete cell eradication was achieved at the highest concentration of 1000 µg/mL across all time points.

Statistical analysis, indicated by P values, shows significant differences ($P < 0.05$) at many points, particularly notable at concentrations of 7.8 µg/mL and higher. Lower concentrations typically did not result in statistically significant changes compared to the control.

Antibacterial activity

The biofilm inhibition by titanium dioxide nanoparticles (TiO_2 NPs) synthesized chemically, compared against a control setting where no TiO_2 NPs were applied. The results demonstrate a distinct variance in effectiveness based on the type of bacterial isolate. Both *Staph. haemolyticus* and *Staph. epidermidis* showed 100% biofilm inhibition when treated with the TiO_2 nanoparticles, indicating that these nanoparticles are highly effective against the biofilms formed by these particular staphylococcal species. Table 4 However, the effectiveness of TiO_2 NPs varied significantly with other bacterial species. For *P. mirabilis*, the biofilm inhibition was only 28.9%, suggesting a markedly lower susceptibility of *P. mirabilis* biofilms to the antibacterial effects of TiO_2 NPs. In the case of *P. aeruginosa*, the nanoparticles were moderately effective, achieving a 61.9% inhibition of biofilm formation. This data

Table 6. Synergism effect of Titanium Dioxide Nanoparticles and Antibiotics Against Various Bacterial Isolates

MO	*Staph. haemolyticus*			*Staph. epidermidis*			*P.aeruginosa*			*P.mirabilis*		
AB	I.F%	TiO_2 NPs	Ab	I.F%	TiO_2 NPs	Ab	I.F%	TiO_2 NPs	Ab	I.F%	TiO_2 NPs	Ab
LEVO	11.76	19	17	-27.77	13	18	93.33	29	15	6.25-	15	16
IPM	300	24	6	-13.04	20	23	47.05	25	17	0.0	15	15
CIP	-20.68	23	29	-54.54	10	22	45	29	20	183.33	17	6
CFM	266.66	22	6	-75	6	24	-68.42	6	19	250	21	6
AML	33.33	20	15	-27.27	16	22	-64.70	6	17	88.88	17	9
NIT	60	24	15	-44	14	25	-64.70	6	17	90.90	21	11
NOR	-12	22	25	-66.66	6	18	18.18	26	22	250	21	6
AK	-16.66	20	24	-26.08	17	23	5.26	20	19	57.14	22	14
AZM	216.66	19	6	-71.42	6	21	-35	13	20	133.33	14	6
MEM	17.64	20	17	-29.16	17	24	80	36	20	15.78	22	19

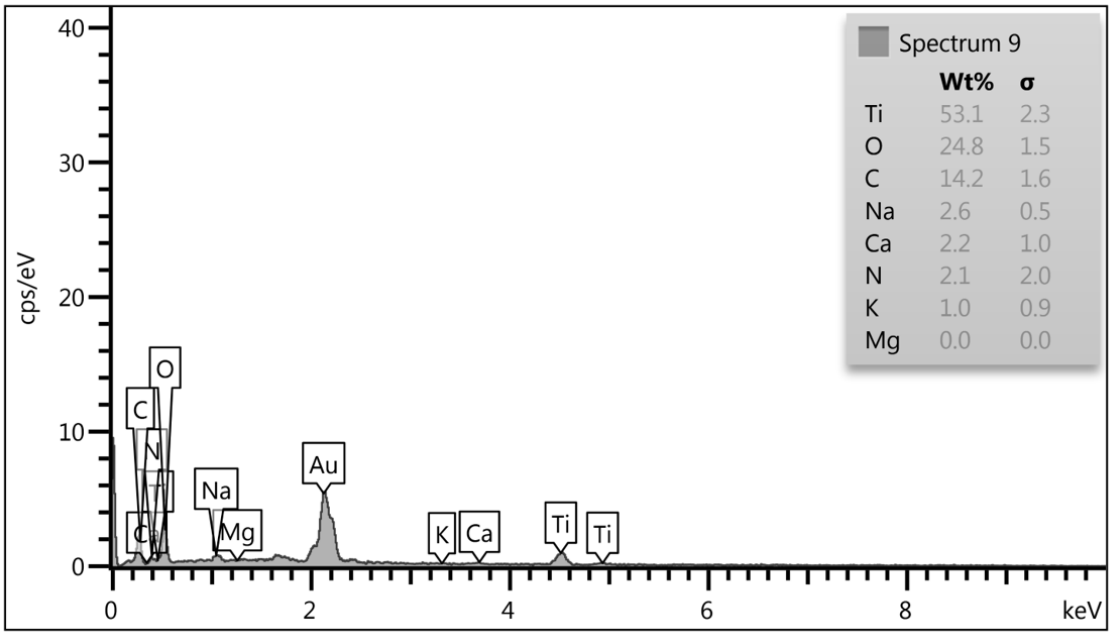

Fig. 5. Elemental dispersive spectrum analysis.

underscores that while TiO_2 nanoparticles can be highly effective against certain bacterial biofilms, their efficacy can differ substantially depending on the specific microorganism. The Antibacterial capacity of TiO_2 nanoparticles (NPs) synthesized chemically at different concentrations against several bacteria. In their studies, they have demonstrated outcomes for *Staph. haemolyticus*, *Staph*. *epidermidis*, *P*. *mirabilis*, and *P*. *aeruginosa*. Bacterial growth is inhibited for each bacterium and the level of inhibition is presented as zones of inhibition in millimeters, with information on three individual measurements and a mean with standard deviation Table 5.

A distinct trend can be observed across the results: the antibacterial activities of the TiO_2 nanoparticles might be substantially lower when their concentration is decreased to 20% from 100%. For example, S. haemolyticus has got more than 25% reduction in zone of inhibition from 28. 33±1. 52 mm at 40% to 14. The maximum concentration of the pollutant is 66±1. 15 mm at 20% concentration. Another bacterium from the Staphylococcus family, *Staph. epidermidis*, shows a similar decrease from 24±1 mm to 13±2 mm with the same concentration gradient Fig. 3.

The results show different degrees of inhibition, with TiO_2 NPs being particularly efficient against *P. mirabilis*, which is one strain against which they exhibit substantial inhibition. On the other hand, depending on the bacterial strain, certain drugs show stronger inhibition than others, indicating inconsistent efficiency. Remarkably, when it comes to preventing bacterial growth, TiO_2 NPs are either more effective than some antibiotics or at least competitive.Table 6 and Fig. 4.

Elemental Dispersive Spectrum(EDS)

Using EDS spectra,the elemental analysis of the chemical compounds was examined. The EDS spectra of bio-mediated TiO_2 NPs are displayed in Fig. 5. The peaks at certain energies on the elemental analysis graph, the height of which is inversely correlated to the element's concentration. Elements like carbon (C), oxygen (O), sodium (Na), and others are identified in the spectrum, with titanium (Ti) displaying the largest weight proportion. The weight percentages and standard deviations for every element are given in the box labelled "Spectrum 9," providing a quantitative examination of the elements.

The elemental data composition and distribution of titanium tetraisopropoxide (TTIP) nanoparticles was obtained through their characterization. Several peaks with varied sizes and intensities were found by the analysis, suggesting that the

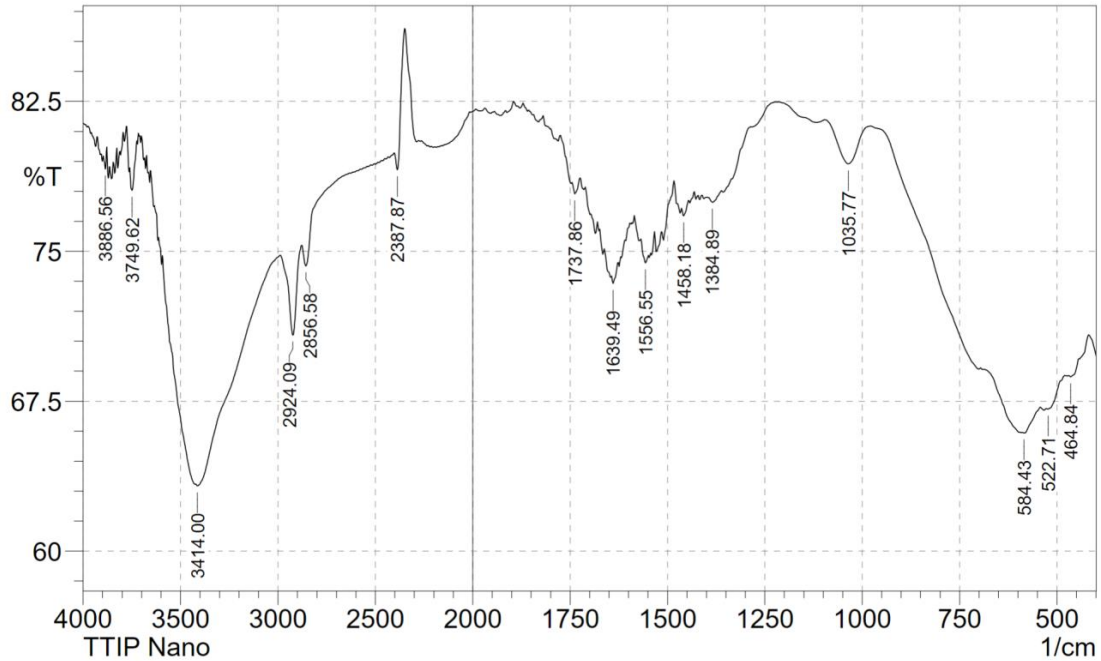

Fig. 6. Elemental composition and distribution of TTIP Nanoparticles based on peak intensity and area analysis

sample included a variety of components. Peak 4 stood out as having the maximum intensity at 1035.77 and a significant corrected area of 0.595, indicating the presence of a large component. Peaks 10 and 12 were prominent in the sample, as seen by their considerable intensities and corrected areas. On the other hand, a few peaks, such peaks 1 and 13, showed reduced corrected areas, suggesting small components or possible problems with baseline correction Fig. 6.

Scanning Electron Microscope

The prepared TiO_2 NPs' SEM images are displayed in (Fig. 7). The surface morphology of chemically synthesized TiO_2 nanoparticles resembles a sphere, while the bio-mediated TiO_2 nanoparticles have a spherical structure as seen in the SEM picture. It was discovered that the average particle size of spherically formed TiO_2 NPs was in the 39 nm range.

The X-ray diffraction results of the study showed that the nanoparticles of TiO_2 were predominantly in the anatase phase, tetragonal, with a calculated particle size of 74 nm and a surface area of 19 m2/g. 16 m^2/g. These results are in agreement with those concluded by Zhang and Banfield (2000) who synthesized anatase TiO_2 nanoparticles with the same structural features and highlighted the role of particles with small sizes in increasing the photocatalytic activity due to the increased surface area [21]. Also, the UV-Vis absorbance spectrum with the blue shift and which relates to band-gap energy changes accord with Zhang et al. (2020) who stated that similar shifts could assist in improving the light absorbing ability of the material [22]. It lost efficacy against Staphylococcus species and P. aeruginosa at MIC of 25 mg/mL while it showed susceptibility against P. mirabilis at very lower concentration of MIC 1. 56 mg/mL.This is in line with those of [23], who also found that different bacteria species exhibited differential resistance to nano- TiO_2, possibly caused by the differences in the cell walls and the metabolic activities. Staphylococcus species shows high resistance level. Hence more concentration of the TiO_2 or combined treatment strategies may be required for better management of the microbes [24].

The TiO_2 nanoparticles' antioxidant activity concentration-dependent increase is remarkable since it increases with the concentration, resulting in a higher antioxidant capacity at higher concentrations. This is the same trend reported by [25], who suggested that the antioxidative stress activity of nanoparticles might be due to their surface properties and ability to remove reactive oxygen species. These characteristics show the possibility of using TiO_2 nanoparticles as protective coatings, as well as in the form of antioxidants in pharmaceutical formulations [26].

The statistically significant dose-dependent toxicity of TiO_2 nanoparticles for HSSCC cell line has been demonstrated, agreeing with the conclusions drawn by the study by Čapek

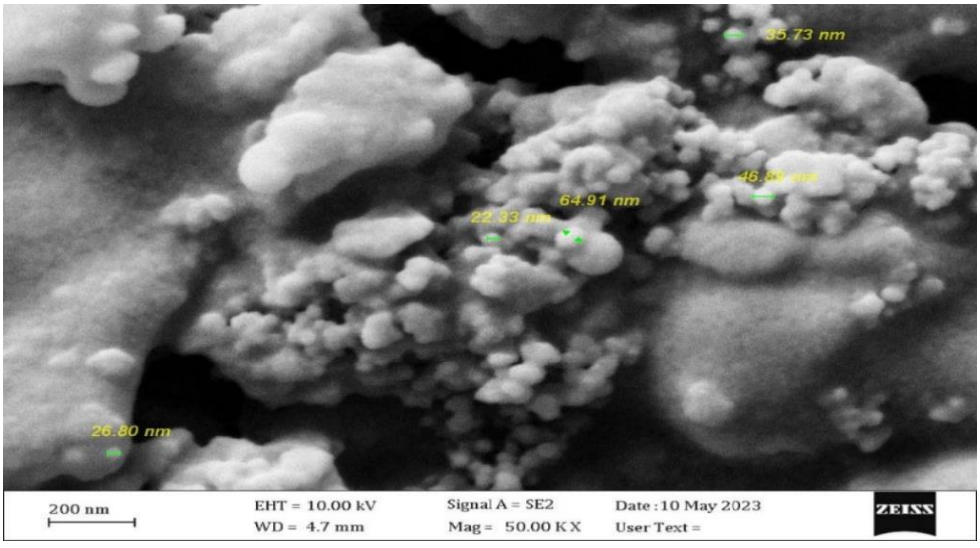

Fig. 7. SEM images of TiO_2 nanoparticles

and Roušar (2021), where such effect has been observed. They have identified and proved the fact that TiO_2 nanoparticles cause apoptosis via the entering of molecular reactive oxygen species and damage of the mitochondrion's functionality [27]. The clearly poisonous effects arise at the higher concentrations (250 µg/mL and 500 µg/mL) signify that TiO_2 nanoparticles can be used as therapeutic medications for cancer treatment, especially because they can discriminatingly then target and destroy cancer cells at the required concentration [28].

The inhibition of the TiO_2 nanoparticles was strain specific with differences such as 100% in *S. haemolyticus* and *S. epidermidis* to 80% in MRSA and *P. aeruginosa.* The fact that the nanoparticle chemistry can act directly on the bacterial cells, similar to what is demonstrated in the study of Jin *et al*. [29], that it may be dependent on its surface chemistry and size is very exciting. This sequential interaction will define the potency of the molecule to isolate biofilm growth and inhibit cell adhesion These criteria are supported by the low suppression rates of *P.mirabilis and P.aeruginosa*, bacteria complicating from the biofilms and becoming more resistant to antibacterial agents due to their structural features [30].

The anitibacterial activity of TiO_2 nanoparticles changes with varying concentrations and strains of bacteria in this study. Such information could provide the basis of optimizing the dosage, which in turn, could further enhance the susceptibility of bacteria. Such an effect matches the scholars' opinion by El Sayed *et al*. They said that nanoparticles' quantity is of utmost significance which provides the required effects, yet does not develop resistance [31].

CONCLUSION

TiO_2 nanoparticles that are used in chemical synthesis to manufacture products are bactericidal, have bacterial isolates such as Proteus *mirabilis*, eukaryotic biofilm cells in their surroundings, hence are clear of any kind of disruption and can be considered to be a remarkably promising development of antimicrobial coating. Additionally, nanomedicine could solve skincare and cancer treatment where it would function in a similar manner of adjusting the particular nanoparticle according to the oxidant stress adjustment. As opposed to that, Visualizing Human Skin Squamous Cell Carcinoma which is as a result of the exposure UV-B radiation the means of how exactly the UV wavelengths' type of radiation can be used on the cancer treatment. Such a treatment is fair because these radiation wavelengths are going to damage only cancer tissues.

CONFLICT OF INTEREST

The authors declare that there is no conflict of intere sts regarding the publication of this manuscript.

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