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Study the effect of adding (TiO_2) nanoparticles prepared using Leaf Extract of (Raphanus sativus L) on the biological activity of some antibiotics

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Abstract

The increased use of antibiotics has led to the development of resistance in many pathogens, representing a major challenge for the treatment of infections. In this study, we explored the bioavailability of selected antibiotics in the presence of nanoscale TiO_2 particles prepared from plant extracts. The aim was to determine whether the addition of these nanoparticles could improve the efficacy of existing antibiotics. TiO_2 particles are synthesized from plant extracts, making them ecologically sustainable and biocompatible. These nanoparticles were characterized by various techniques,TiO_2 nanoparticles was determinedWe got the absorbance at 227 nmwhich is the same as the energy bandgap of commercial TiO_2, such as transmission electron microscopy and X-ray diffractionsample showed a dominant peak at $2\theta = 26.50^\circ$ and 47° which proved the (101) crystallographic plane of anatase and (105) rutile form of TiO_2 NPs, to assess their size, shape, and crystal structure Images from Field Emission Electron Microscopy (FE-SEM) verified the nanoparticles' spherical shape and relatively homogenous size distribution, the EDX spectra clearly demonstrated that the TiO_2NPs manufacturing technique is exceedingly safe and pure as no harmful chemicals were utilized. These findings demonstrated that the production of TiO_2 nanoparticles was successful, we assessed the bioavailability of some commonly used antibiotics in the presence of these nanoparticles. Bacterial growth inhibition tests were carried out using bacterial strains sensitive to the selected antibiotic. The results showed that antibiotic efficacy was significantly enhanced in the presence of TiO_2 nanoparticles dye degradation and bacteriological tests will be performed successively, effectiveness and indicating a promising medicinal performance.

Keywords: Titanium dioxide TiO_2, Biosynthesis, *Raphanus Sativus L*, Nanoparticles,antibacterial activity.

1. Introduction

Nanotechnology is the science concerned with the study of the design, characterization, production, and applications of shapes, devices, and systems that are controlled by shape and size at the nanometer level [1]. Nanoscience deals with the science of materials and technologies with scaled volumes(1-100nm). Through a presentation by Richard Feynman at the meeting of the American Physical Society in 1959 at the California Institute of Technology in his speech entitled (There is Plenty of Room at the Bottom)[2]., Feynman excelled in his lecture as he gave a perception of the possibility of changing the properties of any material and maximizing its features [3]. The term “nanotechnology” was first used by Norio Taniguchi in 1974, although it was not widely known [4]. A lot of studies on nanoscience and nanotechnology have been done all over the world [5]. This led to the discovery of different ways to synthesize new nanomaterials that have different physical and chemical properties than their large non-nano particles [6-7-8]. In fact, these nanomaterials exhibit interesting unique properties Essentially, it opened the door to new generation technologies in electronics, computers, and optics. Biotechnology, Medical Imaging, Medicine, Structural Materials, Aerospace, Energy [9-10-11].

Titanium dioxide (TiO_2) is a white powder extensively used to decontaminate water and food, ensuring environmental and industrial safety, while also serving to protect the skin against harmful radiation [12–16]. To better understand how this metal oxide functions, it is relevant to describe its polymorphic crystal structure [11-13].

The end of the 20th century was marked by scientific and technological developments major ones whose consequences have only recently become apparent. Three factors are the origin of this evolution: a good understanding of the properties of matter on a scale atomic, advances based on molecular methods by which organisms can also operate, and the development of information processes. These factors have led to an increasing unification at the nanoscale of various disciplines sciences, notably physics, chemistry, and biology. Nanotechnology is an emerging field of research and technology that involves manufacturing and engineering of materials, structures, and systems at the nanoscale (at least one dimension) [17].The origins of this movement generally date back to the end of 1959, with the speech founder of Richard Feynman "There is plenty of room at the bottom" [18]during the annual meeting of the American (Physical Society at the California Institute of Technology). The term "nanotechnology" was first coined in 1974 by Norio Taniguchi, professor at the Tokyo University of Science [19]. Nanotechnology is considered an enabling technology through which existing materials, almost all artificial materials and systems, can acquire different properties that make them suitable for many new applications [20].

Prologue Considering the importance of electronic, optical and photo propertiesTiO_2 catalysts, pure and doped, and their uses in various applications. In what follows, we review various studies carried out in this regard, focusing on the nano powder/nanoparticle properties of titanium and its dioxides. Titanium (Ti) is the fourth most abundant metallic structure in the earth's crust (~0.5%) [21]. Other estimates suggest that titanium is the ninth most abundant element in the earth's crust, with an average content of approximately 0.63% [22]. The combination of these physical and chemical properties (corrosion resistance, low bio reactivity) and mechanical makes them attractive for numerous applications in aerospace, medical, industrial, and other fields [21]. The first industrial production of TiO_2began in Norway in 1918. United States and Germany [23].

This study aims to present the different methods of manufacturing nanoparticles, as well as analysis techniques and potential applications in different fields such as medicine, electronics, and the environment. Nanoparticles have properties unique which give them considerable advantages in these areas, but their production and their use also raise safety and impact concerns environmental. We will explore the different methods of

Manufacturing nanoparticles, such as chemical synthesis, lithography, the gas phase method, and many others, as well as analytical techniques such as electron microscopy and mass spectroscopy. We will also examine the potential applications of nanoparticles in areas such as targeteddrug therapy, environmental sensors, and production of green energy. [24-25].

1. Presentation of plants

Radish, Raphanus sativus L., is a biennial vegetable plant of the family of Brassicaceae cultivated for a very long time for their fleshy hypocotyls and consumed raw as a vegetable, the edible part occurring in various taproot forms (long, round). With red, white, black, or pinkish white flesh (the species used in our work) is the swollen underground part of the stem, just above the root. The radish (***Raphanus sativus L***.) may be a descendant of these type radishes (Raphanus rostratus or Raphanus segetum), plants of a very ancient culture [26]**.** Radish (***Raphanus sativus L***.) is a spherical, fleshy, and swollen root with white skin pink. Soft or firm fabric. Hairy leaves directly above, large, with stems deep, with long toothed lobes, the ends of which are larger than the other leaves and very rough. Radishes develop branched stems, 4 to 8 decimeters high, with flowers white, quite small, with four erect petals on the stem, and produce short, swollen in the shape of mung beans (pods). The interior is spongy and contains seeds flattened reds, each housed in a single, rounded depression [26]**.**

Figure 1: TheRadish flowers (*Raphanus sativus L*)

2. Description

Radish (***Raphanus sativus L.)*** belongs to the cruciferous family like cabbage. Today the family is known as the cruciferous family, a name derived from Brassica, meaning cabbage. This means that its flowers have four petals arranged in a cross, which is a typical shape of Brassicaceae (**Figure1**) [27]. The radish has a taproot, stocky, variable in size, moderately vigorous, spherical, with swollen flesh and a pinkish-white rind; soft tissue or morphology (**Figure2**) [28].Radish seedlings are grown in rosettes and elongated to produce peduncles that can reach a height of 50 to 100 cm [28]The radish flowers are white, very small, with 4 erect petals, bearing short and swollen mung bean-shaped fruits (siliques), oblong- lanceolate, swollen at the base, without joints, The interior is spongy and contains flat red seeds, each located in a specific circular depression [29].It is a species that resists early frosts but is damaged by normal winter temperatures [28].

Figure 2: Plant of *Raphanus sativus L*

3. Experimental

3.1 Materials

The Titanium trichloride ($TiCl_3$). with a purity of 90% were attained from Sigma Aldrich company. And you get radish (Raphanus sativus L) in the southeastern oases of Algeria, in

During all stages of preparation, we used double distilled water to wash the Leaf Raphanus sativus L, prepare leaf extracts, and prepare chemical solutions. The formation of TiO_2NPs was mainly diagnosed with JASCO UV spectrometer. The vital functional groups in TiO_2NPs were determined by SHIMADZU FTIR spectrometer. The crystalline properties of TiO_2NPs were confirmed by PROTO XRD BENCHTOP X-ray POWDER diffractometer. The purity of the product was confirmed, and the morphological characteristics were examined in terms of size and shape by an energy dispersive X-ray analyzer (EDX) with SEM equipment a Zeiss SmartEDX type.

3.2 Preparation of aqueous Leaf *Raphanus sativus L* extracts

To prepare the extracts, we washed radish leaves well with tap water to remove any dust and dirt particles, then washed them again with deionized water. It was dried in a well- ventilated room for a week. They were ground to a fine powder **(Figure 3).**and prepared at a concentration of 10% (10 g in 100 ml of double distilled water) and shaken for 2 h at room temperature. The extract was placed in a water bath at 80°C for 45 minutes. The resulting extract was then filtered. The resulting extract was then filtered, and the radish leaf extract was allowed to cool at room temperature and stored at 4°C for later use.

Figure 3: Dry the plant leaves and grind them

3.4 Green Synthesis of TiO_2 NPs

In order to prepare the nanoparticles, 10g of electrolyte $TiCl_3$ was dissolved in 200ml of distilled water. Each solution was stirred using a magnetic stirrer at a temperature of 60°C to obtain aqueous solution homogeneous. A volume of 200 ml of the plant extract is added dropwise to a volume equal to each solution of $TiCl_3$. The mixture was maintained at a temperature between 70 and 80°C for one day with magnetic stirring keep on going. A change in color of the solution to yellow for TiCl3 was observed. These were centrifuged at 15,000 rpm for 20 minutes The pellets were washed several times with distilled water to remove impurities then dried at 90^0C for an hour to remove the water. Finally, a heat treatment was carried out at 550°C for 4 hours (Calcination)in oven a type Nabiertherm(MORE THAN HEAT 30-3000 °C) to obtain powder from TiO_2NPs. Thepowder obtained was characterized by physicochemical methods such as UV visible, IR, DRX, SEM. These steps are summarized in the flowchart presented in **(Figure 4).**

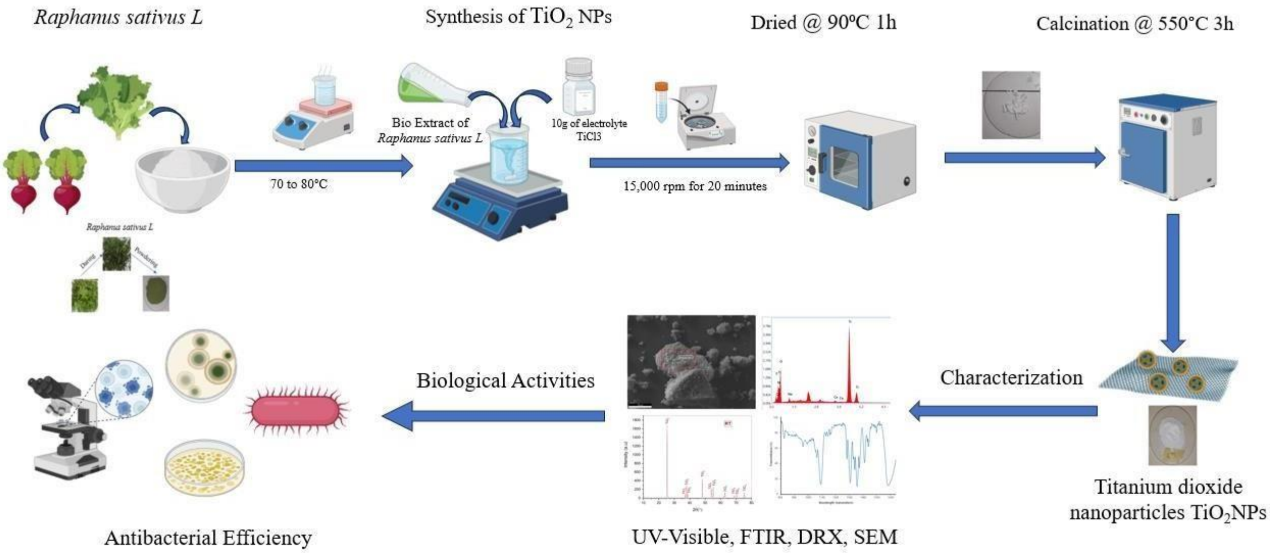


Figure 4:Diagram illustrating the protocol for preparing TiO_2NPs by leaf extract of (Raphanus Sativus L)

4. Mechanism of green synthesis of nanoparticles by leaf extract of plant.

Various plant metabolites, including terpenoids, polyphenols, polysaccharides, alkaloids, phenolic acids, and proteins, play an important role in the biological reduction of metal ions, leading to the production of nanoparticles**.** [33].

In general, the mechanism of green synthesis of metals nanoparticles using plant extracts involves three major phases: activation phase, growth phase, and termination phase**(Figure.5).**

1) The activation phase is the basic step in which metal ions are recovered from their salts by the action of plant metabolites, biomolecules with reductive capabilities. In addition, metal ions change from a mono- or divalent oxidation state to a zero-valence state and transform into nuclei of reduced metal atoms [34].
2) growth phase, which refers to the spontaneous coalescence of nanoparticles in which neighboring small nanoparticles spontaneously combine into larger particles, which is accompanied by an increase in the thermodynamic stability of the nanoparticles [35].
3) The last step of the synthesis process is the termination phase during which the nanoparticles finally reach their maximum possible activity, and this process is affected by the strength and ability of the plant extract to stabilize the metal nanoparticles **[**36].

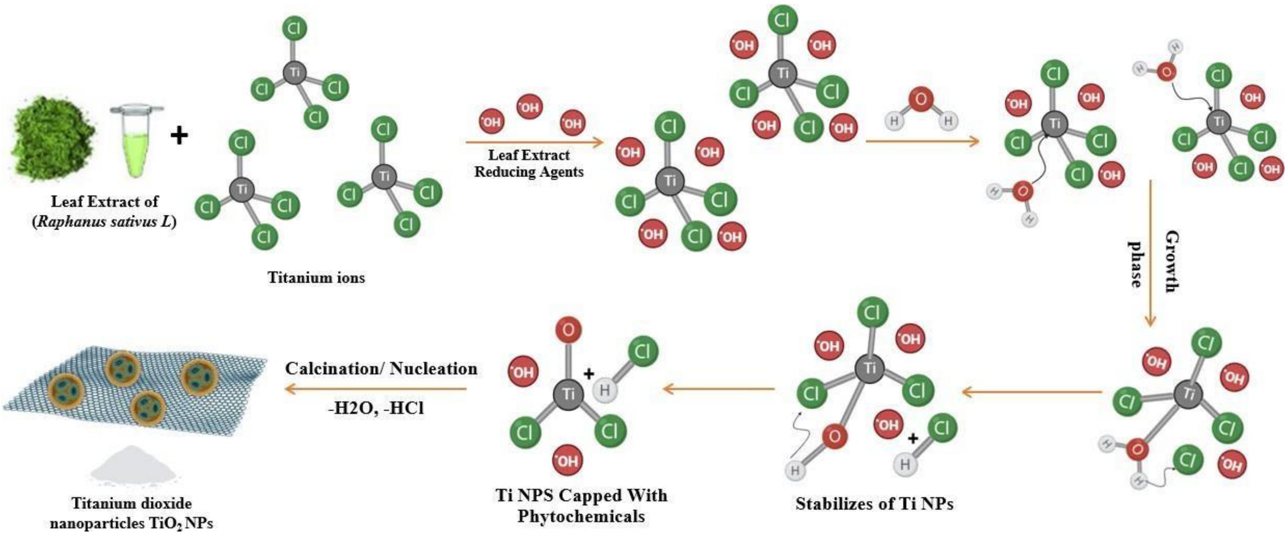
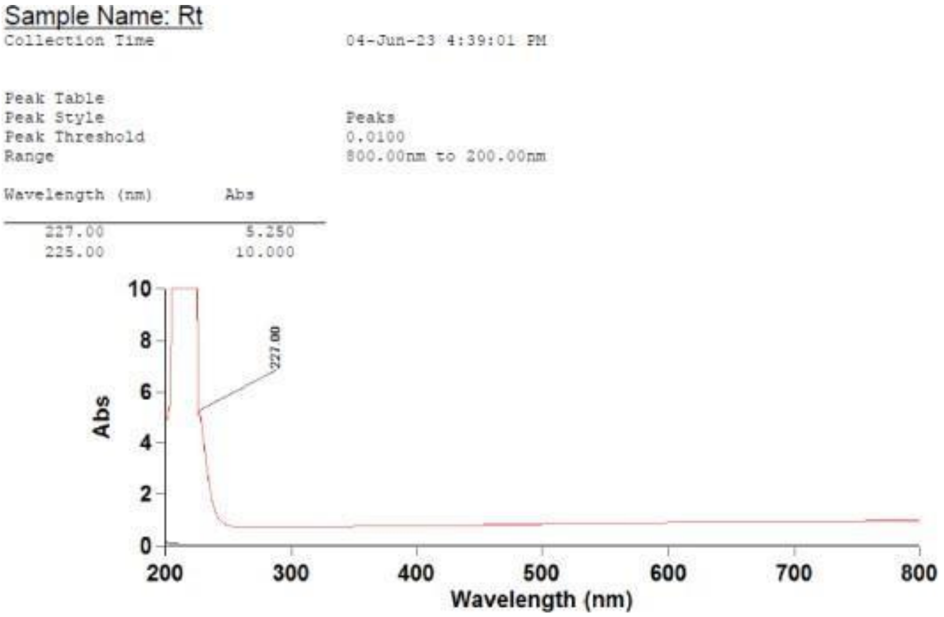


Figure5:Mechanism of plant mediated synthesis of TiO_2 nanoparticles(TiO_2NPs)

5. Results and Discussion

5.1 Diagnosis of TiO_2-NPS by UV-Vis

TiO_2 exists in three crystal structures: rutile, anatase, and brookite. Photocatalytic investigations have typically focused on rutile and anatase phases [37]. Rutile is the TiO_2 phase with the most thermodynamically stable crystal structure while anatase is metastable.[38]. Brookite, on the other hand, is extremely uncommon and unstable. Although the rutile phase has a smaller bandgap (3.0 eV) than the anatase phase (3.2 eV), this phase has higher photocatalytic activity due to the lower electron-hole recombination rate in the anatase crystal structure [39-40]. Anatase and rutile, on the other hand, have been found in certain investigations [41-42-43]. There is some disagreement regarding whether the anatase structure has a larger photon absorption capacity and as such higher activity because of the direct or indirect nature of the bandgap. Anatase possesses a metastable crystal structure, although it only exists in tiny crystal sizes. Conversion from anatase to rutile phase is found when crystal size increases[44-45]. The characteristics that substantially impact the bandgap of the anatase and rutile phases include the synthesis conditions of these catalysts, the structure of the precursor utilized, the oxygen vacancy number, impurities, crystal size, and type of electronic transition [46-50], **(Figure 6)** shows the UV analysis results for TiO_2, the results revealed that absorption peak of the TiO2 NPs dissolved in water is centered at the wavelength of 227 nm which is the characteristic peak of TiO_2. This value (227 nm) being a small can far from that reported in the literature which shows an absorption peak centered at 280 nm. These differences can be attributed to the sensitivity of the UV spectrum to many factors such as the shape, size, and agglomeration of particles [51], It has been concluded from the UV absorbance spectra that TiO2 reacts with intense UV light and instantly act as a photo catalyst through its hydroxyl radical causing breaking of strong covalent bonds [52].



Sample Name: Rt

Collection Time 04-Jun-23 4:39:01 PM

Peak Table

Peak Style Peaks

Peak Threshold 0.0100

Range 800.00nm to 200.00nm

Wavelength (nm)	Abs
227.00	5.250
225.00	10.000

Figure6: UV-Visible spectra of TiO_2 nano particles (TiO_2NPs)

5.2 *Diagnosis of TiO_2-NPs by* Infrared spectroscopy FTIR

Functional groups of a chemicals are determined using Fourier Transform Infrared Spectroscopy (FTIR). At temperatures over zero degrees Kelvin spectrophotometer equipped with an ATR accessory with a resolution of 4 cm-1 in the range of 400-4000 cm-1., objects produce infrared radiation. When infrared radiation hits a substance, it is absorbed and causes chemical bonds to vibrate in the substance [53]. The FTIR spectra of the samples **(Figure7)**Contain bands The infrared spectrum of the base TiO_2 NPs showed a broad band in the range 400-480 cm-1 which is characteristic of Ti-O vibration, which confirms the formation of the metal-Oxygen band [54] and Ti-OH vibration at 1710 cm-1, the peaks at 3210 to 4010 cm-1 in the spectra are due to the stretching and bending vibration of the –OH group.

Agilent Technologies

Sample ID:RT
Sample Scans:16
Background Scans:8
Resolution:16
System Status:Good
Method Name:kennnza
User:UNIV
Date/Time:05/14/2023 9:40:49 AM
Range:4000 - 400
Apodization:Happ-Genzel
File Location:C:\Users\Public\Documents\Agilent\MicroLab\Results\\RT_2023-05-14T09-40-49.a2r

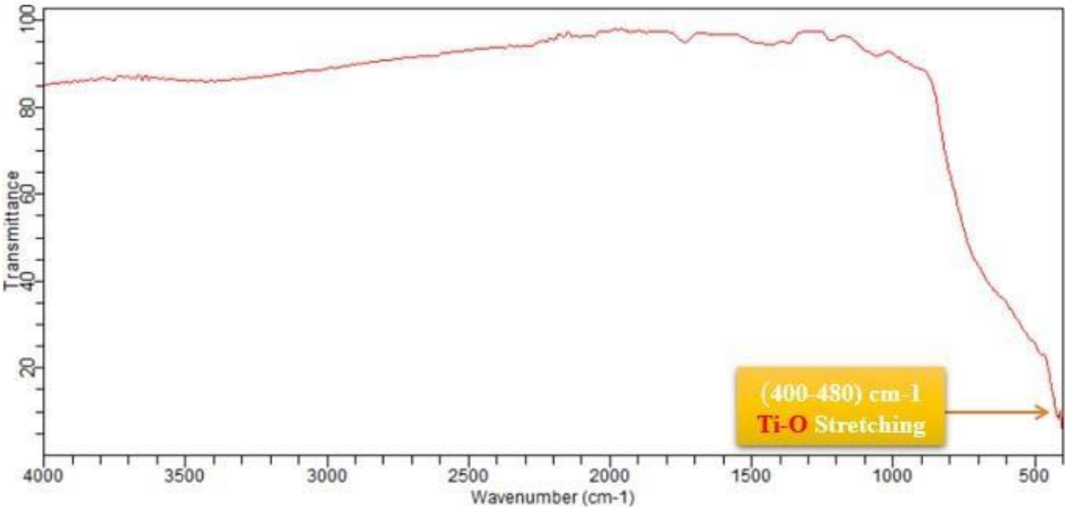
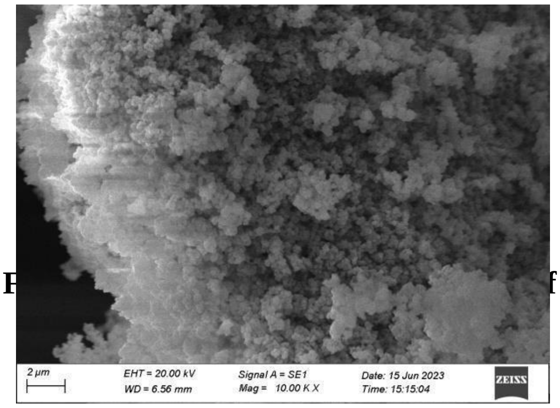


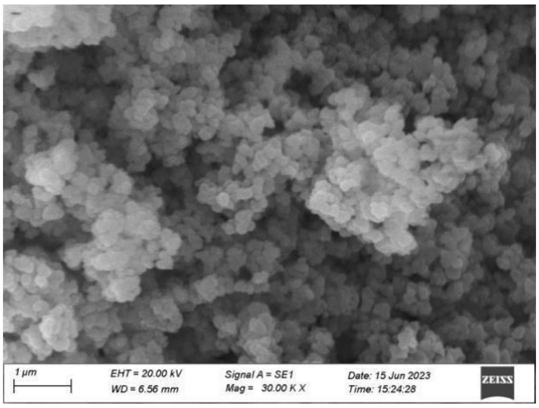
Figure7 : FTIRSpectra of TiO_2 nanostructures in the cm^{-1} region

5.3 *Diagnosis of TiO_2-NPS by Emission Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX)*

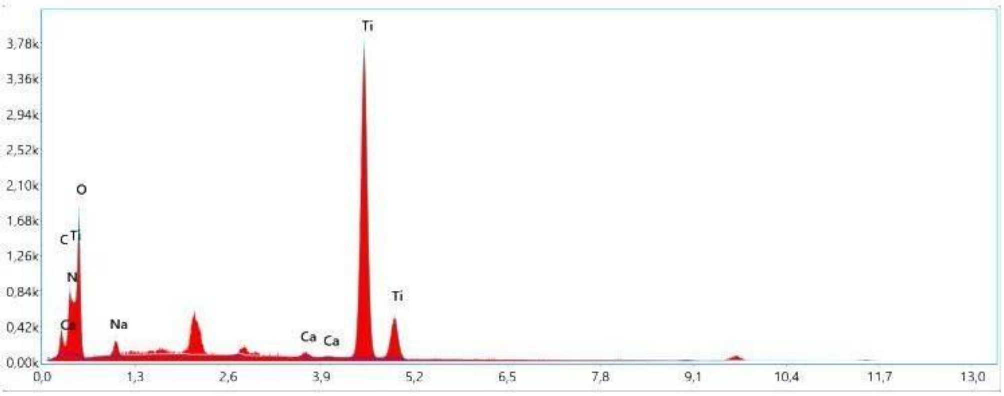
In order to study the morphology of Titanium Dioxide Nanoparticles (TiO_2 NPs), the topographic images, crystal size and shape of the resulting powder are determined using The SEM is derived from scanning electron microscope which we used to analyze the structure, morphology, and grain size of Titanium Dioxide Nanoparticles. The instrument was accelerated at about 20KV and scanned the sample at 6. 56mm.The sample was dispersed in isopropyl alcohol and scanned with different magnifications at 10.00 KX2 µm and 30.00 KX 1 µm. SEM images as given below tell us the grain size for pure titanium dioxide that is in the range from 20-50 nm. (**Figures 8)** show SEM images of two distinct forms of TiO_2 NPs are gathered in rectangular shapes with smaller NPs situated between each two rectangular in a spherical form are given below.



F **f the**



The EDX technique to identify the elements for weight atomic percentage to contents of each materials present by using Field Emission Scanning Electron Microscope (FESEM), The microstructure of the TiO_2NPs was obtained in (**Figure.9**).at low magnifi cation of 10.00KX with a bar scale of 2 µm and 30.00KX with a bar scale of 1 µm which both confirms the presence of pure Titanium with maiger amounts of other elements , the TiO_2 distribution seems to have more enclosures aggregation. A high surface area is attainable with the configuration of the TiO_2 enclosure, The dispersion of nanoparticles on the surface was even when the enclosures area was covered by a large portion area at a magnifi cation of 10.00KX with a bar size of2 µm& 1µm at 30.00 KX . A creation of H+ ions may occur due to the presence of excess H_2O molecules. Van der Waals forces between TiO_2 molecules are lowered through these ions. The results also reveal that the size of EDX spectra clearly demonstrated that the TiO_2NPs manufacturing technique is exceedingly safe and pure as no harmful chemicals were utilized. It is worth noting that, when compared to conventional methods for synthesis of TiO_2 NPs, the proposed approach based on leaf extract of ***(Raphanus SativusL***) is extremely safe and does not require any toxic chemical or complicated synthesis procedures.



Résultats quantitatifs intelligents

Elément	% de masse	% atomique	Intensité totale	Erreur %	Kratio	Z	A	F
C K	3.62	7.00	17.14	11.41	0,0162	1.1532	0.3882	1.0000
N K	2.19	3.63	14.36	13.01	0,0111	1.1285	0.4492	1.0000
O K	43.38	63.02	111.34	11.05	0,0571	1.1067	0.1190	1.0000
NaK	3.12	3.15	16.62	12.89	0,0081	1.0079	0.2566	1.0009
CaK	0.59	0.34	7.39	20.87	0,0060	0.9569	0.9950	1.0711
TiK	47.10	22.85	477.11	1.83	0,4142	0.8680	1.0095	1.0040

Figure. 9 : **EDXMorphologies &Spectrum Analysis of the Synthesized TiO_2 NPs**

5.4 Diagnosis of TiO_2-NPs by XRD

The structure and size of TiO_2particles were studied by XRD technique using an X-ray diffractometer in the 2θ angle range. Figure 10 shows the XRD patterns of TiO_2 NPs synthesized from Radish leaf extract with titanium dioxide, It can be seen that the diffraction peaks are displayed throughout the sample spectrum. There are different crystal planes such as (101), (103), (004), (200), (105), (211), (204), (116), (220), (212) and (215)

reflections,
respectively and confirmed the nanocrystalline nature of the synthesized particles. The XRD

sample showed a dominant peak at 2θ = 26.50˚ and 47˚ which proved the (101) crystallographic plane of anatase and (105) rutile form of TiO_2 NPs (**Figure. 10**). [55].

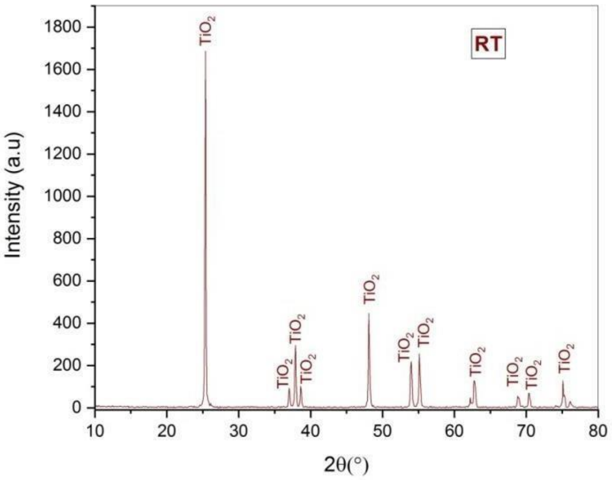
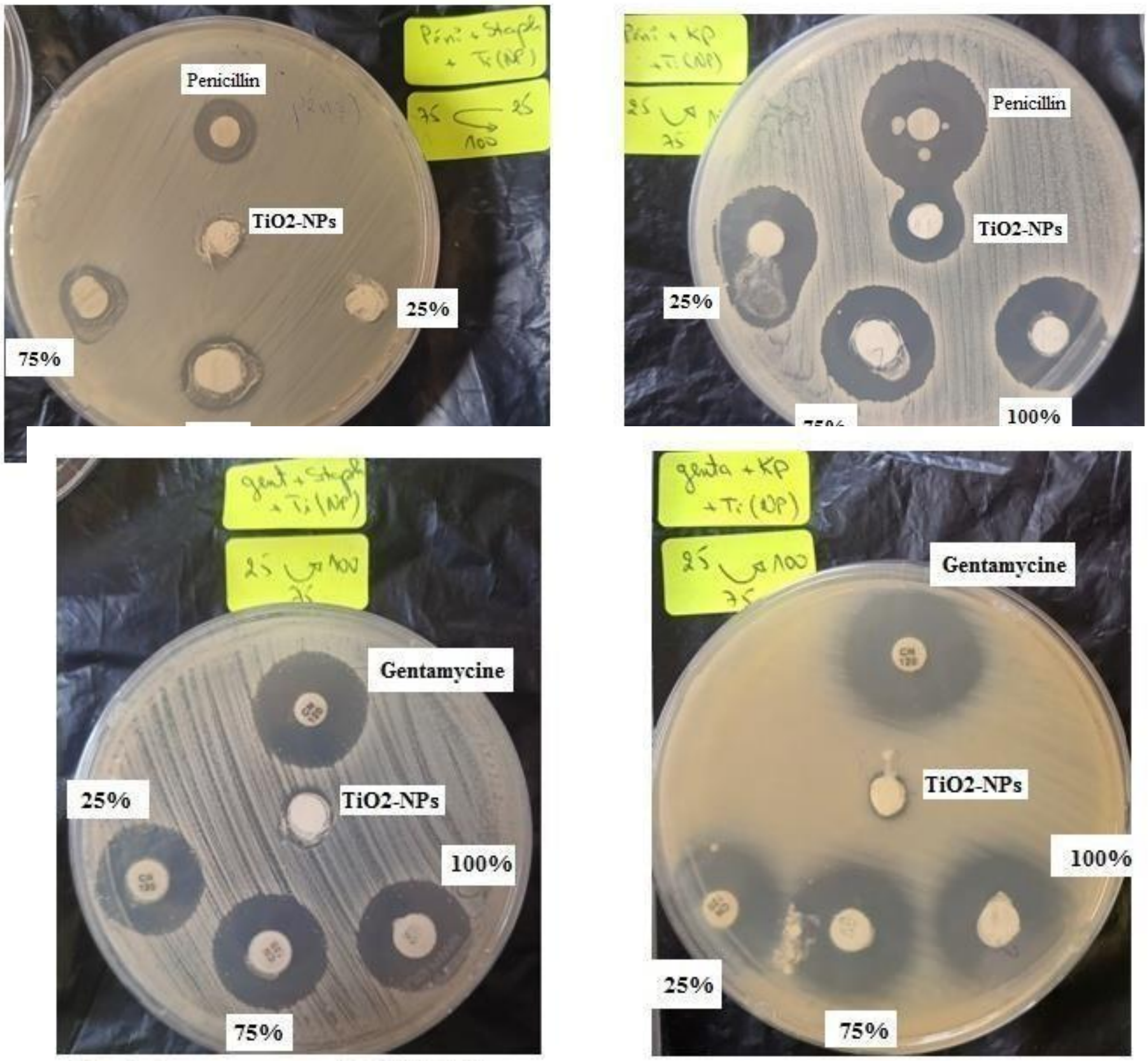


Figure 10: **X-ray diffraction of TiO_2nanoparticules(TiO_2NPs)**

6. Results of the antibacterial activity ofTiO_2nanoparticles

Our experiments carried out with the aim of finding another means of combating bacterial resistance to antibiotics, show that TiO_2nanoparticles applied by the disk method with different concentrations of NPs exert a positive effect on the selected strains. The results of his presented by the figuresZones of inhibition characteristic of the interaction of these ATB with prepared TiO_2NPs appeared after 24 h of incubation of bacterial cultures in the presence of ATB and NPs selected according to the strain. The measurements of the diameters of these zones are presented in **(tables 2)** and**(figure 11)**. According to the recommendations of the Antimicrobial Spectrum Committee of the French Society of Microbiology (CA-SFM), bacteria can be classified into a group belonging to the following categories: Susceptible-Resistant-Intermediate [30]. According to the table below, there were differences in the responses of the antibiotic strains tested. However, Staphylococcus and KP species showed resistance to both bacteria tested.



Staphylococcus aureus ATCC 29213

Klebsiella pneumoniae

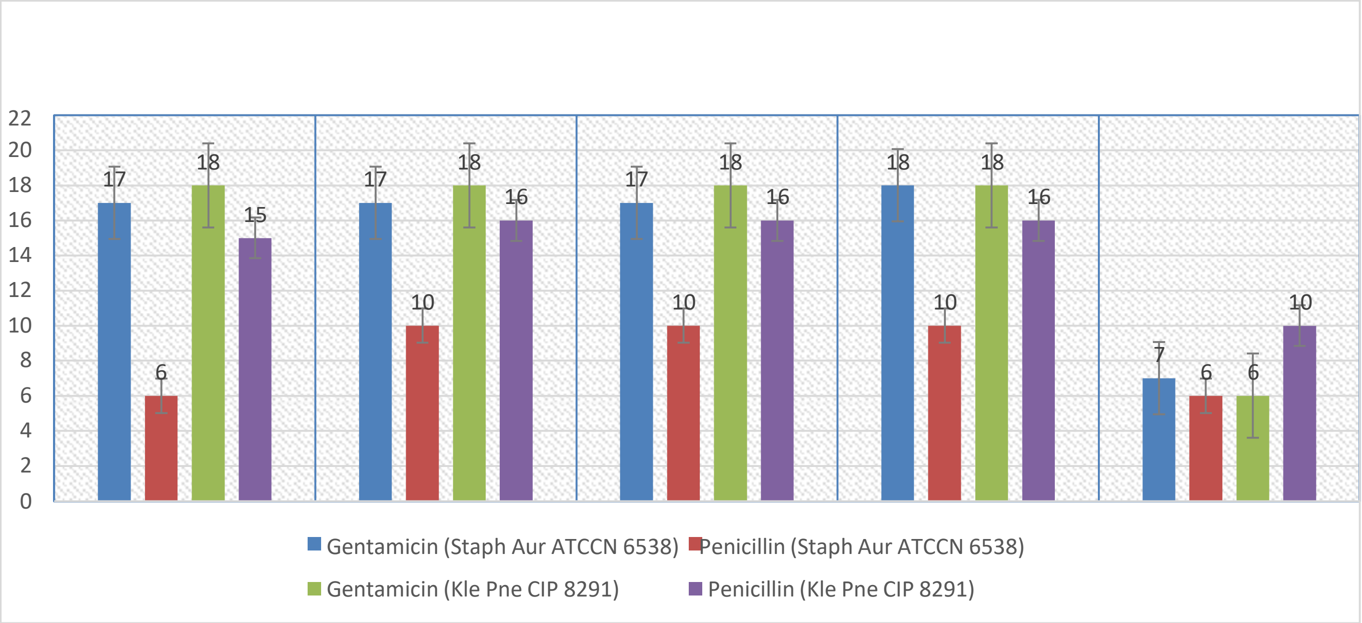
Figure11 : **Antibacterial test of TiO_2NPs (Gentamicin + Staph) and (Gentamicin+ KP)**

Table 2 shows the inhibition zones for Gentamicin 120mg plus TiO_2 NPs and in Gram-(+) bacteria of 18 mm (Staphylococcus) and 18 mm (KP) It is the same. Comparand the zones of inhibition for Penicillin KP (16 mm) showed a larger zone of inhibition than Staphylococcus (10 mm).

Table 2:Antibacterial results for TiO_2 nanoparticles(TiO_2NPs)

Diameter of Disk (mm)		ATB + TiO_2 NPs	ATB + TiO_2 NPs	ATB + TiO_2 NPs	ATB	TiO_2 NPs
		25%	75%	100%	100%	100%
Staphylococcus aureus ATCCN 6538	Gentamicin	17	17	17	18	7
	Penicillin	6	10	10	10	6
Klebsiella pneumoniae CIP8291	Gentamicin	18	18	18	18	6
	Penicillin	15	16	16	16	10

Figure12: Antibacterial results for TiO_2NPs



The inhibition zone reflects the sensitivity of the bacterial species to the inhibitor, and in fact, strains sensitive to antimicrobial agents exhibit larger inhibition radi than the more resistant strains. The results showed that the TiO_2 nanoparticles had an antimicrobial effect in the presence of an inhibition zone. TiO_2 nanoparticles only inhibited bacteria at high enough concentrations. The results also showed that all microbes tested showed greater sensitivity to higher concentrations of nanoparticles. Based on these results, we wanted to study the effect of nanoparticles on antibacterial activity. Therefore, we only studied nanoparticles (TiO_2) with proven antimicrobial activity, using the disk method**.**

7. Conclusions and Outlook

In conclusion, TiO_2-NPs were prepared using Raphanus sativus L. leaf extract using green synthesis technique. Our study focuses on the growing demand for nanoparticles and explores their use in different fields such as cosmetics, pharmaceutical products, catalysts and antibacterial agents. This approach has the advantage of not requiring any solvents or hazardous chemicals. UV-visible spectroscopy, XRD, FTIR, SEM, and EDX methods have demonstrated the successful synthesis of titanium dioxide nanoparticles (TiO2NPs) on natural Raphanus sativus L. leaf extract.

The performance of Raphanus sativus L leaf extract in the green synthesis of TiO_2-NPs was studied, and the results showed that it has the antibacterial effect of TiO2NPs in the presence of inhibition zone of gentamicin 120 mg in addition to TiO_2-NPs and Gram-(+). Bacteria 18 mm (Staphylococcus) and 18 mm (KP) are the same. Comparison of zones of inhibition for penicillin KP (16 mM) showed a larger zone of inhibition than for staphylococci (10 mM), and all microbes tested showed greater sensitivity to higher nanoparticle concentrations. Based on these results, we wanted to study the effect of nanoparticles on antibacterial activity. Therefore, we only studied the effect of adding nanoparticles (TiO2) to some antibiotics on the antimicrobial activity established using the disk method.

The overall data indicated that TiO_2-NPs nanocomposites are promising candidates for biological testing and to evaluate their biological activity for in vitro, in vivo, catalysis, and biomedical applications because they are safe, cost-effective, and biocompatible in nature.

Acknowledgments

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