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Evaluation nanoantibacterial potential of TiO_2 with *M. oleifera* plant extract against food spoilage pathogens

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Abstract

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Foods are exposed to deterioration and spoilage, known as microbiological spoilage, due to the growth and activity of pathogenic microorganisms, particularly bacteria.

The role of titanium particles in inhibiting the growth of many microorganisms was known. Additionally, the use of plants and their extracts, specifically medicinal ones, in preserving various foods has become widespread. The use of nano-titanium and the Moringa oleifera plant against pathogenic bacteria that cause food poisoning in food has not been previously discussed. The current study aimed to investigate the effect of titanium dioxide and dried moringa leaf extract, separately and when mixed, on the growth of *B. cereus* and *K. pneumoniae.* A completely randomized block design was used to implement the experiment, with three replications for each treatment.SEM and AFM were used to investigate nanoparticles characterized by titanium dioxide/DMLE, which improved the physical and granular properties and regular shape of the nanoparticles with a thickness of 1–4 nm. Titanium dioxide / DMLE particles with a size of (1–4) nanometers showed a bacterial antagonism against B.cereus and K.pneumoniae. The minimum inhibitory level was recorded (1.5 mg/ml of TiO_2 + 6 mg/ml of DMLE) and (3 mg/ml of TiO_2 + 12 mg/ml of DMLE), respectively, compared with the minimum inhibitory concentration of titanium dioxide (3, 6 mg/ml) and DMLE, which recorded (24, 48 mg/ml). The synergistic action of titanium dioxide/DMLE resulted in the highest inhibition diameter (14.9 and 14.6 mm) compared to titanium dioxide (13.5 and 12.4 mm) and DMLE (10.3 and 10.0 mm) towards *B. cereus* and *K. Pneumoniae,* respectively.

Keywords: Titanium dioxide; *Moringa oleifera* leaves; SEM; AFM; MIC; pathogenic bacteria

Introduction

Preserving food products from spoilage is a priority for researchers, who explore safe methods to combat deterioration. Spoilage can occur due to various factors, including physical and chemical elements, enzymatic activity, and infections caused by microorganisms such as bacteria, molds, and yeasts (Rawat, 2015).

Nanotechnology is a rapidly evolving and advanced field of science. It depends on the manufacture of nano-sized materials that possess effective chemical, physical, and biological properties based on their size and shape, which leads to their effectiveness and utility in many applications on a vast scale (Morones et al., 2005). This field has recently piqued the interest of researchers and professionals across diverse fields, including pharmaceuticals, medicine, engineering, and food sciences. It aligns with regulations set forth by the Food and Agriculture Organization and holds promise for developing new food products and processes (Ramaswamy et al., 2007). Nanomaterials exhibit numerous physical, biological, and electrical features owing to their small size (1-100 nanometers). The minuscule size of nanoparticles facilitates easy entry into microorganism cells, disrupting vital functions (Abdelghany et al., 2018).

(Hasan et al., 2020) demonstrated that the combination of TiO_2 with the alcoholic extract of Trigonella foenum-graecum contributed to synergistic inhibition against three isolates of Acinetobacter baumannii. Titanium dioxide nanoparticles exhibited a broad-spectrum antibacterial effect, even against antibiotic-resistant strains (Ranj et al., 2023). The effective properties of nanomaterials against pathogenic microbes have made them widely used in the safety and preservation of food from spoilage caused by pathogenic microbes that threaten human health) Baranwal et al., 2018). These nanomaterials can contribute to improving food safety by protecting foods from spoilage and prolonging their shelf life and nutritional value as additives, without altering the content or physical properties of these products (Das et al., 2017). The antimicrobial effectiveness of TiO_2 is attributed to its intrinsic characteristics, including light absorption, low cost, and a high surface-to-volume ratio, which confer effective physicochemical properties. Many researchers suggest that the crystal structure of titanium dioxide, available in three forms (brookite, rutile, and anatase), is responsible for its biocidal action, particularly anatase, which possesses photocatalytic and voltammetric actions to eliminate pathogens (Yang et al., 2002; Ali et al., 2017). Furthermore, utilizing plant extracts in green synthesis to obtain TiO_2 nanoparticles offers advantages such as rapid production, increased reduction potential of TiO_2 ions, enhanced particle stability, simplicity, broad-spectrum effects, and low cost (Nasrollahzadeh et al., 2015). Several researchers (Yahya et al., 2018; Castillo-Henríquez et al., 2020; Hara et al., 2020; Ghosh et al., 2022) have shown that the mixture and synthesis of nanoparticles with plant extracts exhibit antimicrobial potential, among other properties. The Moringa plant, belonging to the family Moringaceae, is often referred to as the perennial tree due to its medicinal properties and versatility. Compounds such as terpenoids, phenols, alkaloids, and flavonoids found in Moringa act as antioxidants, thereby reducing microbial growth (He et al., 2013). In a study by Hariharan et al. (2017), TiO_2 was combined with Cynodon dactylon leaf extract to create TiO_2/Cynodon dactylon, exhibiting potent biocidal activity against E. coli. (Aravind et al., 2021) synthesized green nanocomposites of TiO_2/jasmine flower extract, displaying apparent inhibitory activity against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae, and by (Tandon et al., 2023; Shafie et al.,2024) with leaves of the psidium guava plant and Aloe vera.

Materials and Methods

Preparation of ethanolic extract from dried moringa leaves

The method of Stahl (1969) extraction was followed with some modifications. Moringa leaves were harvested from the national garden belonging to the Ministry of Agriculture and diagnosed by specialists in the field of medicinal plants at the Ministry of Agriculture/National Herbarium. The leaves were thoroughly washed with water to remove impurities and suspended dust, and then left to dry at laboratory temperature with continuous stirring until it was confirmed that they were completely dry. Then, they were ground with a silver-crest electric mill to obtain a fine powder. After this step, 195 mL of 70% ethyl alcohol was added to 25 g of the crushed leaf powder in a 500 mL volumetric flask, accompanied by continuous stirring using a thermal magnetic stirrer for 1 hour at either 40 °C or 55 °C. Then, the mixture was filtered using several layers of cloth with four small holes to get rid of plankton, followed by centrifugation (4000 rpm/min for 20 minutes). The process was repeated three times to obtain a clear solution, which was transferred to a glass beaker to evaporate the solvent using a Rotary evaporator device and obtain a thick extract. Then, it was dried in the oven at 40 degrees Celsius and stored at 4 degrees Celsius in clean glass containers until use.

Preparation of the solutions of nano-titanium dioxide and dried moringa leaves extract

TiO_2 particles were obtained in powder form (NanoShell Company, USA). Basic solutions of TiO_2 and dried moringa leaves extract were prepared to make the necessary dilutions for the experiment as follows:

1. Preparation of TiO_2 solution: Prepared by dissolving 150 mg of titanium powder in 10 ml of distilled water.
2. Preparation of Moringa leaf solution: Prepared by dissolving 1 g of powdered extract in 10 mL of hot distilled water.
3. Preparation of titanium/Moringa leaves solution: Prepared by dissolving 150 mg of TiO_2 with 1 g of dried Moringa leaves extract in 10 ml of hot distilled water.

Study of the surface characterization of titanium dioxide/M. oleifera nanoparticles:

Scanning Electron Microscope (SEM): A SEM device (MIRA3 TESCAN model) with 10,000× magnification was used in Iran to analyze and describe the morphology and shape of titanium dioxide/*M. oleifera* nanoparticles. A small quantity of titanium dioxide/*M. Oleifera* nanoparticles were deposited on a copper plate coated with carbon (a fluffy layer of it), and photographs were taken after the sample dried (Hassan et al., 2019).

Atomic Force Microscopy (AFM): AFM was employed to investigate the shape, surface, size, distribution, and regularity of titanium dioxide/M/M particles. oleifera nanoparticles. The procedure involved placing 0.1 mL of the sample on the slide to create a fluffy film and allowing it to dry for a few minutes before scanning with the AFM machine (Chen et al., 2015).

Determination of the minimum inhibitory concentration of titanium dioxide and dried moringa leaves extract against B. cereus and K. pneumonia.

Multiple concentrations (1.5, 3, 6, 9, 12, 15, 18 mg/mL) of titanium dioxide and (6, 12, 24, 48, 96, 192, 384 mg/mL) of dried moringa leaf extract were prepared. These concentrations were used separately and mixed to determine the minimum inhibitory concentration (MIC) against *B. cereus* and *K. pneumoniae* (isolated from cases of food poisoning at Al-Yarmouk Hospital, Baghdad, Iraq). Whatman filter papers with a diameter of 6 mm, saturated under sterile conditions with the prepared concentrations, were placed on solid Miller-Hinton Agar Media inoculated separately with the two types of bacteria under study. The dishes were then incubated at 37°C for 24 hours. The concentration that caused the most reduction in the number of colonies (the least inhibitory concentration) was determined (Levinson, 2002).

Determination of the inhibitory efficiency of titanium dioxide and dried moringa leaves extract against B. cereus and K. pneumonia.

The inhibitory efficiency of titanium dioxide and dried moringa leaves extract concentrations was tested against *B. cereus* and *K. pneumoniae*, separately and when mixed, using the Agar-Gel Diffusion method. This involved pouring 15 mL of Miller-Hinton Agar Medium into Petri dishes, inoculating after hardening with 100 µL of bacterial isolates, and leaving them to dry at room temperature for 15 minutes. Then, holes with a diameter of 6 mm were made on the surface of the medium using a sterile cork puncture. After that, 100 microliters of the prepared concentrations were transferred to the holes. The plates were left in the refrigerator at 4°C to allow the concentrations to diffuse through the medium, and subsequently transferred to the incubator at 37°C for 24 hours. The apparent areas of inhibition were calculated in mm using a ruler (Wikler, 2007).

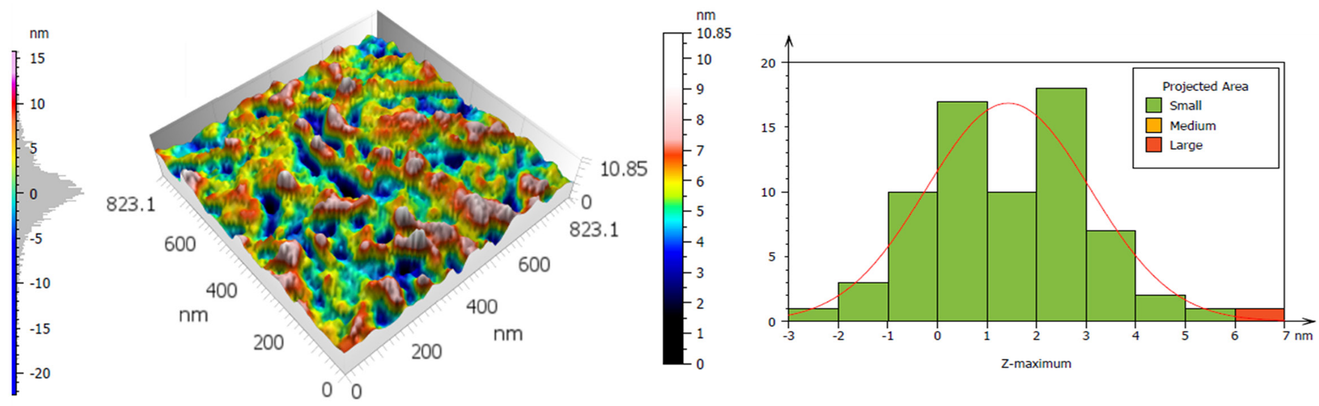
Statistical data analysis

The SPSS statistical program was used to analyze the data obtained from the study at the 0.05 significance level, where averages were compared using the least significant difference test.

The results

Figure 1 (a) shows the topography of titanium dioxide/*M. Oleifera* nanoparticles were examined using the AFM device with a thickness of 10 nm. The size of the nanomaterial ranges from 1 to 4 nm and has a regular spherical shape. According to Figure 1 (b), which indicates the small thickness of the nanomaterial, and based on the examination data, the surface nature is regular and homogeneous, which is an ideal condition for achieving such nano-sizes.

It is noted from Figure 2 that the adhesion of nanopar-



a) b)

Fig. 1 (a). Two-dimensional image of titanium dioxide/ *M. oleifera* nanoparticles by AFM scan
Fig. 1(b). Granular size distribution of titanium dioxide / *M. oleifera* nanoparticle
Source: Authors' own elaboration

ticles to the surface of the plant extract is characterized by nanoscale dimensions in addition to its regular spherical appearance. This observation aligns with findings from (Nabi et al., 2022, and Ahmad et al., 2020) who confirmed the possibility of obtaining nanomaterials made of titanium dioxide through the green combination with extracts of plant materials, exhibiting nanoscale specifications in terms of size, shape, area, smoothness, and antibacterial and antifungal inhibitory capabilities.

Minimum inhibitory concentration of titanium dioxide and dried moringa leaves extract against B. cereus and K. pneumoniae

Table 1 presents the inhibitory action of titanium dioxide and dried moringa leaves extract. The results indicate that titanium dioxide alone exhibited activity, with minimum inhibitory concentrations of 3 and 6, respectively, against *B. cereus* and *K. pneumoniae*. In comparison, moringa extract also showed effectiveness, albeit with higher inhibitory

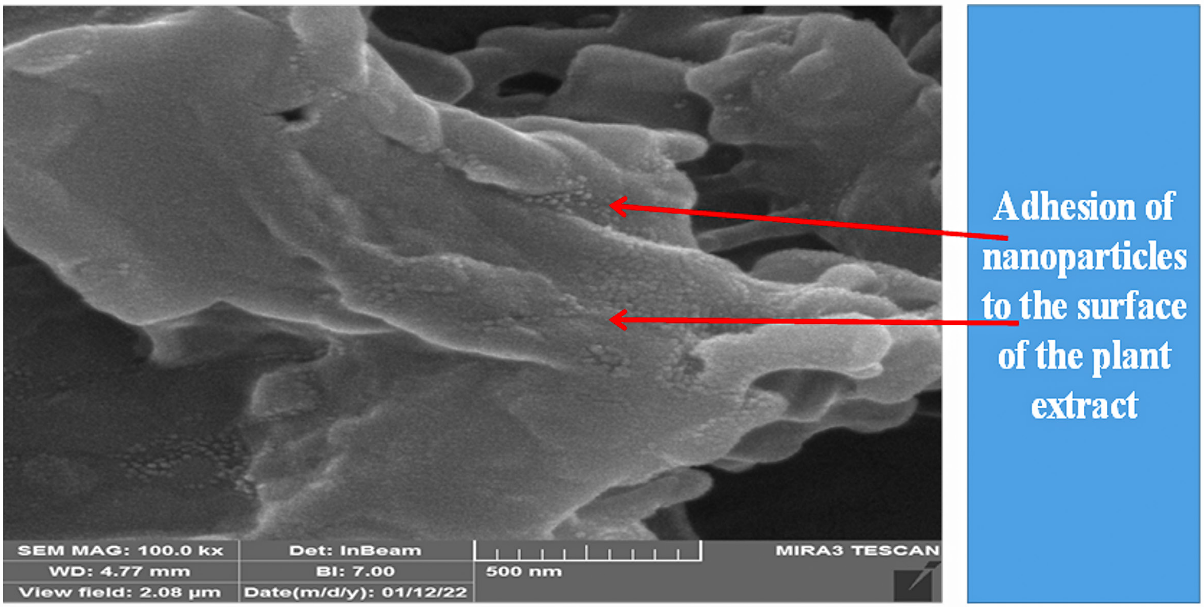


Fig. 2. SEM image magnification of synthesized of titanium dioxide / *M. oleifera* nanoparticles.
Source: Authors' own elaboration

Table 1. The minimum inhibitory concentration of TiO_2 particles and dried moringa leaves extract against *B. cereus* and *K. pnemuniae*

Bacteria's type	Prepared solutions	Minimum inhibitory concentration (mg/ml)						
B.cereus	DMLE	6	12	24	48	96	192	384
		+	+	+	–	–	–	–
	TiO_2	1.5	3	6	9	12	15	18
		+	+	-	–	–	–	–
	TiO_2 + DMLE	6+1.5	12+3	24+6	48+9	96+12	192+15	348+18
		+	–	–	–	–	–	–
K.pneumoniae	DMLE	6	12	24	48	96	192	384
		+	+	+	+	–	–	–
	TiO_2	1.5	3	6	9	12	15	18
		+	+	+	–	–	–	–
	TiO_2 + DMLE	6+1.5	12+3	24+6	48+9	96+12	192+15	348+18
		+	+	–	–	–	–	–

Sensitive (–), Insensitive (+), TiO_2: Titanium dioxide, DMLE: dried moringa leaves extract, TiO_2 + DMLE: Titanium dioxide +: dried moringa leaves extract.
Source: Authors' own elaboration

concentrations of 24 and 48, respectively. However, when titanium dioxide was mixed with the plant extract (1.5 mg/ml of TiO_2 and 6 mg/ml of DMLE) and (3 mg/ml of TiO_2 + 12 mg/ml of DMLE), it demonstrated the most efficient inhibition of the growth of the studied bacteria. This confirms the synergistic action between titanium dioxide and the Moringa extract, with the effectiveness increasing directly with the concentrations of both materials. No other study has explored the inhibitory action of titanium dioxide combined with the Moringa plant. The variation in results of the lower inhibitory concentrations towards the bacteria may be attributed to the resistance or sensitivity of the bacteria in the presence of TiO_2 and the active substances present in Moringa. Gram-positive bacteria exhibited higher sensitivity at all concentrations compared to Gram-negative bacteria, which demonstrated higher resistance (Al-Jubouri, 1990; Adeyinka et al., 2018).

The concentrations of titanium dioxide and dried moringa leaf extract used, whether separately or when mixed (Table 2), resulted in an inhibitory effect, as measured by the diameters of the inhibition zones. These ranged from 10.0 to 14.6 mm towards *K. pneumoniae* and from 10.3 to 14.9 mm towards B. cereus. Significant differences were observed between the types of solutions used, with the TiO_2 + DMLE treatment exhibiting the highest inhibition zone for the two types of bacteria studied. This finding confirms the synergistic action between the nanoparticles and Moringa extract.

Table 2. Inhibitory efficiency (mm) of TiO2 particles and dried moringa leaves extract against *B.cereus* and *K. pneumoniae*

Bacteria's type	Prepared solution	Inhibition zone (mm)
B.cereus	DMLE	10.3^a
	TiO_2	13.5^b
	TiO2+ DMLE	14.9^d
K.pnemuniae	DMLE	10.0^a
	TiO_2	12.4^c
	TiO_2 + DMLE	14.6^d

The variation in the letters indicates that there are differences at $p > 0.05$
TiO_2: Titanium dioxide, DMLE: dried moringa leaves extract, TiO_2 + DMLE: Titanium dioxide + dried moringa leaves extract.
Source: Authors' own elaboration

Discussion

The antibacterial activity of Moringa plant extract against bacteria is attributed to various organic compounds, including benzyl isothiocyanate, alkaloids, flavonoids, saponins, glycosides, steroids, phenols, chlorogenic acid, caffeic acid, hydroxycinnamic acid, and coumarins. Moringa leaves are a storehouse of nutrients and vitamins. Additionally, peptides work directly to inhibit the growth of pathogenic bacteria by preventing the formation of the cell membrane or by producing enzymes that affect vital cellular activities (Bukar, 2010). Other research also refers to plant extracts as a rich source of phytochemicals, which are considered a controlling factor in the growth of microbes due to their chemical diversity. Plants contain numerous phytochemicals such as flavonoids, alkaloids, and phenols, which provide antimicrobial and oxidative effects (Alibi et al., 2021). Several explanations and theories have been developed regarding titanium's antagonistic pathway towards microorganisms (Xie and Hung, 2019).

TiO_2 nanoparticles (NPs) disrupt microbial cells in two ways. Firstly, in the presence of UV light, TiO_2 NPs tend to be in an oxidation state, leading to stimulated oxidation and reduction reactions. This results in the formation of various reactive oxygen species (ROS), including free radicals, superoxide, H_2O_2, and OH. These compounds encourage biocidal activity by degrading phospholipids, lipopolysaccharides (LPS), and proteins, leading to the destruction of biomolecules such as DNA, cellular proteins, and enzymes (Liao et al., 2020; Padmavathy et al., 2011). Nanoparticles are also found to be highly effective in inhibiting cellular enzymes and DNA replication. This role is through their association with electron-donating groups such as carboxyl, hydroxyl, and sulfur groups. They also cause holes in bacterial cell walls, leading to increased cell membrane permeability and thus cell death.

The second way, in the absence of UV irradiation, involves the generation of electromagnetic attractions between TiO_2 NPs and the microbe's surface due to the positive charge of the nanoparticles and the negative charge of the microbes. This stimulates the interaction of TiO_2 with thiols, indoles, amides, hydroxyls, and carbohydrate groups that donate electrons, resulting in increased bacterial cell wall permeability due to the formation of pores, and subsequently, cell death (Egmi et al., 2018). another explanation for the mechanism of action of titanium oxide nanoparticles is as an effective antibacterial that nanoparticles can penetrate the bacterial cell, damage its DNA, and release antimicrobial ions which will bind to the electron donor groups containing sulfur, oxygen, or nitrogen elements in the molecules which will inhibit adenosine triphosphate (ATP) synthesis and DNA replication, which will eventually lead to cell death (Li et al., 2004).

Many mechanisms possessed by nanomaterials are complex due to their diverse characteristics, which affect the growth and life of pathogenic organisms. These include the inhibition of cell membrane formation, penetration of the cell wall, and oxidative stress in cells (Matai et al., 2020).

The synergistic effects of both plant extracts and nanomaterials in combating or reducing the impact of pathogenic bacteria that cause food spoilage will lead to enhanced food safety and prolonged shelf life of food products. This will contribute to the development of these products and their positive impact on human health, while also promoting sustainable growth. The integration of nano- and plant-based antibiotics in food products has significant implications for public health and the food industry (Sharmin et al., 2021; Wang et al., 2017).

Conclusions

Food pollution is considered one of the oldest and most widespread types of pollution, as the bacteria that cause it secrete various toxins that result in pathological symptoms, which can be severe and even fatal, in addition to significant material and economic losses. Nanomaterials are considered highly effective against germs due to their ability to penetrate bacterial cell membranes, release free oxygen radicals, and reduce their effects, in addition to inhibiting vital processes of microorganisms such as glycolysis and protein transfer across the membrane. Nanoparticles also affect cells by interacting with the outer part of the plasma membrane. This interaction alters the structure of the membrane and its permeability, leading to the breakdown of the plasma membrane and its accumulation in the cytoplasm. Consequently, it interferes with the fundamental processes of cell growth, ultimately inhibiting its growth. Plant extracts, through their active substances, inhibit the role of pathogenic bacteria and their toxic effects by producing a broad spectrum of secondary metabolic compounds, such as steroids, flavonoids, glycosides, and alkaloids, which in turn possess therapeutic or antimicrobial properties**.**

The use of TiO_2 molecules and dried Moringa leaves extract contributed to an apparent inhibitory effect when used alone against *B. cereus* and *K. pneumoniae*. This inhibitory effect increased and was more pronounced when they were mixed, as it directly increased with concentration, indicating a synergistic role of both. This study is the first of its kind in terms of content, encompassing knowledge of the inhibitory action of titanium dioxide and dried Moringa leaf extract (DMLE) against Gram-positive and Gram-negative bacteria, as well as their role in preventing food spoilage and various cases of food poisoning. This opens up prospects for their use as alternatives to inhibit microorganisms and preserve food, thereby prolonging its shelf life.

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