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In vitro testing of biomedical applications of biosynthesized titanium nanoparticles using *Saccharopolyspora spinosa*

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Abstract

The antibacterial, antifungal, antiviral, antioxidant, and antitumor properties of biosynthesized titanium nanoparticles from isolated and identified *Saccharopolyspora spinosa* were tested *in vitro*. Investigation of ultrastructural changes in microbes treated by TiO_2 nanoparticles. Elucidation of the role of titanium nanoparticles in enhancing the apoptotic rate of tumour cells and ensuring their minimal cytotoxicity. Actinomycete was isolated from animal compost specimen in starch nitrate agar medium and identified using cultural and morphological features. Actinomycete was tested to synthesize TiO_2 nanoparticles where *S. spinosa* were the most successful isolate to produce nanoparticles and its identification has been done by genetic tools and deposited in genebank**.** Titanium dioxide nanoparticles were characterized by UV, XRD, EDX, FTIR, TEM, particle size and zeta potential. Antioxidant action of TiO_2NPs was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Antitumor action of TiO_2NPs versus A549 cells. Apoptotic rate was evaluated using flow cytometry. Antibacterial, antifungal and antiviral roles of TiO_2NPs were determined. The alteration in the most affected microorganisms were examined using transmission electron microscope. *S. spinosa* was isolated and identified using various morphological and molecular tools and deposited in gene bank with accession number (OP315279.1). White suspension of TiO_2 nanoparticles could be seen with spherical shape. TiO_2 nanoparticles has promising antioxidant impact with IC_{50} = 78.35 ± 2.59 µg/ml, as well as antitumor action versus A542 cells with IC_{50}= 96.12± 3.64 upon acceleration of apoptotic rate. TiO_2 nanoparticles has minimal toxicity versus Vero cells with CC_{50} = 402.94± 14.76 µg/ml. TiO_2 nanoparticles has promising antimicrobial impact versus *Proteus vulgaris*, *Candida albicans* and Heptitis A virus which confirmed by ultrastrucute examination. *S. spinosa* have the capability to produce TiO_2 nanoparticles with pleotropic biomedical applications to be verified by *in vivo* studies.

Keywords:Saccharopolyspora spinosa; Titanium nanoparticles*;* antioxidant; antitumor; antimicrobial; antiviral

1. Introduction

Nanoparticles are emerging swiftly in the modern period and have drawn amazing attention because of their significant therapeutic and manufacturing applications [1]**.** The toxic impact of nanoparticles, their conceivable absorption methods, and their detrimental effects on living things still need to be better understood [2]. Moreover, the production of nanoparticles chemically intensifies their harmful impact on the environment and living organisms [3, 4]. The biological-mediated production of nanoparticles using biological sources offers one potential means of minimizing these negative consequences [5, 6].

Since their creation in the 1990s, titanium dioxide (TiO_2) nanoparticles (NPs) have been used in a variety of biomedical sectors, including the preparation of medicinal drugs [7-10]. Current studies have concentrated on using nanoscale titanium, which has a diameter of less than 100 nm, to create non-toxic and efficient tools [11, 12]. Specifically, nanoscale TiO_2

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particles have been used in numerous biomedical applications due to their unique therapeutic features, which include resistance and biocompatibility with body tissues [13, 14]. NPs may have a variety of non-specific effects on intracellular organelles and extracellular microenvironments. There hasn't been much research done on how metal oxide NPs affect the cellular microenvironment and tissue physiology. For appropriate therapies to be developed, a thorough investigation of the anticancer action is necessary [15-18].

Saccharopolyspora is a genus that belongs to the Pseudocardiaceae family [19-21]. It could be isolated from different habitats, while soil is *Saccharopolyspora*'s important source [22-24]. Several bioactive secondary metabolites can be produced by *Saccharopolyspora* species [25, 26]. A family of insecticides known as spinosyns is derived from *Saccharopolyspora spinosa*. They cause the nicotinic acetylcholine receptors to become active, acting as neurotoxins [27].

The titanium nanoparticles used in this work were produced by *S. spinosa*. The formed nanoparticles were thoroughly described, and *in vitro* testing of their antioxidant, anticancer, antimicrobial, and properties was conducted to demonstrate their possible therapeutic usefulness.

2.Experimental:

2.1. Acquisition of specimen for isolation of actinomycete

Using a serial dilution method, actinomycete isolate was obtained from the animal compost specimen [28]**.** Starch-nitrate agar, Inorganic-trace salt- starch agar, Glycerol asparagine agar, Yeast extract- malt extract agar, Oatmeal agar, Tryptone yeast extract broth, and Peptone yeast extract iron agar media (ThermoScientific ^{TM}) were the media used for the isolation. Specimen was placed on three plates, which were incubated for 7 days at 30 °C. Actinomycete on the plates had their growth periodically monitored. On the basis of color, dryness, toughness, and convexity, the colonies that formed were chosen and cleansed [29]**.** For additional investigation, the pure colonies were selected, separated, sub-cultured, cleaned, and maintained at 4 °C in starch nitrate agar slants.

2.2. Assessing isolated actinomycete capacity to produce titanium dioxide nanoparticles

Actinomycete specimen was allowed to develop in 100 ml starch nitrate broth medium for 7 days at 30 °C with 150 rpm shaking. This culture was considered the origin culture. A volume of 75ml of starch nitrate broth medium was added after 25ml of the original culture had been diluted four times. This diluted culture solution was again allowed to grow for another 7 days. 20 ml 0.0025M [$Ti(OH)_2$] titanium tetraisopropoxide was added to the broth culture and maintained in a bath for ~20min at 60°C. A white deposition was seen at the bottom of the flask, suggesting the beginning of the conversion. At 30° C, the actinomycetes-containing broth culture was incubated. After 12-48 hours, a clearly coalescent white cluster floating in the media in the broth culture was found, demonstrating the formation of TiO2 NPs [30-32].

2.3. Characterization of TiO_2-NPs

The biosynthesis of TiO_2 nanoparticles in the broth medium was tested by recording UV absorbance by spectrophotometer (JENWAY 6305, Germany). The crystalline structure of the TiO_2 nanoparticles were detected using X-ray diffraction (XRD) (Bruker, Germany) with CuKα radiation (1.5406 Å) in the 2θ scan range of 10-90°. TEM was conducted using (JEOL1010, Japan) to test the shape and size of the nanoparticles. FTIR spectrum of TiO2 nanoparticles was detected on Fourier Transform Infrared spectrophotometer (Bruker, Germany) in the region of 4000 to 500 cm-1. The EDX microanalysis was shown using an X-ray micro-analyzer (Oxford 6587 INCA) connected to a JEOL JSM-5500 LV scanning electron microscope. Particle size and zeta potential were detected using Zetasizer Nano ZS [33-35].

2.4. Identification of actinomycete producing TiO_2 nanoparticles

According to its cultural and morphological traits, the strain that can manufacture Ti-NPs was recognized [36-37].

On a starch agar slant, an actinomycete was cultivated for seven days. The starch nitrate broth was inoculated with 2 ml of the spore suspension and cultured on a rotary shaker for 3 days at 200 rpm and 30 °C. Using a PCR Product extraction kit, genetic material from grown organisms was purified (Qiagen, Valencia). The DNA sequences were isolated using an Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). A BLAST® analysis (Basic Local Alignment Search Tool) was first carried out to determine sequence identity to GenBank accessions. The primers used in the experiment were F: AGAGTTTGATCMTGGCTCAG3 and R: TACGGYTACCTTGTTACGACTT[38]. The phylogenetic tree was produced using the MegAlign module of Lasergene DNA Star version 12.1 Phylogenetic analyses was done using maximum

likelihood, neighbor joining and maximum parsimony in MEGA6 [39, 40].

2.5. Surface Examination of actinomycete producing TiO_2 nanoparticles

Scanning electron microscopy (JOEL, Japan) was used to examine structure of fresh culture of *S. spinosa*, according to Sayed et al., [41].

2.6. Testing antioxidant action of produced TiO_2 nanoparticles

DPPH test was applied to test antioxidant role of prepared TiO_2 nanoparticles where DPPH reagent was added to specimen to initiate the reaction and left for 30 minutes at 37°C, then measured at 515 nm. Ascorbic acid was used as standard [42, 43].

2.7. Antitumor assay of produced TiO_2 nanoparticles

The biosynthesized TiO_2 nanoparticles were examined for cytotoxic impact on A549 (adenocarcinomic human alveolar basal epithelial cells). Cells were allowed to attach for 24 hours until confluence, after which they treated with specimens at concentrations from 500 to 15.63 µg/mL and left for 24 hours at 35 °C. Then, the fresh medium was incorporated and treated with 100 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide)] solution (6 mg/mL) for 3 h at 37 °C. Absorbance was detected at 560 nm using a microplate reader (SunRise TECAN, Inc.,CA, USA). The images were captured by a digital camera combined with an inverted microscope (Olympus, Tokyo, Japan) [44, 45].

2.8. Flow cytometry analysis

Annexin V/propidium iodide staining kit was used to examine the apoptotic rate. (5×10^6 cells) for both Control and treated Cells were examined. Cells were washed with PBS (phosphate buffer saline), centrifuged, and floated in annexin V binding buffer (BD Biosciences, USA) containing annexin V-FITC at 4 ℃ in the dark for 30 min. Dot plots for both specimens were detected using a flow cytometer (BD Biosciences) [46].

2.9 Cytotoxicity assay

The Vero cells ($1x10^4$) were plated in 96-well dishes with 100 µL of DMEM (Dulbecco's Modified Eagle Medium) growth media. After being implanted for 24 hours, monolayer cells were transferred using a pipette into 96 micro-titer plates (Falcon, USA). The addition of fresh DMEM medium with varying sample concentrations was then followed by repeated two-fold dilutions of the test specimen. The plates were kept moist with 5% CO_2 for 48 hours at 37 °C. After crystal violet staining, absorbance at 580 nm was measured [47].

2.10. Antimicrobial and Minimal inhibitory concentration (MIC) of produced TiO_2 nanoparticles

The antimicrobial activity of TiO_2 nanoparticles was tested using diffusion method to determine the inhibition zone of specimen against Gram-positive bacteria, *B. subtilis* (ATCC6633), and *S. aureus* (ATCC25923), as well as Gram-negative bacteria, *E. coli* (ATCC25922), *Proteus vulgaris* (ATCC 13315, (yeast) *C. albicans* (ATCC 10231), and filamentous fungi *Aspergillus fumigatus* (RCMB 002008). Besides, to determine MICs of TiO_2NPs, the efficient concentrations were diluted repeatedly and evaluated for the most affected microorganisms (i.e with highest inhibition zones) [48, 49].

2.11. Antiviral screening of produced TiO_2 nanoparticles

The impact of TiO_2 nanoparticles versus HAV was tested. The virus was propagated and assed in confluent Vero cells. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose with eight wells per dilution and 20µl of inoculum per well [50, 51].

Monolayers of Vero cells ($2x10^5$ cells/ml) attached at the bottom of micro-titer plate were incubated for 24 h at 37 °C in a humidified incubator with 5% CO2. The plates were washed with fresh DMEM and challenged with various doses of HAV virus, and then the cultures were simultaneously treated with the tested sample in a fresh maintenance medium; following this, they were incubated at 37 °C for 48 h. After the incubation period, the viability of the cells was determined by MTT assay [52-54].

2.12. Ultrastructure examination

Both control and treaded cells (*Candida albicans* and *Proteus vulgaris*) using TiO_2 nanoparticles were fixed by glutaraldehyde for 3 hours. The specimens were then immersed in 2% osmium tetroxide for 2.5 hours, and the blocks were stained in 1% uranyl acetate and dehydrated with a different levels of ethanol. The specimens were incorporated in resin. The specimens were processed by an ultra-microtome (Leica, Germany), and the slices then examined on a transmission electron microscope (JOEL1010, Japan) [55].

For negative staining, the virus or treated virus samples by TiO_2 nanoparicles or standard drug were deposited in copper grids (EMS, PA, USA). Excess liquid was blotted away with filter paper; samples were contrasted using uranyl acetate. The grids were washed in distilled water and dried on filter paper, then examined using electron microscopy to detect virucidal activity [56].

2.13. Statistical Analysis

All Experiments were performed three times, with the mean and ±SD representing the outcomes. A statistical study was carried out utilizing (GraphPad prism, version5, USA). $P \leq 0.05$ was considered to be a statistically significant value when using the T-test analysis.

3.Results

3.1. The Cultural, morphological features of the actinomycete isolate

The culture and morphological features of the isolate were seen on different media as shown in (**Tables 1, 2) and (Fig. 1).** The aerial hyphae of the isolate were white, while the substrate mycelia were yellow brown on starch nitrate agar. White, spiny, non-motile spores could be seen of the isolate.

(Fig. 1) Culture Plate of actinomycete isolate.

Table (1) Cultural characteristics of the actinomycete isolate growing on different culture media.

Type of media	Growth	Color of substrate mycelium	Color of substrate mycelium	Color of diffusable pigments
Starch nitrate	Very good	White	Yellow	None
Inorganic-trace salt- starch agar	Good	Whitish pink	Yellow	None
Glycerol asparagine agar	Good	White	Yellow brown	None
Yeast extract- malt extract agar	Good	Yellowish white	Pale brown	None
Oatmeal agar	Moderate	White	Yellow	None
Tryptone yeast extract broth	Weak	White	Pale yellow	None
Peptone yeast extract iron agar	Good	Whitish pink	Yellow brown	None

Table (2) Morphological characteristics of the actinomycete isolate.

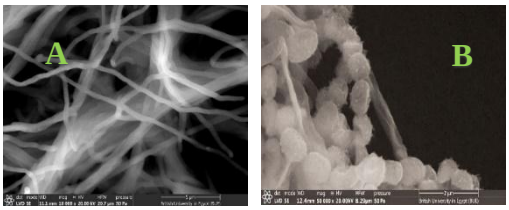
Morphological Characteristics	**Results**
Spore chain	**Rectus-flexibilis**
Spore surface	**Spiny**
Spore mass color	**White**
Motility	**Non motile**
Color of substrate mycelium	**Yellow**
Diffusible pigment	**None**

3.2. Sequencing analysis and generation of phylogenetic tree

The obtained sequence was compared with the sequence of *Saccharopolyspora* sp. to illustrate the identification of the isolate by alignment of sequence. Gel Electrophoresis was applied to detect PCR product as seen in **(Fig. 2A)**, the alignment process illustrated that this isolate was belong to *Saccharopolyspora spinosa* by 99% as shown in **(Fig. 2B).** The sequence was submitted to the GenBank with accession number of: OP315279.1 (https://www.ncbi.nlm.nih.gov/nuccore/op315279)

3.3 Surface examination

Scanning images revealed that the isolate have various features where rectus-flexibilis spiny spores could be seen as depicted in **(Fig. 3A, B)**



(Fig. 3) Scanning electron micrograph of actinomycetes isolate (A) The rectus-flexibilis shape of the spore chain (10000X), (B) The spiny spore surface (50000X).

3.4. Description of TiO_2NPs

i. Examination by naked eye

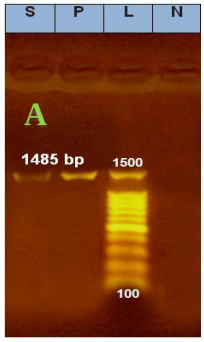
The biosynthesis of Titanium dioxide nanoparticles by white suspended particles in the tube as shown in (**Figure 4A).**

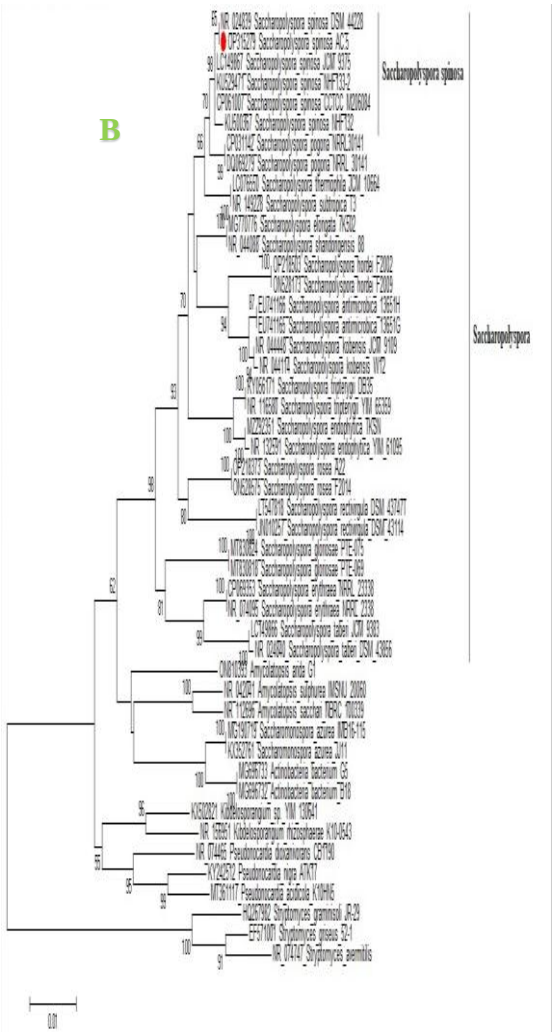
ii. UV-visible spectroscopy

UV screening from 200- 900 nm to investigate the presence of TiO_2 nanoparticles where a peak could be seen at a wave length of 350 nm as depicted in (**Figure 4B).**

iii. Examination using transmission electron microscopy

Biosynthesized TiO_2 nanoparticles could be examined using transmission electron microscope. Spherical nanoparticles could be seen with average diameter of 23.3 nm as shown in **(Figure 4C, Table 3)**.

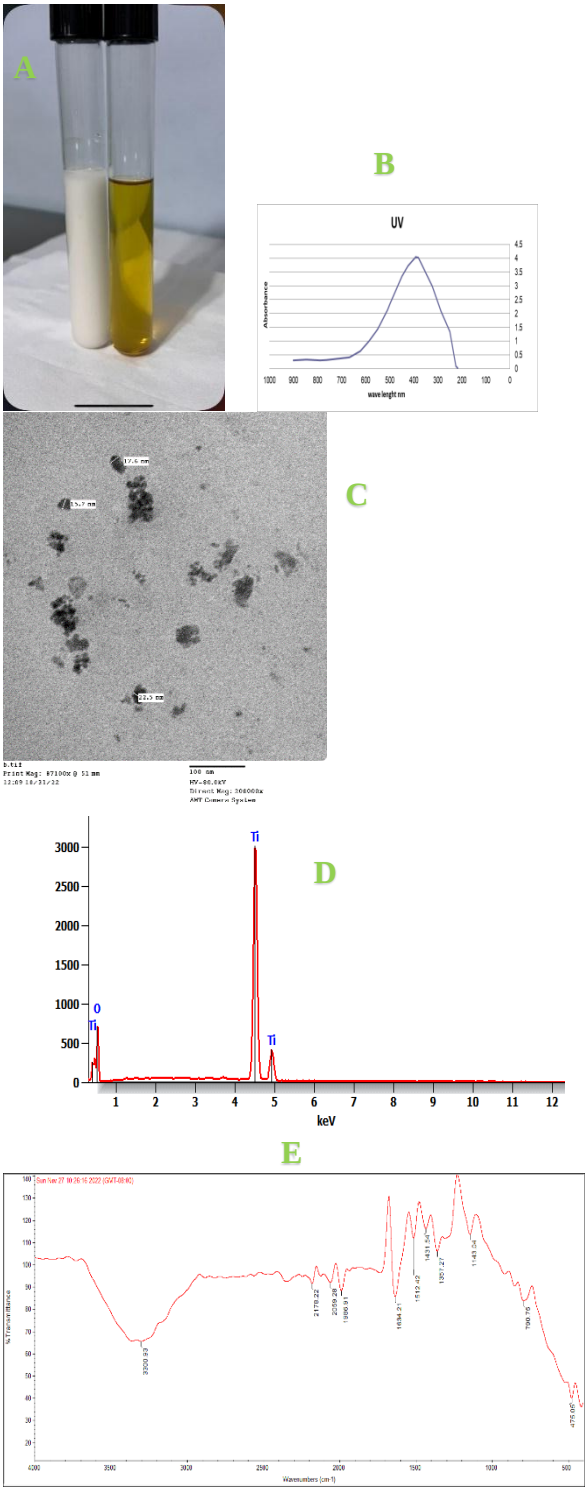




(Fig. 2) (A) Agarose gel electrophoresis of extracted DNA *Saccharopolyspora spinosa. (*L: Ladder, P: Positive control, N: Negative control, (1, 3) duplicates of *S. spinose*; (B)Phylogenetic tree of *S. spinosa* upon applying neighbor analysis.

iv. Testing of energy dispersive spectroscopy

Titanium and oxygen elements could be seen with percentages of 73.49±1.2 and 26.51±0.63, respectively as major peak upon analysis of EDX as depicted in **(Figure 4D, Table 3)**



(Fig. 4) Characterization of biosynthesized TiO_2 nanoparticles using *S. spinosa* (A) Visual examination of white suspension of TiO_2 nanoparticles relative to control; (B) UV-visible spectrum of TiO_2 nanoparticles; (C) Spherical nanoparticles by TEM; (D) EDX testing; (E) FTIR examination.

Table (3) Various percentages of elements (%) by applying EDX testing and different dimensions of TiO_2 nanoparticles synthesized by *S. spinosa* by TEM (mean ± SD).

EDX	Means of elements % ± Standard Deviation of mean (SD)				
Elements	Ti		O		
%	73.49±1.2		26.51±0.63		
TEM					
	Count	Mean	Minimum	Maximum	S.D
TiO_2NPs size	14	23.3	22.5	24.0	1.04

v. FTIR examination

FT-IR examination was applied to characterize functional groups of synthesized TiO_2-NPs using *S. spinosa* metabolites as shown in **(Figure 4E).** While FT-IR spectra of biosynthesized TiO_2-NPs illustrated various featured peaks 475.05, 790.75, 1143.04, 1357.27, 1431.54, 1512.42, 1634.21, 1966.91, 2059.28 and 2178.22 cm−1. A big broad peak at 3300.93 cm−1. 1634.21 may be belong to primary amine N–H bond. Peaks at 2059.28, 2178.22 corresponding to C=C and O–H, while the peaks at 3300.93 could be related to synthesis of titanium dioxide. Thus, TiO_2-NPs has been formed by different functional groups in filtrate to reduce titanium to titanium dioxide NPs.

vi. XRD examination

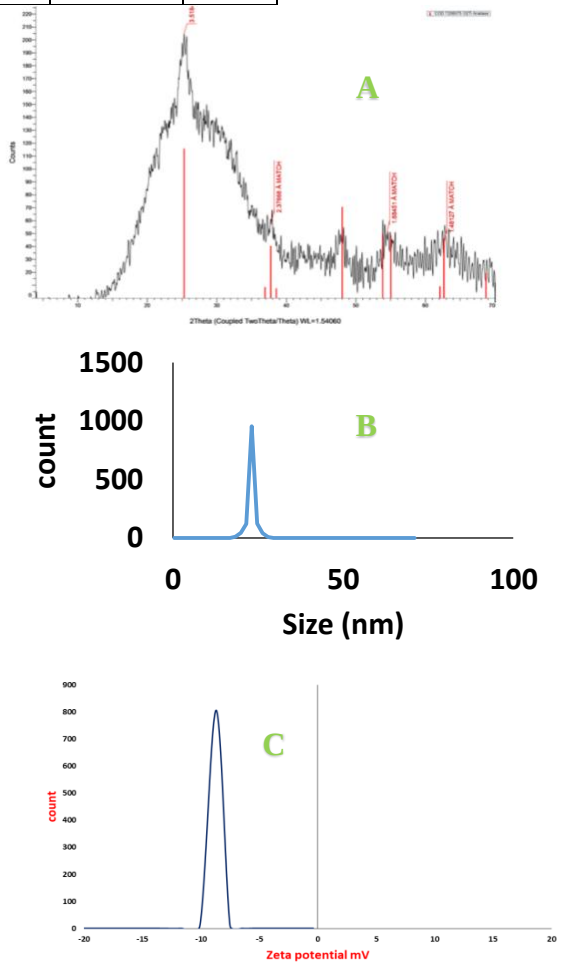
XRD testing could be seen **(Figure 5A)** represented the structure of synthesized TiO_2-NPs. The peaks of 2 Theta degree are 25.00, 38.72, 48.00, 54.22, 63.71, 46.2036 and 69.59 relative to TiO_2NPs synthesized. XRD analysis showed various peaks as debris of synthesis process and processed by temperature.

vii. Particle size

The particle size distribution of TiO_2 NPs could be seen in (**Figure 5B)**. The particle size of the TiO_2 NPs have range in size from 15 to 25nm which is a similar results of TEM, while zeta potential could be seen at -10 as shown in (**Figure 5C)**. Different particles could be seen with different sizes.

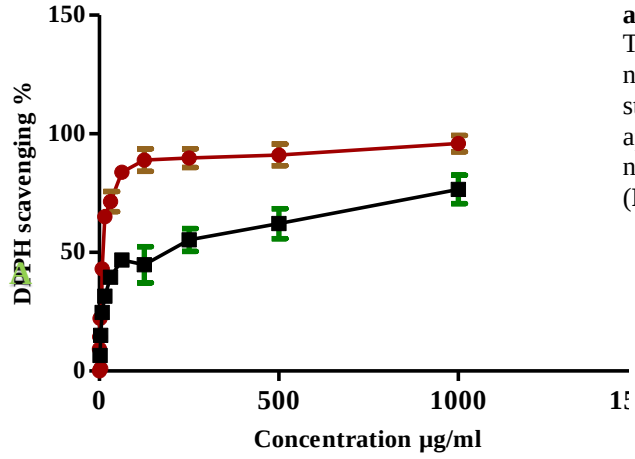
3.5. Antioxidant results

Antioxidant role biosynthesized TiO_2 nanoparticles were investigated using DPPH assay where nanoparticles have IC_{50} = 78.35 ± 2.59 µg/ml and while, Ascorbic acid had IC_{50} = 10.21 ± 0.77 µg/ml upon applying DPPH as illustrated in (**Fig. 6**).



(Fig. 5) Characterization of biosynthesized TiO_2 nanoparticles using *S. spinose* (A) XRD analysis; (B) Particle size analysis (C) Zeta potential.

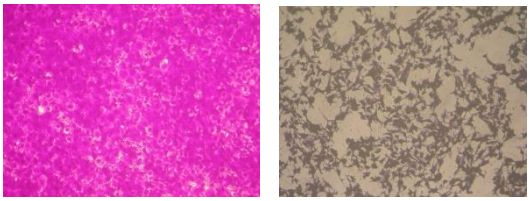
These results revealed that TiO_2 nanoparticles have promising antioxidant role.



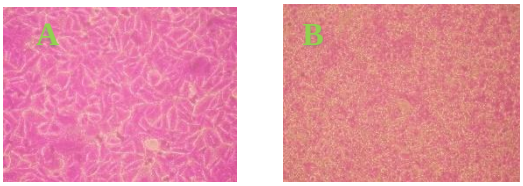
(Fig. 6) Antioxidant activity graph of TiO_2NPs versus ascorbic acid where for TiO_2NPs (Black line) IC_{50}= 78.35 ± 2.59 µg/ml; While ascorbic acid (red line) IC_{50} = 10.21 ± 0.77 µg/ml (Data are represented as means ± S.D).

3.6. Anticancer impact and cellular toxicity of TiO_2NPs

It was shown that the biosynthesized TiO_2 nanoparticles has promising anticancer impact versus A549 cells with IC_{50} value = 96.12± 3.64 µg/ml as depicted in (**Fig. 7**). Furthermore, testing biosynthesized TiO_2 nanoparticles on Vero cells reveled to assure its biosafety showed minimal effect on cells with CC_{50} value = 402.71 ± 14.76 µg/ml revealed its potency and possibility for using it in different applications as depicted in (**Fig. 8**)



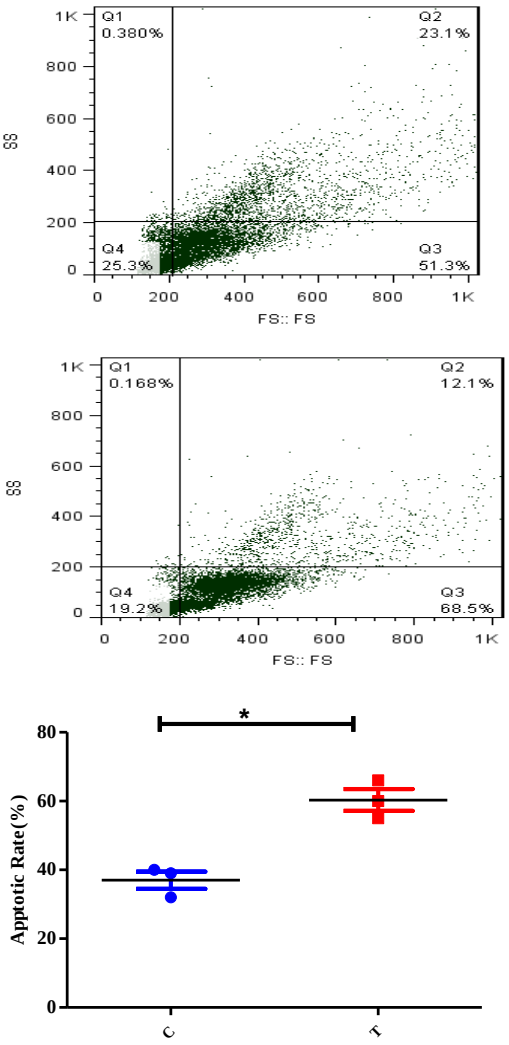
(Fig. 7) Inverted microscopy images for antitumor action of TiO_2NPs versus A549 cells; (A) Control Cells; ((B) Treated cells using TiO_2NPs where IC_{50}= 96.12± 3.64 µg/ml (Magification =100x).



(Fig. 8) Inverted microscopy images for cytotoxic impact of TiO_2NPs versus Vero cells; (A) Normal Cells; (B) Treated cells using TiO_2NPs where CC_{50}= 402.94± 14.76 µg/ml (Magification =100x).

3.7. Elucidation the role of TiO_2NPs in apoptosis

To illustrate the role of biosynthesized TiO_2 nanoparticles versus A549 cells by Annexin-V staining kit where a dramatic elevation (P<0.05) of apoptotic rate of A549 cells treated by TiO_2 nanoparticles relative to untreated cells as shown in (**Fig. 9**)



(Fig. 9) Flow cytometric analysis of TiO_2NPs using Annexin V staining kit (A) Non treated A549 Cells; (B) Treated cells using TiO_2NPs; (C) Statistical analysis between control (C) and treated cells (T) where (*) $p \leq 0.05$ revealing a significant increase in apoptotic rate of cancerous cells upon treatment using TiO_2NPs.

3.8. Antimicrobial results for TiO_2NPs

It could be noticed that TiO_2 nanoparticles has the most promising inhibition zone 17.4.0±1.2mm versus *Proteus vulgaris* (ATCC 13315) with MIC of 125 µg/ml. Furthermore, the inhibition zone of TiO_2 nanoparticles against *Candida albicans* (ATCC 10231) was 15.3±0.9 mm with MIC of 625 µg/ml as depicted in (**Tables 4, 5).**

Table (4) Investigation of antimicrobial action TiO_2 nanoparticles on various tested microorganisms.

Smaples / Microgrnasms	Ti Nps		Control
	Mean	S.D	
FUNGI			Ketoconazole
Aspergillus fumigatus (RCMB 002008)	NA	-	17
Candida albicans (ATCC 10231)	15.3	±0.9	20 ±0.4
Gram Positive Bacteria:			*Gentamycin*
Staphylococcus aureus (ATCC 25923)	10.4	±0.8	24 ±0.7
Bacillus subtilis (ATCC6633)	12.1	±0.6	26 ±0.6
Gram Negatvie Bacteria:			*Gentamycin*
Escherichia coli (ATCC 25922)	13.7	±1.1	30± 0.5
Proteus vulgaris (ATCC 13315)	17.4	±1.2	25 ±0.6

The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 µl was tested), ATCC: American type culture collection :RCMB: Regional Center for Mycology and Biotechnology ;(Positive control for fungi : Ketoconazole: 100 µg/ml); (Positive control for bacteria: Gentamycin: 4µg/ml); *NA: No activity.

Table (5) Minimum Inhibitory concentration (MIC) for the most affected microorganisms**).**

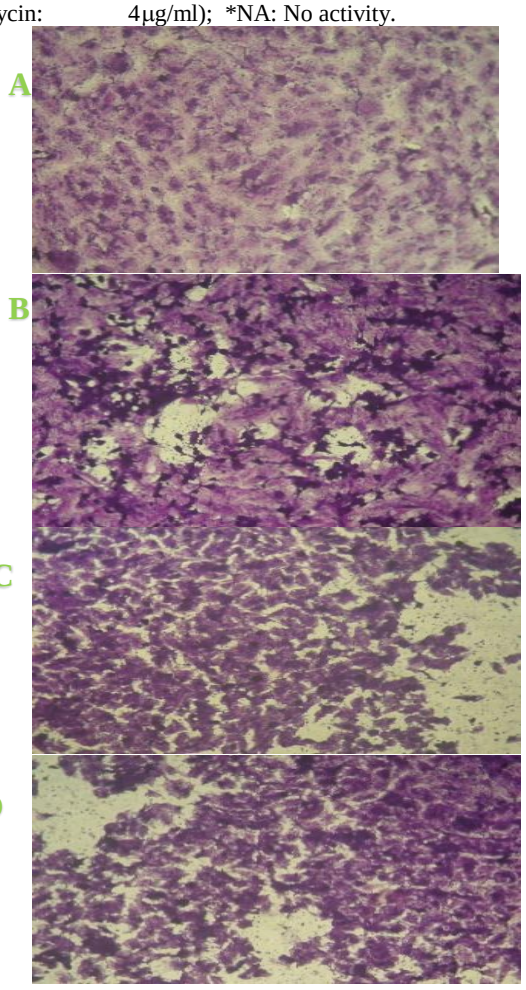
Microorganism/ treatment	**Ti NPs** (µg/ml)
***Candida albicans* (ATCC 10231)**	625
***Proteus vulgaris* (ATCC 13315)**	125

4.9 Antiviral role of TiO_2NPs

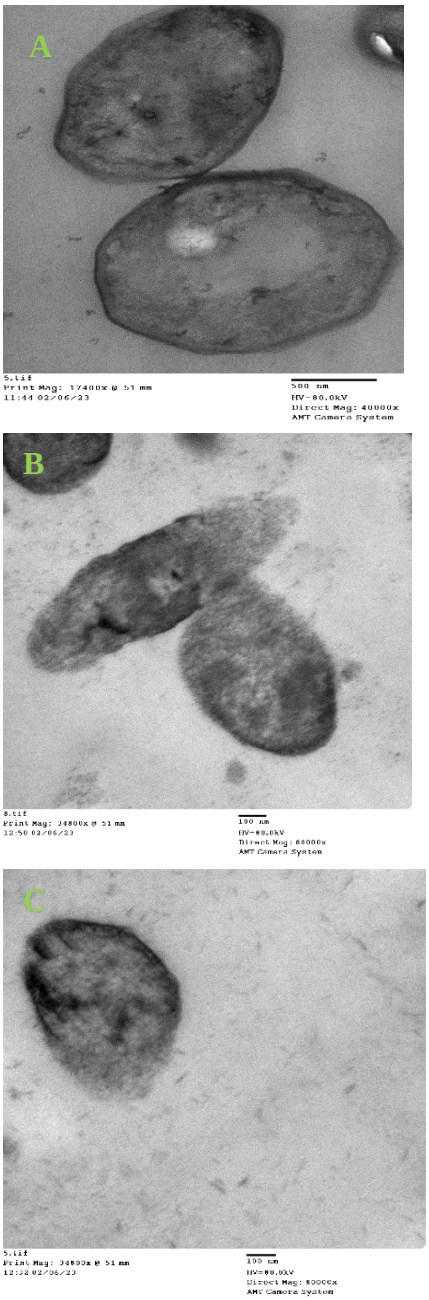
The antiviral impact of the TiO_2NPs versus Hepatitis A virus (HAV) when tested at maximum non-cytotoxic level was 37.24 ±1.7% with CC_{50} of 402.71±14.76 µg/ml relative to standard drug(Amantadine) which was 83.24±3.18% with CC_{50} of 5.67±0.41 µg/ml as depicted in (**Figure 10)**

4.10. Electron microscopy results

Antibacterial impact of TiO_2NPs versus *Proteus vulgaris* (ATCC 13315) was confirmed by electron microscopic examination as shown in (**Figure 11)**. Untreated *Proteus vulgaris* could be seen as organized cells with smooth outer surface layer and large vacuoles as well as well-structured internal organelles as shown as shown in (**Figure 11A**). While, treatment of *Proteus vulgaris* with TiO_2NPs destruct outer surface of bacterial cells and lysis to cellular organelles as shown in (**Figure 11B**) with similar impact that has been done by standard drug which shown in (**Figure 11C**).

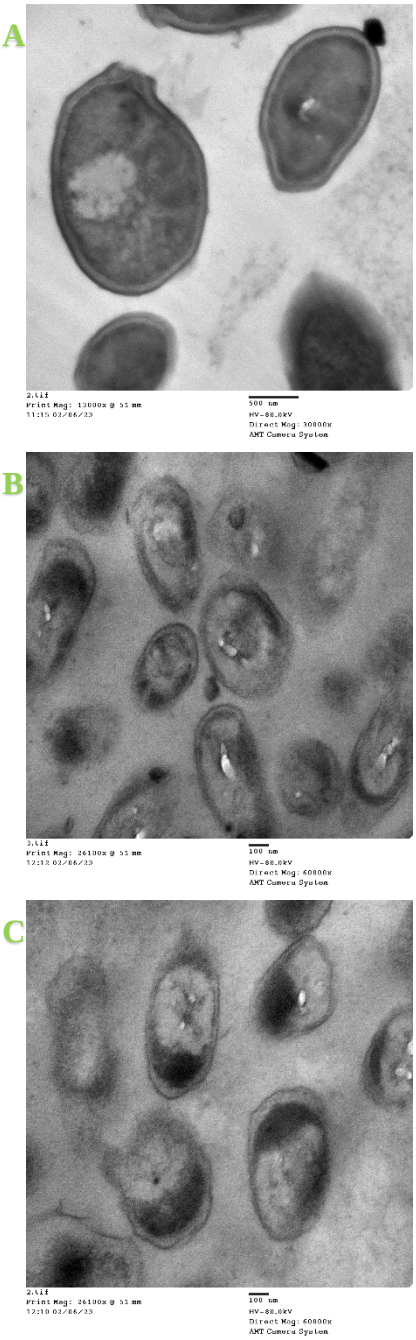


(Fig. 10) Antiviral impact of TiO_2NPs Where (A) Uninfected Vero cells; (B) Vero cells infected by HAV virus; (C) Vero cells infected by HAV virus and treated by TiO_2NPs; (D) Vero cells infected by HAV virus and treated by Standard drug (Magnification 100X).



(Fig. 11) TEM examination of *Proteus vulgaris* (A) Control cells; (B) Treated by TiO_2 NPs and (C) Treated by standrad dug.

While, anti-candida impact of TiO_2NPs versus *C. albicans* (ATCC 10231) were examined by transmission electron microscope as shown in (**Figure 12).** A thick and smooth outer surface of *C. albicans* in untreated cells (**Figure 12A**). While treatment either using standard drug or TiO_2 NPs lead to destruction of *C. albicans* cellular structure as shown in (**Figure 12B, C**) respectively.



(Fig. 12) TEM examination of *Candida albicans* (A) Control cells; (B) Treated by TiO_2 NPs and (C) Treated by standrad dug.

Furthermore, antiviral impact of TiO_2NPs versus HAV were detected as shown in (**Figure 13).** Where untreated virus could be seen as spherical particles. While treatment using either standard drug or TiO_2 NPs lead to destructed surface of virions as shown in (**Figure 13B, C**) respectively.

(Fig. 13) TEM examination of Hepatitis A virus (A) Control virus; (B) Treated by TiO_2 NPs and (C) Treated by standrad dug.

4.Discussion

The use of nanomaterials as medicines has become a cutting-edge method for slowing the progression of several deadly diseases [57]. Green nanoparticles were far less hazardous to cells than chemical nanoparticles, indicating that they are secure and might be applied broadly in therapeutic settings [58]. Several studies have demonstrated that pathogenic bacteria evolved protective mechanisms that made them more challenging to cure, such as resistance genes or genetic modifications, leading to prolonged infection with a higher death rate [59]. Because antimicrobial medications are so widely used, nosocomial infections have evolved from bacteria that were easily treated to bacteria that are quite resistant to treatment. The use of nanoparticles in the treatment against pathogenic microbes is currently being investigated as a potential antibiotic substitute [60].

Due to their unique physicochemical characteristics, titanium dioxide nanoparticles are widely used in catalysts, composite materials, beauty products, semiconductors, cuisine, paintings, plastics, skincare products, and sewage treatment systems [61]. Growing fear over TiO_2 NPs' possible effects on flora and wildlife has increased as a result of their presence in the environment [62]. Many microorganisms were used to create TiO_2 nanoparticles with antimicrobial impact [63].

In the present investigation the isolated *S. spinosa* was used to synthesize TiO_2 nanoparticles from titanium tetraisopropoxide. It has been reported that *S. spinosa* frequently produces tetracyclic macrolides that are powerful versus target insects with a high safety profile [64]. Kaur et al., [65] reported that TiO_2 NPs can be employed as inducers of soil mico-nutrients and microbiological movements at trace amounts.

In this work *S. spinosa* was identified using cultural, morphological and genetic tools and generation of phylogenetic tree indicating 99 % of similarity to this actinomycte. Several investigators employed molecular markers to pinpoint certain actinomycete strains that produced naturally helpful compounds [66]. The biosynthesized nanoparticles have been visually seen in the contained with white suspended particles and examined using UV-visible spectrophotometer to detect its wave length of 350 nm and spherical particles with mean diameter of 23.3 nm where Albukhaty et al., synthesize nanoparticle with diameter 14 nm with many promising applications [67].

Various methods of analysis including XRD, FTIR, EDX, particle size and zeta potential illustrated the size and functional groups of synthesized TiO_2 nanoparticles as documented tools of analysis [68]

In the present work titanium dioxide nanoparticles have shown promising antioxidant activity. In same line with

Akinola et al., [69] who reported anti-oxidative impact of titanium dioxide nanoparticles synthesized by *Cola nitida* extract. Furthermore, Mikusova et al., [70] illustrated the role of TiO_2 NPs on antioxidant defence in the blood of mice which impact immune system.

In the current investigation TiO_2 nanoparticles have efficient antitumor impact versus A549 cell line with minimal cytotoxicity to be applied with a notable safety profile. Behnam et al., [71] illustrated that TiO_2 NPs were efficient in overcoming skin tumour as therapeutic application in animals. Besides, Al-Shabib et al., [72] used *Withania somnifera* to produce TiO_2 NPs with successive toxicity versus HepG2 cell line.

In this work study titanium dioxide nanoparticles have promising antibacterial action versus *P. vulgaris* by lysis the outer surface of bacterial cells as seen by transmission electron microscopy. In same line with Khashan et al., [73] who reported impact of TiO2 Nanoparticles Prepared by Laser Ablation versus *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Besides, Nair et al., [74] illustrated that essential oil-loaded TiO_2 nanoparticles have synergistic antibacterial action.

In this investigation prepared TiO_2 nanoparticles showed successive anti- *C. albicans* role and destruct candidal cells. Seddighi et al., [75] evaluated antifungal action of iron- oxide nanoparticles versus various *Candida* strains showing its effective role in antifungal action. Besides, Shang et al., [76] confirmed the role of sheets of TiO_2 on polyurethane to kill - *C. albicans.*

The obtained TiO_2 nanoparticles showed efficient antiviral role versus HAV. There are many suggesting mechanisms of antiviral role direct viral interactions that stop the virus from infecting the cell and/or interactions with receptors or cell surfaces that stop the virus from penetrating host cells and/or blocking viral replication, or other programs that stop the virus from spreading [77]. Additionally, Miyauchi et al. [78] showed that the catalyst containing titanium dioxide decrease viral pathogenicity through its capping impact.

The present results reported the different possible applications of nanoparticles prepared by biological protocols as reported by other investigators [79-81].

5.Conclusions

The current work revealed that that *S. spinosa* could be used in synthesis of titanium oxide nanoparticles with antioxidant impact, antitumor impact versus alveolar cancer cell line activity by enhancing apoptosis, antibacterial impact versus *P. vulgaris* anti- *C. albicans* and anti-HAV impact for future *in vivo* and pharmaceutical applications.

6.Conflicts of interest

"There are no conflicts to declare".

7.Formatting of funding sources

Non applicable

8.Acknowledgments

Non applicable

9.References

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