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Green Synthesis, Characterization and Antimicrobial Activity of Titanium Dioxide Nanoparticles Using Laser Ablation Technique

Abstract- *Titanium dioxide nanaoparticles (TiO_2 NPs) were prepared using laser ablation technique by ablation of titanium target immersed in distilled de-ionized water (DDW) by Q-switched pulsed Nd:YAG laser of 1064 nm, 1 Hz, (490) mJ and 700 pulse. UV-visible spectrophotometer and Transmission Electron Microscopy (TEM) were used to characterize the optical and morphological properties of prepared nanoparticles, respectively. The absorption spectrum of TiO_2 NPs was at ultraviolet-region (214 nm) due to Surface Plasmon Resonance (SPR), and the particles size distribution of the prepared nanoparticles ranged from 30 to 100 nm. The antifungal activity of TiO_2 NPs was carried out against Microsporum canis. TiO_2 NPs showed significant inhibitory activity especially at high concentrations and high exposure times with microbial pathogenesis.*

Keywords- *M.canis*, TiO_2 NPs, Q-switched pulsed Nd:YAG laser.

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1. Introduction

Pathogenic *Microsporum canis* (*M.canis*) is a fungus called dermatophytosis which has the ability to invade keratinized tissue in domestic animals and human causes a superficial cutaneous infection in cornified skin layers, nails and hairs [1]. The main clinical appearances are regular alopecia with squamosis and erythema. The transmission of zoophilic dermatophytes coming from the environment and the haircoat of infected animals [2,3].

Nanoparticles (NPs) have unique chemical, biological, electronic and optical properties compared with bulk materials. For example, electrical conductivity, electrical resistivity, strength, hardness, diffusivity, chemical reactivity and biological activity of NPs are different to bulk counterparts [4,5]. Therefore, nanomaterials are candidate in many applications, such as medicine, microelectronics, heterogeneous catalysis, nonlinear optics, and gas sensor technology [6,7].

Titanium dioxide NPs (TiO_2 NPs) are a favorable material and used in various fields due to its high photo catalytic activity, high stability dielectric properties, and low cost. TiO_2 NPs have special magnetic, chemical and optical properties and they have toxic effects so used in dental implants, pharmaceutical products, cosmetics, catheters and packaging [8,9,10].

NPs synthesized using a laser ablation technique has lately regard a promising technique for several advantages: straightforward method, absence of surfactant (green technique), simple synthesis of NPs in liquid, pure colloidal NPs, and NPs synthesized by one–step [11]. The laser parameters, such as pulse width, pulse energy, pulse repetition rate, and laser wavelength effect on the characterization of the synthesized NPs, furthermore, the confined liquid is an important parameter [12].

2. Materials and Methods

I. Sample Preparation

Titanium plate was provided by Danyang Xinli Alloy Company, China. The metal purity (98.4%) was investigated using Energy dispersive X ray fluorescence (ED-XRF) model XEPOS as shown in Figure 1 and Table1. Titanium plate was cut into square-shaped plates with 1cm × 1cm dimensions. Each plate was polished using emery paper and cleaned with ethanol and DDW and to be ready for synthesis process.

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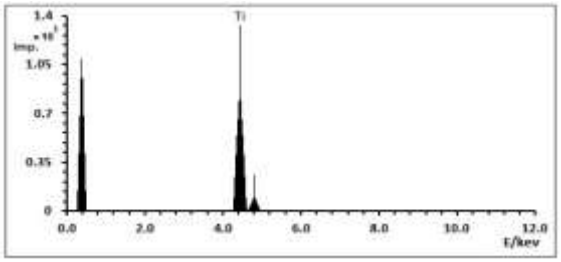


Figure 1: ED-XRF spectrum of titanium sheet

Table 1: Titanium analysis using ED-XRF

Symbol	Element	Concentration	Abs error
Mg	Magnesium	<0.027 %	0.0 %
Al	Aluminum	<0.0060 %	0.0 %
Si	Silicon	<0.0021 %	0.0 %
P	Phosphorus	<0.0011 %	0.00052 %
S	Sulfur	<0.0020 %	0.0 %
Ti	Titanium	98.40 %	0.14 %
V	Vanadium	<0.043 %	0.0 %
Cr	Chromium	<0.0032 %	0.0 %
Mn	Manganese	<0.0031 %	0.0022 %
Fe	Iron	0.0585 %	0.0043 %
Co	Cobalt	0.0052 %	0.0028 %
Ni	Nickel	0.0101 %	0.0021 %
Cu	Copper	0.0058 %	0.0015 %
Zn	Zinc	0.0121 %	0.0016 %
As	Arsenic	0.00364 %	0.00047 %
Zr	Zirconium	<0.043 %	0.0 %
Nb	Niobium	<0.0055 %	0.0 %
Mo	Molybdenum	0.469 %	0.045 %
Ag	Silver	0.0144 %	0.0078 %
Cd	Cadmium	0.0093 %	0.0050 %
Sn	Tin	<0.00035 %	0.0 %
Sb	Antimony	0.0095 %	0.0095 %
W	Tungsten	<0.0015 %	0.0 %
Pb	Lead	<0.00090 %	0.0 %
Sum of concentration		99.00 %	

II. Preparation of TiO_2 NPs

TiO_2 NPs were prepared using pulsed laser ablation (PLA) in DDW. Titanium plate with 1cm × 1cm dimensions was fixed at the bottom of cylindrical glass vessel containing 5mL from DDW. The immersed target was irradiated by Q-switched pulsed Nd:YAG laser at ambient pressure and room temperature. The details of laser beam parameters are summarized in Table 2 and the schematic diagram of experimental set up is shown in Figure 2.

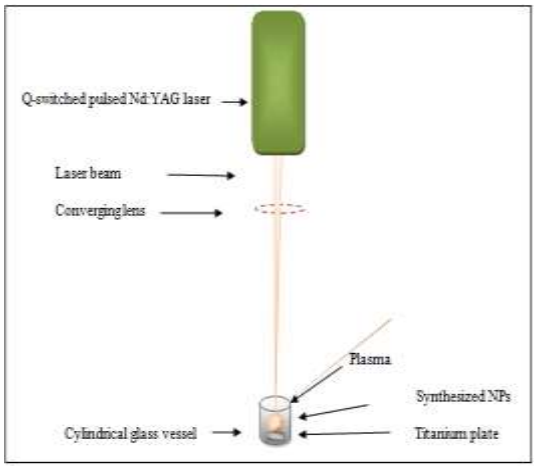


Figure 2: laser set up system

Table 2: The details of laser parameters of nanosecond pulsed Nd:YAG laser that used in this investigation

Laser parameters	Details
Wavelength	1064 nm
Laser energy	490 mJ
Repetition rate	1 Hz
Pulse duration	10 ns
Number of pulses	700 pulses
Focal length	10cm

III. Effect of TiO_2 NPs on M.canis

M.canis was diagnosed in Postgraduate Lab of Biological Department/Collage of Science/ Al Mustansiriyah University. The cellular structure of fungal was investigated using light microscope with digital camera/ XSZ-N107/ Korea.

The effect of prepared TiO_2 NPs against pathogenic fungus *M.canis* was studied using Pour plate method [13] with modification. The effect of TiO_2 NPs was tested by direct exposure of 1 µL of 1×10^5 cell/ml of fungal cell suspension with different concentrations (37, 75 and 150) µg/mL of prepared TiO_2 NPs for 1, 2 and 3 hrs. Sabouraud's dextrose agar (SDA) was prepared by dissolution 65mg/mL of SDA powder in 1000 mL of DDW supplemented with 10µg/ml amoxicillin and 5µg/mL cycloheximide then 25mL of the prepared SDA was poured in two Petri-dishes. Each Petri-dish was punched with 6 mm diameter and loaded with 1 µL of 1×10^5 cell/mL of fungal cell suspension and other holes were loaded with the treated fungal cell suspension (treated group) then incubated at 28ºC for six days. The colonies diameter of treated and untreated group was measured at third and sixth day. The inhibition rate (%) of *M.canis* was expressed as follows:

$$\text{Inhibition rate (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right)\times 100$$

Control = untreated colony diameter.

IV. Statistical Analysis

All data were statistically evaluated using SPSS version 16, ANOVAI and presented as M±SD with $p\leq0.05$ being Least Significant Differences (LSD).

3. Results and Discussion

I. Optical properties of TiO_2 NPs

The absorption spectra, type and concentration of the prepared TiO_2 NPs were determined by UV-Vis spectrophotometer.

The laser beam was irradiated at the immersed titanium target in 5 mL of DDW. The focal length, the number of applied pulses and the repetition rate and were 10 cm, 200 pulse and 1 Hz respectively

The laser beam was absorbed by the metal and exited with electrons. This process lead to smashing electron bonds followed by rapid ionization and vaporization in the liquid thus high temperature and pressure plasma created and the ionized atoms accelerate due to collisions inside the plasma [14,15]. Strong shockwave was generated and the plasma expanded adiabatically and mixed with the surrounding liquid [16]. Thus, visible metallic vapor appeared upon the metal surface and TiO_2 NPs colloidal formed so the liquid changed to gray color as shown in Figure 3. The peak position of the absorption spectrum of TiO_2 NPs was at ultraviolet-region (around 214 nm) due to SPR as shown in Figure 4.

Figure 3: TiO_2 NPs colloidal

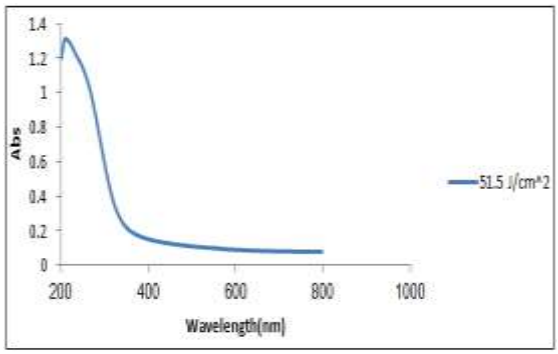
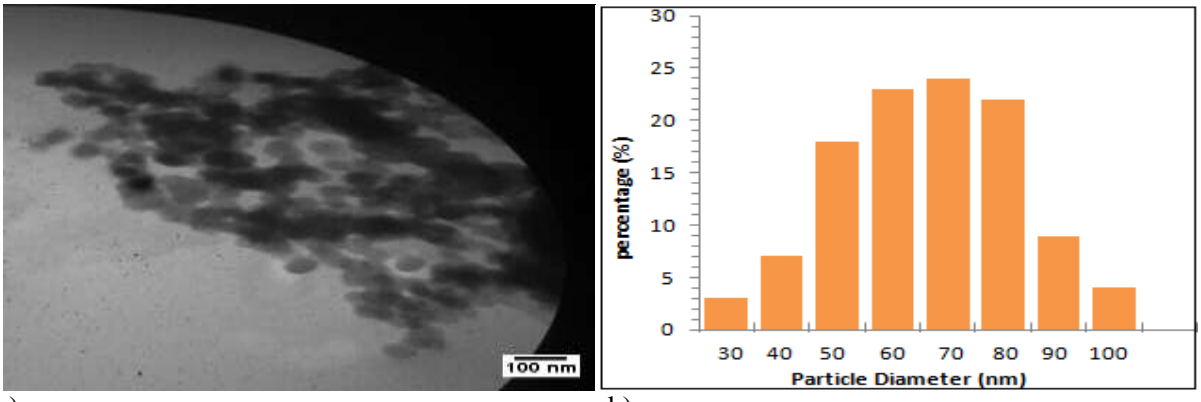


Figure 4: The absorption spectrum of TiO_2 NPs at 200 pulse, repetition rate=1 Hz, λ=1064nm and 43.4 JL/m^2 fluence

II. Morphological properties of TiO2 NPs

Morphology and the distribution of particle size of TiO2 NPs were characterized using Transmission Electron Microscopy (TEM). Figure 5 shows the crystalline shape of TiO2 NPs prepared using 1064 nm generated by Q-switched pulsed Nd:YAG laser and the particle size distribution which ranged from 30 to 100 nm.



a) b)

Figure 5: (a) The morphology and (b) the particles size distribution of TiO_2 NPs with 700 pulse at 490 mJ, 1Hz and 1064nm.

II.Antifungal effect of TiO_2 NPs

The antifungal effect of various concentrations (37, 75 and 150) µg/mL of prepared TiO_2 NPs was tested on the viability of the dermatophyte *M.canis*. The structure of fungal cell is shown in Figure 6. The *M.canis* fungal colonies diameter that their cells were treated with TiO_2 NPs reduced while the control group naturally grew fast as shown in Figure 7. The concentrations of TiO_2 NPs and the exposure times were the main factors that affected on the viability of pathogenic *M.canis*. Increase in the concentrations and the exposure times of TiO_2 NPs leading to significant decreasing in the colony diameter as shown in Table 3. The findings demonstrated that TiO_2 NPs

activity was increased with increasing in the TiO_2 NPs concentration and exposure time.

The antifungal activity of TiO_2 NPs attributed to their special properties such as high surface area, small size of particles that is similar to the biomolecules size, shape, coatings and charge of particles surface [10]. Chemical reactions occur between NPs and biomolecules cause reactive oxidative Stress (ROS) caused by hydrogen peroxide and hydroxyl radicals and contain superoxide radical (O_2^-), induce an imbalance in the macrobiomolecules leading to disruption in the cell membranes, proteins, enzyme, phospholipids, nucleic acids and DNA [17,18].

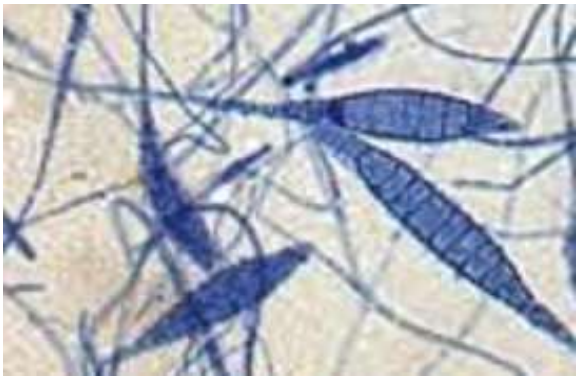


Figure 6: *M.canis* cellular structure

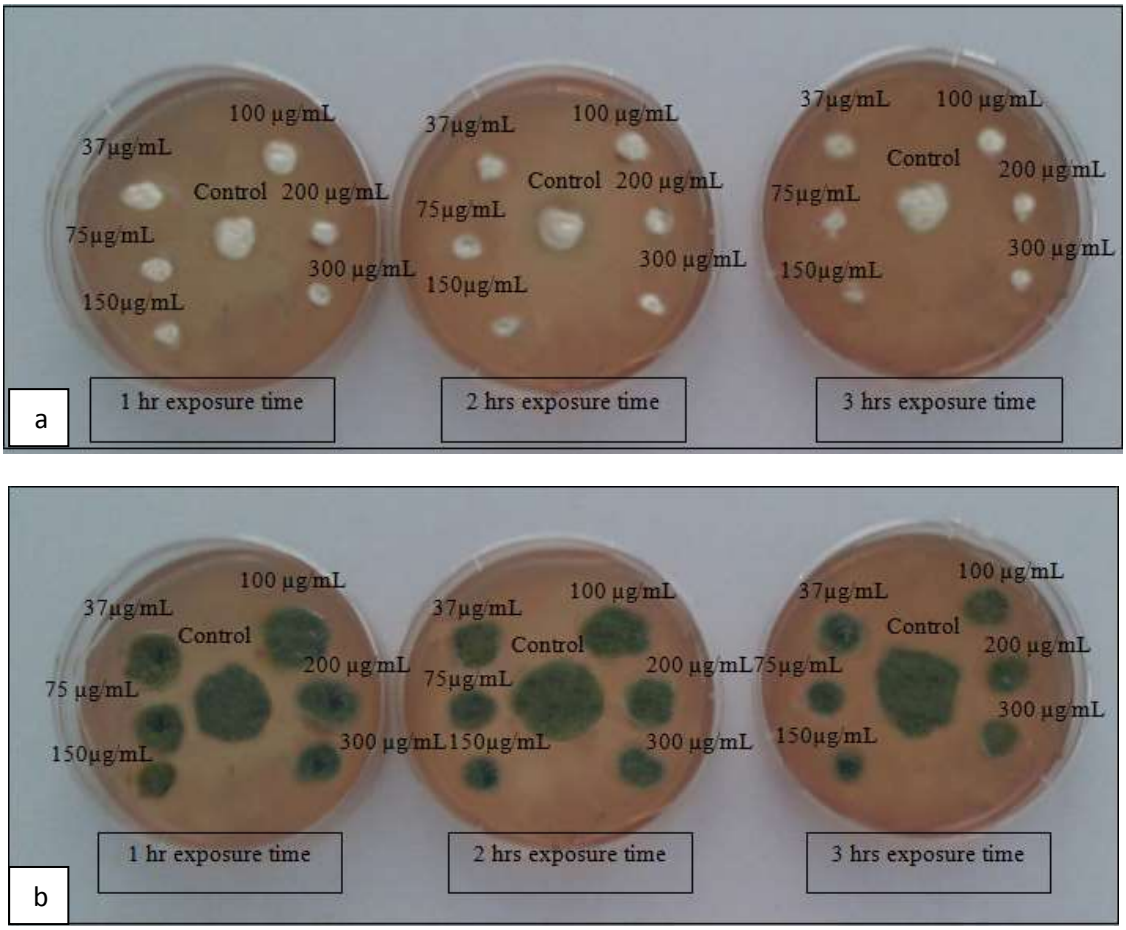


Figure 7: (a: The colonies diameter of control group and group of *M. canis* treated with different concentrations of TiO_2 NPs at different exposure times after three days incubation time and (b: the colonies diameter of control group and group of *M. canis* treated with different concentrations of TiO_2 NPs at different exposure times after six days incubation time.

Table 3: Colony diameter in centimeter of 1 µL of 1.0×10^5 cell/ mL of *M.canis* treated with different concentrations of synthesized TiO_2 NPs at different period times.

Times / Con µg/mL	Three-days incubation time			Six-days incubation time		
	1 hr exposure period	2 hr exposure period	3 hr exposure period	1 hr exposure period	2 hr exposure period	3 hr exposure period
0.0	1.3±0.1a	1.23±0.057a	1.16±0.08a	2.6±0.17a	2.6 ±0.28a	2.6 ±0.28a
37	0.56±0.057b	0.53±0.057b	0.46±0.057b	1.36±0.15b	1.3±0.1b	1.23±0.05b
75	0.53±0.057b	0.51±0.028b	0.43±0.057b	1.3±0.017b	1.26±0.11b	1.06±0.011b
150	0.41±0.028c	0.38±0.028c	0.33±0.057b	0,86±0.15c	0.66±0.057c	0.6±0.1c

- Each number represent M±SD of three replicate.
- Various letters in each column represent significant differences at ($p\leq 0.05$).

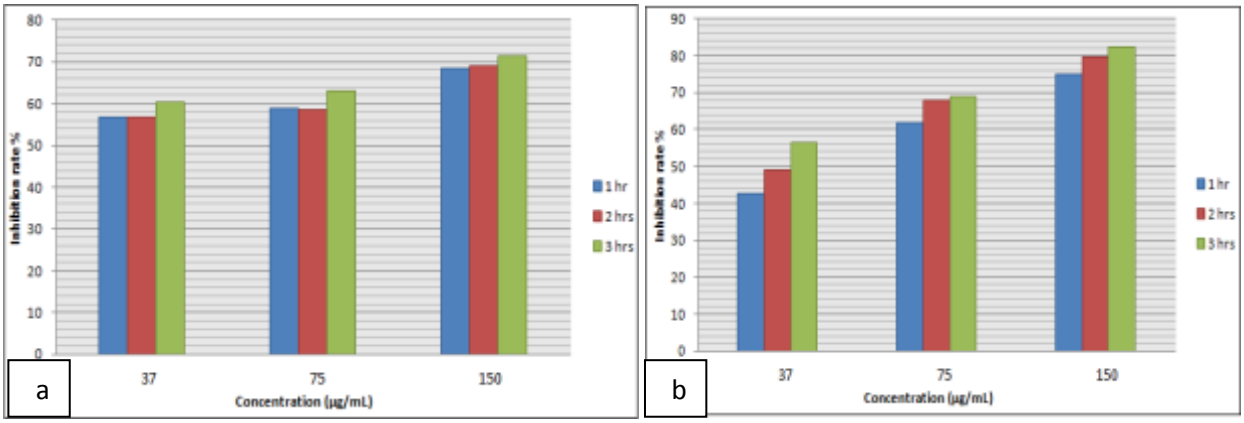


Figure 8: *M.canis* inhibition rate at different concentrations of TiO_2 NPs (0.0, 37, 75 and 150) µg/ mL and exposure periods (1 hr, 2 hrs and 3 hrs) at (a) three days incubation period (b) six days incubation period

4. Conclusion

Finding showed that the ablation via Q-switched Nd:YAG laser has adequacy to prepare TiO_2 NPs with 214 nm absorption spectrum, nearly spherical particles and suitable particle size (30-100nm). In vitro assay showed that prepared TiO_2 NPs have antifungal activity against *M.canis* viability by increasing the inhibition rate of colony diameter significantly at 150 µg/mL and 3 hrs.

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