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Copper Oxide NPs: Synthesis and their Anti-Dermatophyte Activity against Trichophyton rubrum

Abstract-*Trichophyton rubrum (T.rubrum) is a pathogenic dermatophyte that can causes fungal infection in keratinized layer of the human tissues such as skin, hair and nails. This work was carried out to study the antifungal activity of CuO nanoparticles (CuO NPs) on the viability of T.rubrum. CuO NPs have been synthesized by pulsed laser ablation of copper target immersed in liquid media using Q-switched pulsed Nd:YAG laser with 1064 nm. The optical properties and the surface charge of CuO NPs colloidal were characterized using UV–Vis spectrophotometer and Zeta potential techniques. UV–Vis spectrophotometer exhibited two peaks of absorption of CuO NPs colloidal: sharp peak at 200 nm and another peak at 630 nm. Zeta potential technique showed negative charge of CuO NPs colloidal (-28.16 mV). The morphological properties of CuO NPs such as particle size, shape and particle size distribution were characterized using Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM), the shapes were spherical and the particle size distribution was inhomogeneous which ranges between 20 to 180 nm. The synthesized CuO NPs presented suitable fungistatic activity against T.rubrum and its highest growth-inhibitory effectiveness was at high concentration (100 µg/ml) and high exposure time (3 hrs). Moreover, the inhibition rate of T. rubrum progressively increased with increasing CuO NPs concentration and exposure time.*

Keywords- Anti-dermatophyte Activity, CuO NPs, *T. rubrum*, Q-switched pulsed Nd:YAG laser.

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1. Introduction

Metal NPs exhibit unique and fascinating properties so they have a vast range of useful applications such as nanophotonic devices, sensors, optoelectronics devices, solar cells, storage of information, catalysis, drug delivery, cancer treatments and as antimicrobial agents [1,2]. Copper NPs have distinctive catalytic activity so they use in disinfecting and antimicrobial activity against the microorganisms such as bacteria, fungi and viruses [3,4].Pulsed laser ablation in liquid is a simple, non-catalyst and fast technique to fabricating and synthesizing pure colloidal metal NPs[5,6]. It includes the ablation of a metal target immersed in a liquid media with high power laser energy. The laser parameters such as laser wavelengths, laser pulse duration, repetition rate effect on the productivity and the particle size of the produced NPs [7,8]. The antimicrobial properties of NPs attributed to their size, shape, concentration, solubility, surface charge, stability and high surface area. The small size and high surface to volume ratio of NPs are the main properties for using it as antimicrobial agent. The size of the metal NPs is similar to the size of most biological molecules so the metal NPs easily penetrate the cellular membrane of the pathogenic microbe [9,10]. *T. rubrum* is a filamentous group of fungi called dermatophytes that responsible of causes most frequent dermatophytosis worldwide [11,12]. These fungi can invade the keratinized tissues in the epidermis and cause a variety of skin infections , including foot infection (tinea pedis), ringworm (tinea capitis) ,jock itch (tinea cruris) and nails infection (onychomycosis)[13,14].

2. Materials and Methods

Copper sheet was provided from Danyang Xinli Alloy Company, China .The sheet was cleaned and washed in ethanol and double distilled de-ionized water to remove the rust and impurities. The high purity Copper sheet (about 98.55%) was investigated by using XRF technique (model XEPOS) as shown in Figure 1 and Table 1.The sheet was cut to square-shaped sheets with dimensions of 1× 1 cm^2 be ready for NPs fabrication process .

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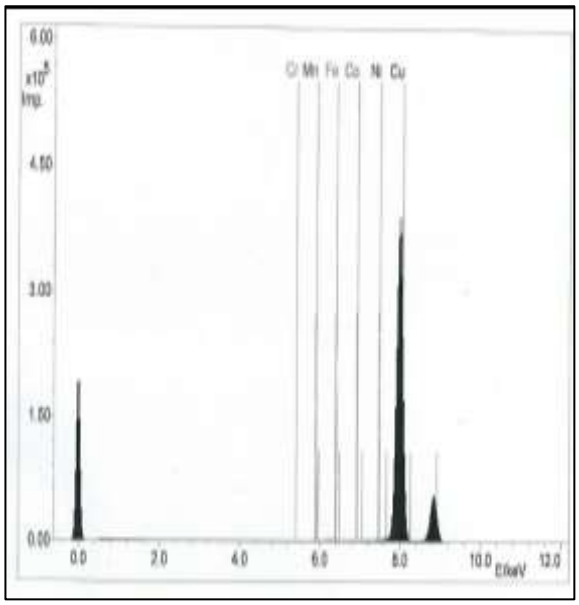

Figure1: XRF spectrum of Copper sheet

Table 1: Copper analysis by XRF

Z	Symbol	Element	Concentration		Abs. Error	
12	Mg	Magnesium	< 0.031	%	(0.0)	%
13	Al	Aluminum	0.0584	%	0.0080	%
14	Si	Silicon	< 0.0025	%	(0.0)	%
15	P	Phosphorus	< 0.0015	%	(0.0)	%
16	S	Sulfur	< 0.0020	%	(0.0)	%
22	Ti	Titanium	0.00514	%	0.00076	%
23	V	Vanadium	< 0.0020	%	(0.0)	%
24	Cr	Chromium	0.0443	%	0.0045	%
25	Mn	Manganese	0.0333	%	0.0031	%
26	Fe	Iron	0.0337	%	0.0023	%
27	Co	Cobalt	0.0253	%	0.0015	%
28	Ni	Nickel	0.0961	%	0.0058	%
29	Cu	Copper	98.55	%	0.12	%
30	Zn	Zinc	< 0.0046	%	(0.0)	%
33	As	Arsenic	< 0.00029	%	(0.0)	%
40	Zr	Zirconium	< 0.023	%	(0.0)	%
41	Nb	Niobium	< 0.0019	%	(0.0)	%
42	Mo	Molybdenum	0.152	%	0.017	%
47	Ag	Silver	0.02120	%	0.00368	%
48	Cd	Cadmium	< 0.00074	%	(0.0)	%
50	Sn	Tin	< 0.0015	%	(0.0)	%
51	Sb	Antimony	< 0.0023	%	(0.0)	%
74	W	Tungsten	< 0.0086	%	(0.0)	%
82	Pb	Lead	< 0.0011	%	(0.0)	%
Sum of concentration			99.00	%		

I. Pulsed laser ablation technique to synthesis Copper NPs

Copper piece was immersed in 5ml from double distilled de-ionized (DDDW) contained in a glass container and focused by Q-switched pulsed Nd:YAG laser as shown in Figure 2 .The characterizations of laser beam parameters are summarized in Table 2.

II. Isolate of dermatophyte

T. rubrum was obtained as diagnosed pathogen from Biological Postgraduate Lab, College of Science, Baghdad University.

III. Stocking and maintenance of T. rubrum isolate

Sabouraud's dextrose agar supplemented with (5µg/ml) cycloheximide and (10µg/ml) amoxicillin was used for stocking and maintenance of dermatophyte isolate, these media was prepared according to the manufacturer's instructions on the can, by dissolved 65gm of media powder in 1000ml DDDW, then the media sterilized by autoclave at 121ºC for 15 min.

IV. Influence of CuO NPs on T. rubrum viability

T. rubrum suspension (1× 10^5) cell/ml was exposed to different concentrations of prepared CuO NPs (25, 50 and100) µg/ml at (1, 2 and 3) hrs, all plates were incubated at 37ºC for seven days. Colony diameter was measured at both third and sixth days.

V. Statistical Analysis

The values were statistically analyzed by using SPSS software version 16. ANOVAI and least significant differences LSD at $p \leq 0.05$ was used to determine the significance between groups as well as descriptive analysis was used to calculate the percent of inhibition rate.

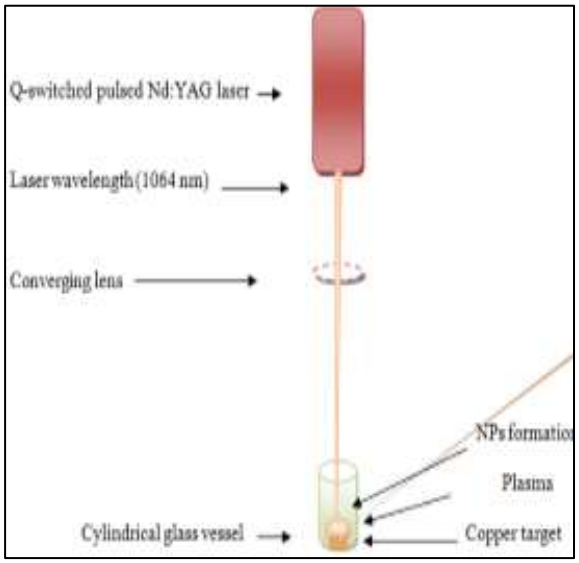

Figure 2: laser set up system

Table 2: Characterizations of laser beam parameters

Laser beam parameters	Characterizations
Laser fluence	43.4 J/cm^2
Pulse duration	10 ns
Number of pulses	500 pulse
Repetition rate	6 Hz
wavelength	1064 nm (Fundamental wavelength)

3. Results and Discussion

I. Optical properties

Absorption spectrum shape, peak position, type, homogeneity and productivity of the prepared colloidal were determined by using UV-Visible absorption spectrophotometer (Shimatzu SP8001).

Laser wavelength, laser fluence, number of laser pulses, repetition rate and the focal length were 1064 nm, 43.4 J/cm^2, 500 pulse,6 Hz and 10 cm respectively, as mentioned in Table 2.

The laser energy focused on the Cu metal sheet immersed in 5 ml DDDW. Initially the electrons in the electron subsystem absorb the laser energy, subsequently is transferred to the lattice and the electron hole (carriers) can absorb photons from incident laser beam by a phenomenon called Inverse Bremsstrahlung [15].

High pressure and temperature plasma was generated by absorption mechanisms followed by formation of shock waves, cavitation bubbles, when the pressure inside the cavitation bubbles reaches the maximum value the bubbles were collapsed (cooling phase of plasma), and the NPs diffused through the liquid media [16].

When the laser pulses were increased, the number of created particles in the solution increased and transparent solution changed to green colloidal (increase in the productivity of CuO NPs) accordingly increase in the colloidal concentration. UV-Vis spectrophotometer showed two peaks of absorption spectrum; sharp peak in the ultraviolet region (around 200 nm) due to the interband transition and the second peak at visible region (around 630nm) agrees with a green color of CuO NPs due to surface plasmon resonance oscillation as shown in Figure 3.

II. Zeta Potential

Figure 4 shows negative zeta potential of the prepared CuO NPs colloidal (-28.16 mv) by using Zeta potential analyzer (Zeta plus) device.

III. Morphology of CuO NPs

A. Transmission Electron Microscopy (TEM)

Particle size distribution and morphology of CuO NPs were characterized by Transmission Electron Microscopy (TEM) type CM10 pw6020, Philips-Germany. Spherical particles shape was obtained and particle size distribution ranged between 20 and 180 nm as shown in Figure 5 a and b.

B. Atomic Force Microscopy (AFM)

The surface morphology analysis was determined using an atomic force microscope (AA3000 Scanning Probe Microscope SPM, tip NSC35/AIBS). Figure 6 show 2-D, 3-D surface morphologies of prepared CuO NPs and the granularity distribution chart. The average dimeter was 103.65 nm.

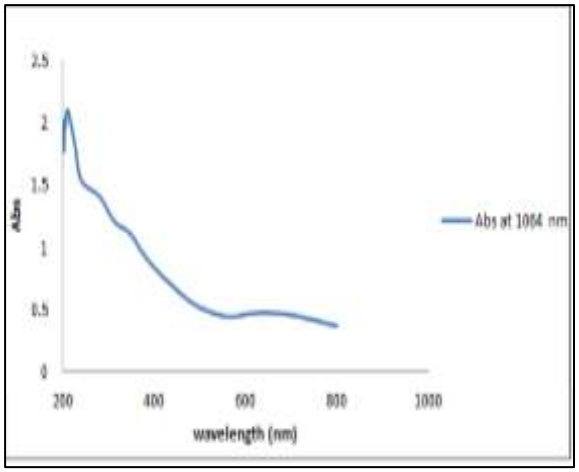

Figure 3: Absorption spectrum of CuO NPs prepared by PLA

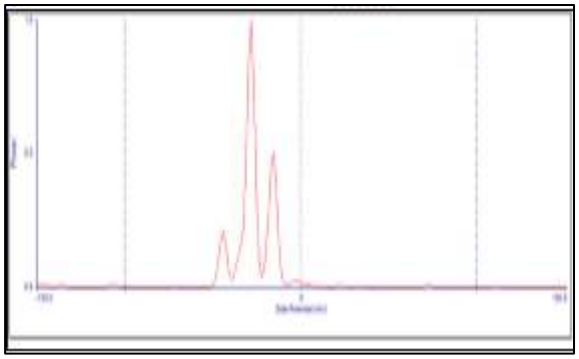

Figure 4: Zeta potential of prepared CuO NPs

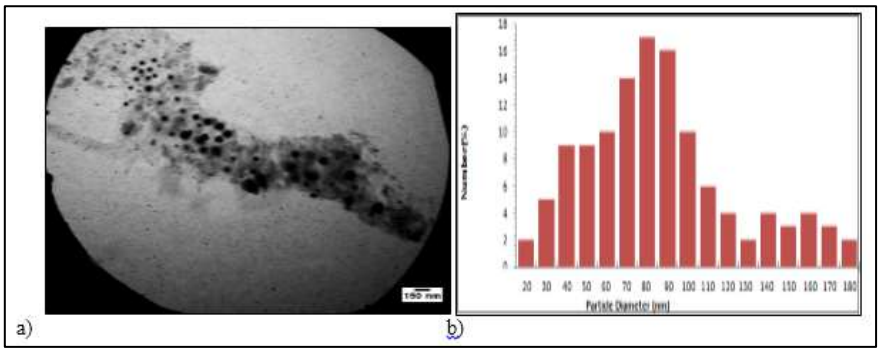

Figure 5: a) TEM image of CuO NPs prepared by PLA b) particle size distribution of CuO NPs

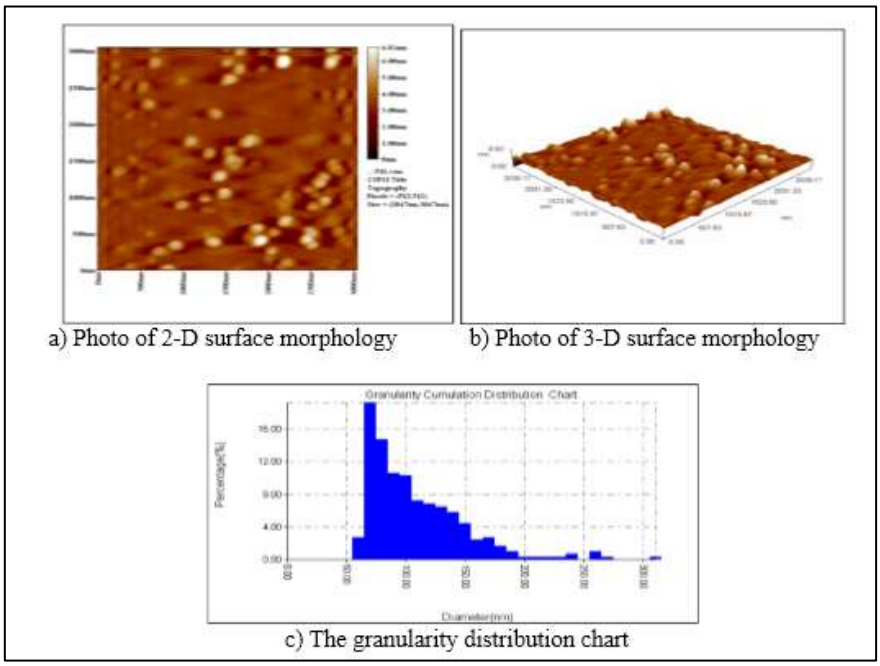

Figure 6: a AFM photos of 2-D surface morphology of prepared CuO NPs b AFM photo of 3-D surface morphology of prepared CuO c shows the granularity distribution chart

C. Fungistatic activity of CuO NPs

The fungistatic activity of CuO NPs was performed against *T. rubrum* pathogenic. *T. rubrum* of 1×10^5cell/ ml cell suspension was exposed to different concentrations (25,50 and 100) µg/ml of prepared CuO NPs at different exposure times (1 ,2 and 3) hrs then incubated in SDA for three and six days as shown in Figure 7 a and d. Comparing with the control groups and the lower CuO NPs concentrations, CuO NPs at 100 µg/ml showed a high growth inhibition effectiveness against *T. rubrum* colonies diameters, and significant growth inhibition was observed at the third exposure time(3 hrs exposure time) as shown in Figure 7 a_3 and b_3. Prepared CuO NPs showed a high growth-inhibitory activity against *T. rubrum* colonies at high concentrations and high exposure times and there was statistically significant inhibitory impact compared with the control groups as shown in Table 3. Moreover the inhibition rate of fungal colonies diameters that their cell suspension was exposed to prepared CuO NPs and incubated for three and six days progressively increased at (1 ,2 and 3) hrs exposure times as shown in Figure 8. The antifungal effect of CuO NPs attributed to their structure and intrinsic properties (physicochemical properties) such as the small particle size, composition, and high surface area to volume ratio, solubility, pH, concentration and copper ions. The NPs easily penetrate and rupture the microorganism membranes and disrupt the *cellular* organization and viability. Metallic ions of NPs generate oxidative stress (ROS) by free radical generation such as hydroxyl radicals through nano-bio interaction and may drive secondary membrane damage, restrain protein action and cause DNA damage [9,17,18].

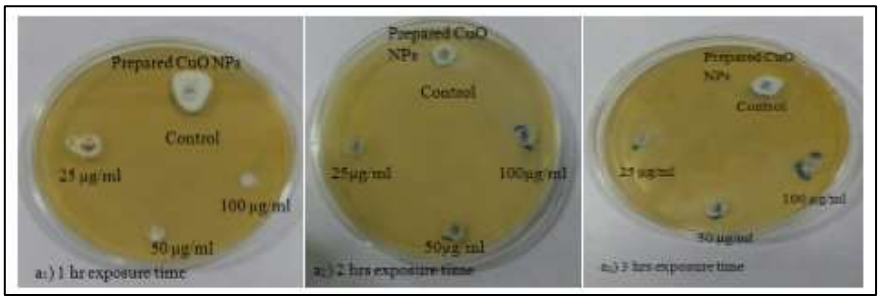

Figure 7: a) The colonies diameters of control group and treated group of *T. rubrum* at different exposure times (a_1, a_2 and a_3) after three days incubation time

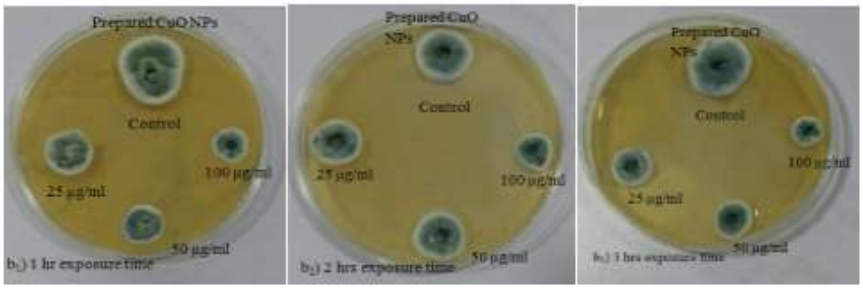

Figure 7: b) The colonies diameters of control group and treated group of *T. rubrum* at different exposure times (b_1, b_2 and b_3) after three days incubation time

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

Table 3: Colony diameter in centimeter of 1×10^5cell/ ml of *T. rubrum* treated with different concentration of prepared CuO nano- particles at different exposure time

Times	Three-day incubation time			Six-day incubation time		
Con µg/ml	1 hr exposure time	2 hr exposure time	3 hr exposure time	1 hr exposure time	2 hr exposure time	3 hr exposure time
0.0	1.13±0.32a	0.93±0.057a	1.06±0.37a	2.5 ±0.25a	2.3±0.26a	2.6 ±0.31a
25	0.53±0.15b	0.41±0.057b	0.36±0.028b	1.4±0.36b	1.06±0.11b	1±0.1b
50	0.46±0.057c	0.38±0.076c	0.33±0.057c	1.2±0.26c	1.03±0.057b	0.96±0.057b
100	0.46±0.057c	0.36±0.057c	0.31±0.027c	1.06±0.2d	1±0.1b	0.9±0.1b

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

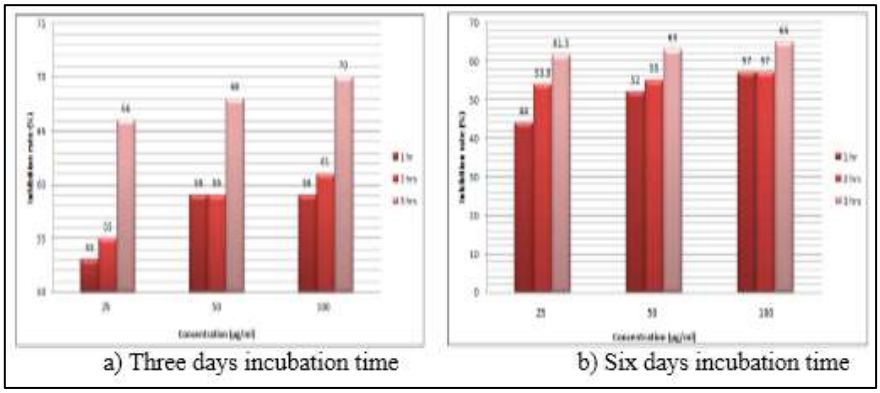

Figure 8: Inhibition rate of exposed T. rubrum to prepared CuO NPs at different concentrations (25, 50 and 100) µg/ ml and exposure times (1 hr, 2hrs and 3 hrs) (a). Three days incubation time (b). Six days incubation time

4. Conclusion

Findings demonstrated that the ablation via Q-switched pulsed Nd:YAG laser technique has efficiency to produce CuO NPs colloidal with two peaks of absorption spectrum(sharp peak in 200nm and another peak in 630 nm), spherical particles shape, suitable particle size (20-180nm) and negative zeta potential (-28.16). In vitro study exhibited that these CuO NPs have potential

effect on viability of *T. rubrum* by increasing their inhibition rate, significantly at highest concentration (100 µg/ml) and highest exposure time (3 hrs) .

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References

[1] J. Ramyadevi, K. eyasubramanian, A. Marikani, G. Rajakumar, A. Abdul Rahuman, "Synthesis and Antimicrobial Activity of Copper Nanoparticles," *Materials Letters*, Vol. 71, 114–116, 2012.

[2] C. Chen, Chan, Tzu-Min, Wu,Yi-Jhen , Chen,Jia-Jin, "Review: Application of Nanoparticles in Urothelial Cancer of the Urinary," *Bladder, J. Med. Biol. Eng*, Vol.35, 419-427, 2015.

[3] M. Muniz-Miranda, C. Gellini, A. Simonelli, M. Tiberi, F. Giammanco, and E. Giorgetti, "Characterization of Copper Nanoparticles Obtained by Laser Ablation in Liquids," *Applied Physics A*, Vol. 110, 829-833, 2013.

[4] D. Longano, N. Ditaranto, N. Cioffi, F. Di Niso, T. Sibillano, A. Ancona, A. Conte, M.A. Del Nobile, L. Sabbatini and L. Torsi, "Analytical Characterization of Laser-Generated Copper Nanoparticles for Antibacterial Composite Food Packaging," *Analytical and Bioanalytical Chemistry*, Vol. 403, 1179–1186, 2012.

[5] AK. Ali, "One-Step Synthesis of Copper Oxide Nanoparticles Using Pulsed Laser Ablation in Water: Influence of the Laser Wavelengths on Optical Properties," *Engineering and Technology Journal*, Vol.31, Part B, No.7, 2013.

[6] A.K. AL-Ogaili, A.K. Ali and TH. Ali, "Preparation of Silver Nanoparticles and Study the Optical and Antibacterial Properties," *Engineering and Technology Journal*, Vol.33, Part B, No.3, 2015.

[7] A. Hamad, L. Li, and Z. Liu, "A Comparison of the Characteristics of Nanosecond, Picosecond and Femtosecond lasers Generated Ag, TiO2 and Au Nanoparticles in Deionized Water," *Applied Physics A*, Vol. 120, 1247–1260, 2015.

[8] M. Maciulevičius, A. Vinčiūnas, M. Brikas, A. Butsen, N. Tarasenka, N. Tarasenko, and G. Račiukaitis, "Pulsed-Laser Generation of Gold Nanoparticles with on-Line Surface Plasmon Resonance Detection," *Applied Physics A*, Vol. 111, 289-295, 2013.

[9] Y. Chang, M. Zhang, L. Xia, J. Zhang, and G.Xing, "The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles," *Materials*, Vol.5, 2850-2871, 2012.

[10] A. Sirelkhatim, S. Mahmud, A.Seeni, NH. Kaus, L.C. Ann, SK. Bakhori, H. Hasan, and DW. Mohamad, "Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism," *Nano-Micro Lett*, Vol.7, No.3, 219–242, 2015.

[11] L.A. García-Madrid, MR. Huizar-López, L. Flores-Romo, and AE. Islas-Rodríguez, "Trichophyton rubrum Manipulates the Innate Immune Functions of Human Keratinocytes," *Eur. J. Biol*. Vol.6, No.6, 902-910, 2011.

[12] V.K. Bhatia and PC. Sharma, "Epidemiological Studies on Dermatophytosis in Human Patients in Himachal Pradesh, India," *Bhatia and Sharma Springer Plus*, Vol. 134, No.3, 2014.

[13] F. Aala, R. Nulit, U.S. Yusuf, and Rezaie, "Conventional and Molecular characterization of *Trichophyton rubrum*," *African Journal of Microbiology Research*, Vol. 6, No. 36, 6502-6516, 2012.

[14] T.O. Adejumo and B.S. Amidala, "Control of Dermatophyte-Causing Agents (Trichophyton Mentagrophytes and Trichophyton Rubrum) Using Six Medicinal Plants," *Journal of Medicinal Plants Research*, Vol. 3, No. 11, 906-913, 2009.

[15]GC. Messina, "In Liquid Laser Processes Nanoparticles Synthesis and Manipulation," University of Catania, Ph.D Thesis, 2012.

[16] M. Dell'Aglio, R. Gaudiuso, O. De Pascale, and A. De Giacomo, "Mechanisms and Processes of Pulsed Laser Ablation in Liquids During Nanoparticle Production," *Applied Surface Science*, Vol. 348, 4-9, 2015.

[17] Yah, CS and Simate, GS, "Nanoparticles as Potential New Generation Broad Spectrum Antimicrobial Agents," *DARU Journal of Pharmaceutical Sciences*, 23:43, 2015.

[18] G. Shobha, V. Moses, and S, Ananda "Biological Synthesis of Copper Nanoparticles and its Impact - a Review," *International Journal of Pharmaceutical Science Invention*, Vol.3, No.8, 28-38, 2014.

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