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## Anticandidal Activity of Green Synthesized Zinc Oxide Nanoparticles Using Lemon Peel Extract

Reham Said Metwally<sup>1</sup>, Zeinab M.H. Kheiralla<sup>1</sup>, Sanaa M. Ashour<sup>1</sup>, Sanaa S. Zaki<sup>1</sup>

<sup>1</sup> Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

### Abstract

Nanobiotechnology has developed as an effective technology for developing antimicrobial nanoparticles in an environmentally safe manner. In this study, green synthesized zinc oxide nanoparticles (ZnO NPS) from zinc acetate solution by using lemon peels aqueous extract was characterized by UV–Visible Spectroscopy, High-resolution Transmission Electron Microscopy (HR-TEM) and Dynamic Light Scattering (DLS). Anticandidal activity was investigated against three clinical multidrug resistant *Candida* species including two *Candida albicans*, one *Candida glabrata* and one *Candida krusei* using four antifungal agents by disc diffusion method and antifungal activity of ZnO NPS was assayed by disc diffusion method and determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Characterization studies revealed that the synthesized nanoparticles have rod shape with sizes of 13.58 - 30.70 nm. Notably, high rates of resistance were observed with the four tested antifungal agents against all *Candida* species and the antifungal activity of the synthesized ZnO NPS against *Candida* species were exhibited, with a maximum zone of inhibition of  $24.5 \pm 0.5$  mm against *C. glabrata* followed by *C. albicans* ( $19.5 \pm 0.5$  mm) and *C. krusei* ( $16.0 \pm 0.0$  mm). MIC and MFC for all *Candida* species were 0.25 and 0.5 mg/ml respectively. The cytotoxic data indicates that ZnO NPs have half maximal inhibitory concentration (IC<sub>50</sub>) value =  $230.12 \pm 9.34$  µg/ml on normal human lung fibroblast cell line (MRC5). In conclusion, the study elucidates that lemon peels mediated green synthesized zinc oxide nanoparticles have antifungal activity against different *Candida* species. So that it can be developed as a novel medicine for the treatment of *Candida* associated infections in the near future.

**Keywords:** Lemon fruit peel, ZnO nanoparticles, Green synthesis, Anticandidal activity.

### Introduction

Candidiasis is the most common fungal disease that affects immunocompromised and healthy people around the world [1,2,3,4]. There are several types of candidiasis such as mucosal candidiasis, cutaneous candidiasis, onychomycosis and systemic candidiasis [5,6,7]. Candidemia

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\*Corresponding author: Reham Said Metwally, Botany Department, Faculty of Women for Arts, Science and Education, Ain-Shams University, Cairo, Egypt.

E-mail: [reham.sa3eed@gmail.com](mailto:reham.sa3eed@gmail.com)

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which is another infection caused by *Candida spp.* is the most common nosocomial infection, representing 15% of bloodstream infections and 50-70% of systemic fungal infections with high mortality rate (49%) in immunocompromised patients worldwide [8,9,10,11]. *Candida spp.* are the fourth leading cause of hospital acquired infection, *Candida albicans* is the most common cause of all types of candidiasis [12], non-albicans species include *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*. *Candida glabrata* is the second or third most prevalent cause of candidiasis after *Candida albicans* [13].

Azoles are the preferred and most frequently used drugs among the available antifungal agents for treatment of candidiasis. Depending on the infection type, the anatomical site of infection and the susceptibility profile of species, other antifungals as echinocandins, polyenes, nucleoside analogs and allylamines can also be used [14,15,16]. Fluconazole (a type of azole) is often preferred in treatments of candidiasis due to its low toxicity and cost, in addition to availability in different formulations [17]. However, there are many reports in the literature on the development of resistance among *Candida spp.*, especially in relation to azoles. The need of the hour is the development of a new effective medicine, as the phenomenon of resistance caused the appearance of new fungal infections and facilitated the resurgence of the existing ones as well. So that, the control of *Candida* infections is a challenge in the modern clinic and the search for new medical treatments is fundamental to face the challenge [18].

The use of nanoparticles to prevent *C. albicans* growth is one example of an innovative strategy. Nanomaterials showed enhanced unique properties [19,20] and are widely employed as antimicrobial agents like antifungal and antibacterial activities against different types of diseases causing pathogens [21,22,23,24]. Among all types of nanoparticles, metal oxide nanoparticles have high antimicrobial activity due to their increased surface area to volume ratio [25].

Zinc oxide nanoparticles (ZnO NPs) have a wide range of applications in numerous sectors of science, and they are also economical to manufacture, safe, easy, and ecofriendly [26,27]. Due to their distinctive features, as UV light absorption, they are widely employed in health care commercial items [28]. In comparison to other antibacterial agents, ZnO NPs offer outstanding stability, resilience and biocompatibility [13]. Zinc oxide nanoparticles are generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA, 21CFR182.8991) and are potentially applicable to treat infectious diseases [29]. Green synthesis of zinc oxide nanoparticles is of great importance, and it has many advantages, such

as requiring moderate reaction conditions, requiring no toxic chemicals, cheap in cost and ecofriendly compared to expensive physical and chemical methods that have many disadvantages such as high temperature and pressure requirements, toxic effects that damage the environment, etc. [30,12]. So, green synthesis is very desirable.

The antibacterial efficacy of crude extracts against clinically significant bacterial strains has been documented for *Citrus limon* (*Rutaceae*), widely known as lemon. It is an important medicinal plant, used mostly for its alkaloids, which have anticancer effects [31]. Metal and metal oxide NPs, such as gold NPs, silver NPs, and zinc oxide NPs, have been successfully produced using citrus fruit or peel extracts [32]. Thence, the present study has investigated the green synthesis and characterization of ZnO nanoparticles of lemon peels, evaluated the anticandidal potency against three clinical *Candida* species and investigated the cytotoxicity activity of the synthesized nanoparticles.

## Materials and Methods

### 1. Preparation of plant extract:

Fresh healthy lemon fruits were collected from local market, Cairo, Egypt. Green and yellow lemon fruits' peels were used for synthesis of zinc oxide nanoparticles (ZnO NPs). Fruits' peels were rinsed under running water then, it was washed with double-distilled water, cut into small pieces, and dried in the oven at 50 °C for 24h then, homogenized to fine powder and stored in a properly labelled bottle for further use. Five grams of powdered peels of lemon were boiled in 100 ml sterile distilled water at 100°C for 1h. After that, the extracts were cooled, filtered with muslin cloth and then filtered with Whatman No. 1 filter paper and kept in the refrigerator at 4 °C for further tests [33].

### 2. Green synthesis of zinc oxide nanoparticles

Fifty ml of lemon peels extract was mixed with 1 M zinc acetate solution, after 10 min of stirring 0.2M of NaOH solution was added drop by drop to the mixture to adjust the pH until reach to 10. The mixture was stirred for 1 h at room temperature then, the mixture was placed in water bath on a hot plate with magnetic stirrer for 2 h at 75 – 80 °C up to change in the colour. Change of colour from yellowish green to white precipitate indicating the formation of ZnO NPs. The resultant ZnO NPs were separated out by centrifugation at 4000 rpm for 15 min and dried in a hot air oven at 50 °C for 1 h [34].

### 3. Characterization of the Synthesized Zinc Oxide Nanoparticles

#### 3.1- UV-Vis spectrophotometric analysis

UV-Visible Spectroscopy for preliminary characterization of synthesized nanoparticles has proven to be a highly effective approach for nanoparticle investigation and depended on shape, size and distribution of nanoparticles. Knowing the surface Plasmon resonance property of nanoparticles. The green synthesized nanoparticles were characterized by UV-Visible Spectrophotometer (Labomed model UV-Vis Double beam spectrophotometer) with the wavelength ranging from 200 to 800 nm, that the surface was subjected to the measurement of absorbance [35].

#### 3.2- High resolution transmission electron microscope

The size and morphology of the formed nanoparticles were studied using a High Resolution Transmission Electron Microscope (HRTEM) model JEOL JEM-2100 (Japan) at the Egyptian Petroleum Research Institute. Transmission Electron Microscope samples were prepared by sonicating the powder in ethanol and evaporating one drop onto a holey carbon coated cooper grid film [36].

#### 3.3- Dynamic Light Scattering (DLS)

The average size of particles and particles distribution were recorded on Zetasizer ZS Model, Malvern, UK. This technique measures the diffusion of particles moving under Brownian motion. Measurement of size as a function of concentration enables the calculation of  $kD$ , the DLS interaction parameter [37].

### 4. Microbiological Experiments

#### 4.1- Fungal cultures:

Three clinical identified *Candida* species including two *Candida albicans*, one *Candida glabrata* and one *Candida krusei*, that were isolated from urine samples collected from inpatients in Nephrology Department at Theodor Bilharz Research Institute Hospital, Giza, Egypt, were used [38]. Working cultures were kept at 4 °C on universal agar slants [39,40]. For long term preservation, the cultures were stored in vial tubes containing 1 ml aliquot plus 20% glycerol at -20°C.

#### 4.2- Antifungal susceptibility test

*Candida* isolates were grown at 37°C for 24 h on Sabouraud Dextrose agar (Oxoid) plates. Each *Candida* isolate was suspended in sterile saline solution (0.85% NaCl) to obtain 0.5 McFarland suspension. Plates of Muller Hinton agar (Oxoid) supplemented with 2% glucose and 0.5 µg/ml methylene blue were inoculated using a sterile swab to produce a confluent lawn of growth. Four antifungal disks (Oxoid) were applied, Fluconazole (**Flu**) 25 µg, Itraconazole (**Itr**) 10 µg, Miconazole (**Mcz**) 10 µg and Voriconazole (**Vor**) 1 µg. Plates were incubated at 37 °C for 24 h after which zones of inhibition were measured [41]. The interpretive criteria for the antifungal susceptibility test results were those published by [42].

**Table (1): Interpretation of the *in vitro* antifungal susceptibility test**

Group	Mode of action	Antifungal	Symbol	Disk concentration	Resistant (R) mm	Intermediate (M) mm	Susceptible (S) Mm
Azole	affecting cell growth and proliferation	Miconazole	MCZ	10 µg	≤11	19-12	≥20
		Fluconazole	FLU	µg25	≤14	18-15	≥19
		Itraconazole	ITR	µg10	<13	22-14	≥23
		Voriconazole	VOR	1 µg	≤13	16-14	≥17

#### 4.3- Assessment of antifungal activity of zinc oxide nanoparticles

The antifungal activity of ZnO NPs was evaluated against the tested three *Candida* species by agar well diffusion assay according to [43] with slight modification. The inoculum of overnight cultures was prepared to a turbidity equivalent to 0.5 McFarland. Afterwards, the tested yeasts were uniformly swabbed over Muller Hinton agar plates' surface. Using a sterile cork borer, 6 mm wells were made and filled with 100 µl of 1mg/ml of ZnO NPs suspended in dimethyl sulfoxide (DMSO) and 100 µl of DMSO and plant extract as a negative and positive control respectively. The plates were incubated at 37°C for 48 h then the diameter of inhibition zones was measured in millimeter and recorded. All assays were carried out in triplicate.

#### 4.4- Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Minimum inhibitory concentration was recorded as the lowest concentration of antifungal agent that prevented the visible microbial growth as indicated by the absence of turbidity in line with the control. Minimum fungicidal concentration is the lowest concentration of antifungal agent that kills >99.9% of the fungal cells of the initial microbial population. Based on the method of [44], the double dilution method was used to determine MIC of the tested microorganisms. The dosing range of ZnO NPs used was between 125 and 1000 µg/ml. Each tube was inoculated with 100 µl of an overnight culture of appropriate yeast suspension with turbidity equivalent to 0.5 McFarland. Tubes containing Sabouraud Dextrose broth inoculated with *Candida* suspensions were used as positive control while tubes containing Sabouraud Dextrose broth with ZnO NPs were used as negative control. All tubes were incubated at 37 °C for 48h. After incubation, the MFC value was determined by subculturing a loop full from all tubes in which no visible *Candida* growth was observed on Sabouraud Dextrose agar plates that were incubated at 37°C for 48 h [45].

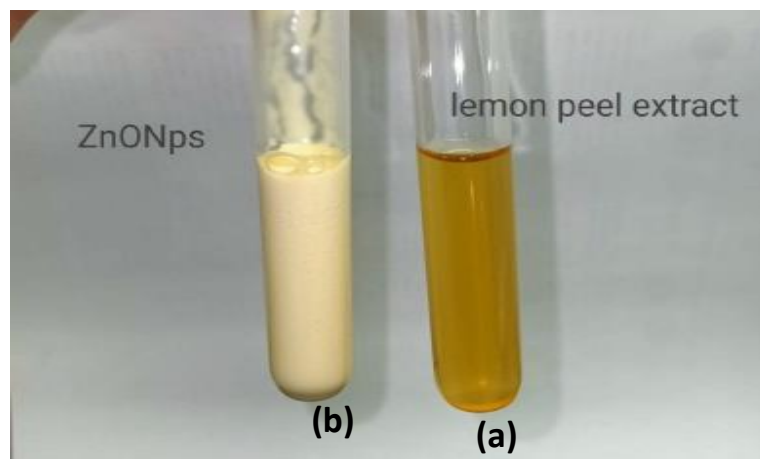
#### 4.5 - Cytotoxicity assay:

The *in vitro* cytotoxic activity evaluation of the synthesized ZnO NPs was carried out in normal human lung fibroblast (MRC- 5 cell line) by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay according to the procedure adopted by [46,47]. The cells were seeded in 96-well plates (Falcon, Albany, NY, USA) at the density of  $1 \times 10^4$  viable cells / well in 100 µl of Dulbecco's modified Eagle's culture medium supplemented with 10 % fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/ml gentamycin and incubated in a humidified incubator with 5 % CO<sub>2</sub> at 37°C for 24h to allow cell attachment. Following attachment, the medium was replaced with complete medium (150 µl /well) containing different concentrations of ZnO NPs (1–500 µg/ml) and incubated for 48 h. Control cells were incubated without test samples. Following treatment, the cells were washed with phosphate buffered saline and incubated with 100 µl /well fresh medium containing 5 mg/ml MTT. The MTT-containing medium was removed after 4 h of incubation in dark condition. After that, the MTT formazan was dissolved by the addition of DMSO (100 µl /well). Then, the optical density was determined at 590 nm using an ELISA plate reader (SunRise, TECAN, Inc, USA). Cell viability was calculated by the following equation:

(%) =  $[(\text{ODt}/\text{ODc})] \times 100$  where ODt is the mean optical density of treated wells and ODc is the mean optical density of untreated cells.

## Results and Discussion

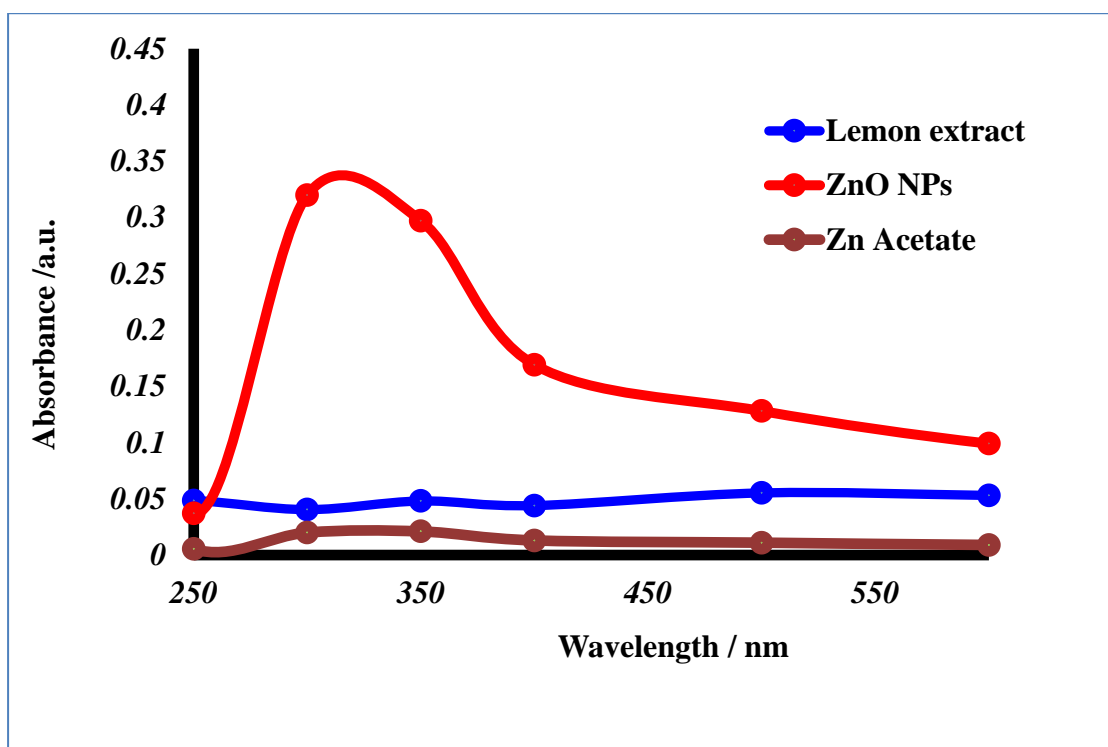
For zinc oxide nanoparticles synthesis, when lemon peels' extract is added to a 1M zinc acetate solution, the colour of the reaction solution changes. Lemon peels extract changed into yellowish white precipitate which represents the synthesis of ZnO NPs (Fig. 1 a & b). Lemon peels' extract was used as a reducing agent as well as a surface stabilizing agent for the synthesis of ZnO NPs.



**Fig. 1:** Visual observation of zinc oxide nanoparticles synthesis: (a) lemon peels extract  
(b) yellowish white precipitate of zinc oxide nanoparticles.

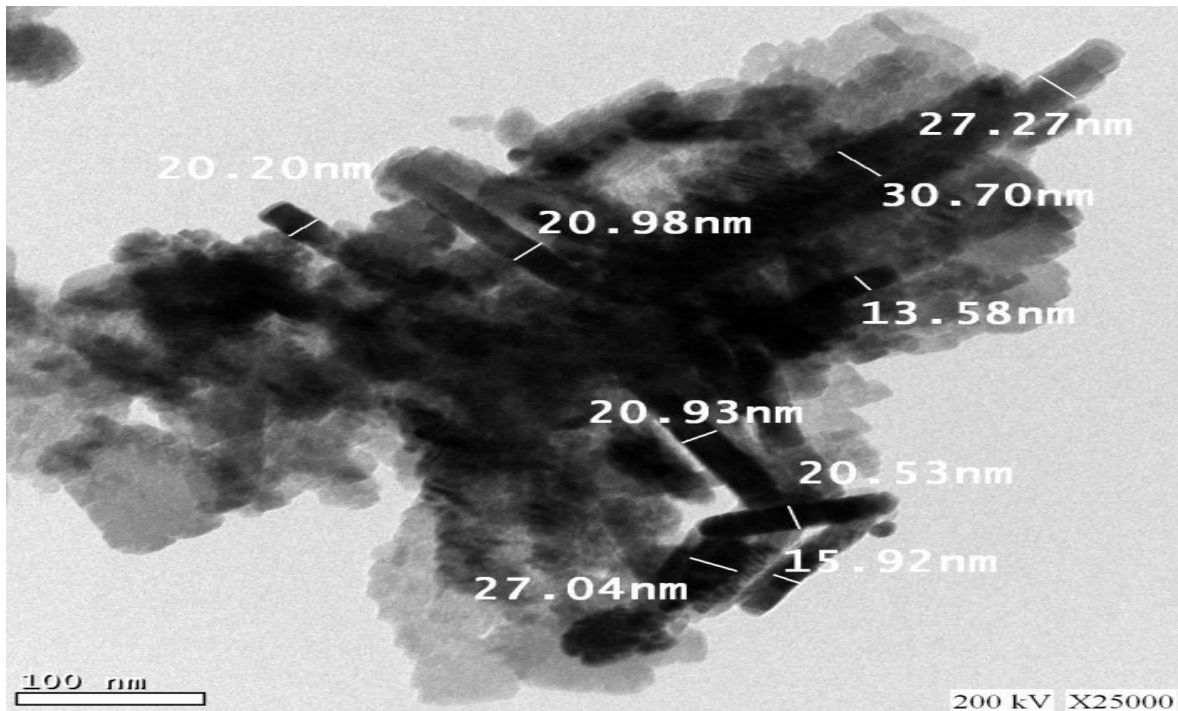
The optical property of nanoparticles is one of the important aspects for the characterization of its structure and properties. The optical absorption spectra of green synthesized zinc oxide nanoparticles were measured using a UV-Vis spectrophotometer at different wavelengths ranging from 300 to 500 nm. **Fig. (2)** shows the UV-vis absorption spectrum of zinc oxide nanoparticles. The spectrum showed the absorbance peaks at 320 nm which is corresponding to the characteristic band of zinc oxide nanoparticles. Similar results have been reported on the green synthesis of ZnO NPS using *Aloe vera* leaf extract [48]. [45] reported that zinc oxide nanoparticles exhibited a characteristics absorption peak in the range of 250 –700 nm.





**Fig. 2:** Ultraviolet-visible spectra of lemon extract, zinc oxide nanoparticles synthesized using lemon peels' extract and zinc acetate.

The size and morphology of the synthesized ZnO NPs characterized by TEM is given in **Fig. (3)**. The HRTEM image revealed the size of ZnO NPs to be in the range of 13.58 to 30.70 nm. ZnO NPs are rod-shaped with well-defined morphology. Characterization of ZnO NPs revealed that all NPs were stable, well dispersed, smooth and the agglomeration might be due to the preparation technique. The particles were placed on a copper grid and allowed to dry, which promotes agglomeration [49, 50]. The size of the obtained ZnO NPs was smaller than that of ZnO NPs synthesized by *Aloe barbadensis* and *Citrus aurantifolia* extracts, size was in the range of 25 to 40 nm and 50 to 200 nm, respectively [51,52].



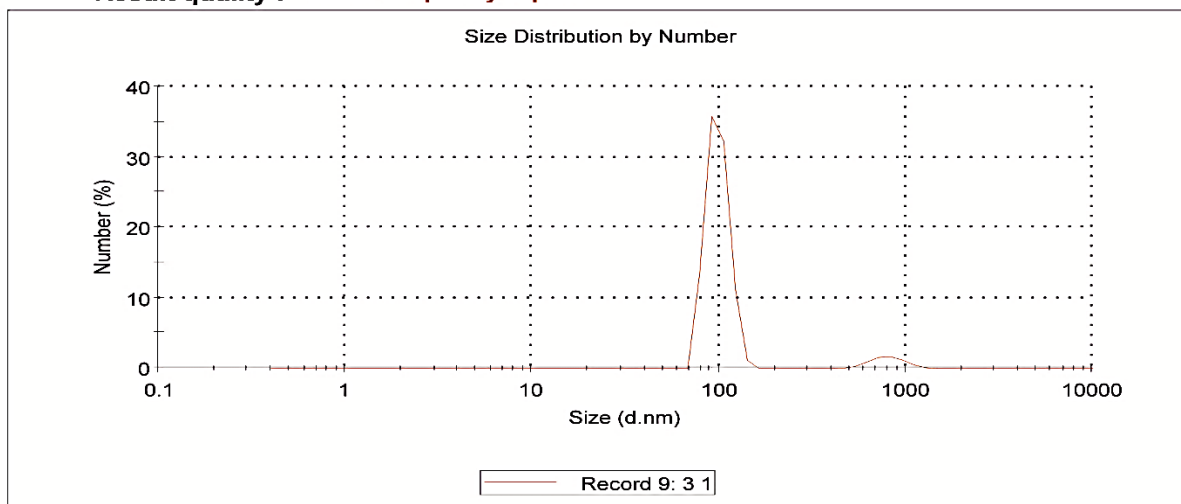
**Fig. 3:** High resolution transmission electron microscope (HRTEM) spectrum image of zinc oxide nanoparticles synthesized using lemon peels' extract.

Figure 4 (a and b) demonstrates the DLS analysis experimental values and the zeta potential of produced ZnO nanoparticles, respectively. The hydrodynamic size of the particles was determined using Dynamic Light Scattering and was found to be 98.77 nm with polydispersity index (PDI) of 0.476 for the aqueous preparation of ZnO NPs as the graph shows, the particle size is polydisperse and is larger as compared to TEM images. The larger size of the ZnO NPs observed via DLS is due to the biasness of the technique toward measurement of larger particles (**even aggregate**) [53]. Zeta potential analysis determines the surface charge of synthesized nanoparticles (-41.1mv). The negative surface charge on particles is due to the high binding affinity of the extract compounds on metallic ions conferring the particles' dispersion stability and preventing from aggregation [54,55].

Results

	Size (d.nm):	% Number	Width (d.nm...)
<b>Z-Average (d.nm):</b> 1036	<b>Peak 1:</b> 808.4	6.4	163.9
<b>Pdl:</b> 0.476	<b>Peak 2:</b> 98.77	93.6	13.59
<b>Intercept:</b> 0.910	<b>Peak 3:</b> 0.000	0.0	0.000

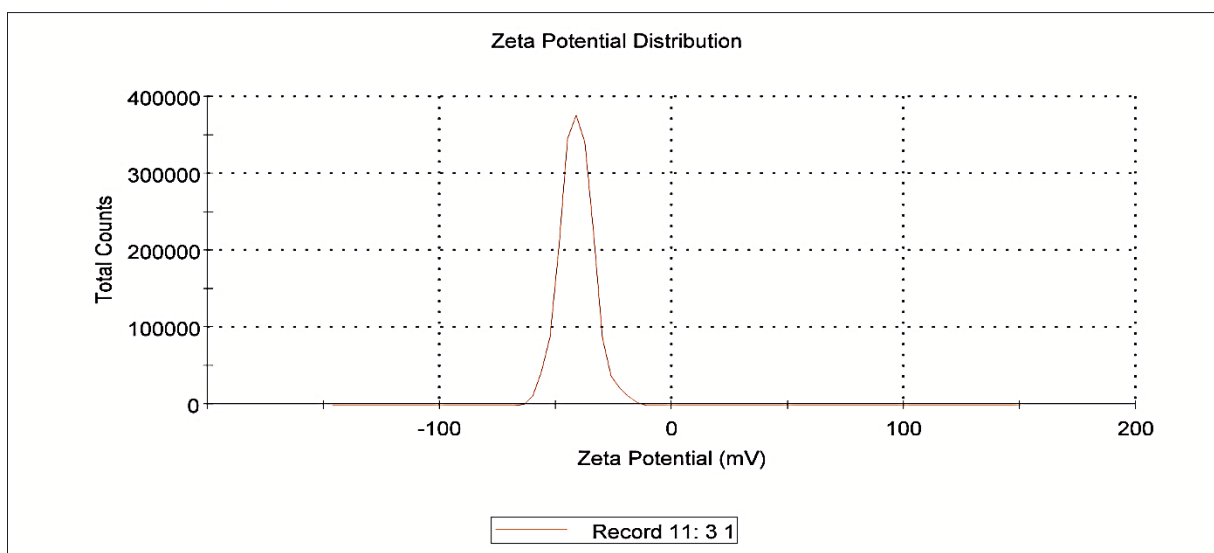
**Result quality :** Refer to quality report



Results

	Mean (mV)	Area (%)	Width (mV)
<b>Zeta Potential (mV):</b> -41.1	<b>Peak 1:</b> -41.1	100.0	7.26
<b>Zeta Deviation (mV):</b> 7.26	<b>Peak 2:</b> 0.00	0.0	0.00
<b>Conductivity (mS/cm):</b> 0.111	<b>Peak 3:</b> 0.00	0.0	0.00

**Result quality :** Good



**Fig. 4:** (a) The size distribution of synthesized ZnO NPS using lemon peels' extract by Dynamic Light Scattering (DLS) and (b) Zeta potential value of prepared ZnO NPS.

The present work showed high rates of resistance to the tested antifungal agents. The two *C. albicans* and *C. krusei* were resistant to Fluconazole, Itraconazole and Voriconazole while *C. glabrata* was resistant to all the tested antifungals (**Table 2**).

The rapid global spread of resistant *C. albicans* clinical isolates and novel antimicrobial drug families have a short life assurance, necessitating the development of new anticandidal agents [13]. Researchers are increasingly focusing on nanomaterials looking for new leads to develop better nano-antimicrobial medications for MDR strains of *C. albicans*. In the present study we assessed the anticandidal activity of ZnO NPs against three MDR *Candida* species. The results of antifungal sensitivity of ZnO NPs synthesized using *Citrus limon* peels' extract against the three different *Candida* species are presented in **Table (2)**. Results clearly demonstrate that lemon peels mediated synthesized zinc oxide nanoparticles shown significant activity against all the fungal strains. A maximum zone of inhibition ( $24.5\pm 0.5$  mm) was recorded for *C. glabrata* followed by *C. albicans* ( $19.5\pm 0.5$  mm) and minimum zone of inhibition ( $16.0\pm 0.0$  mm) was recorded for *C. krusei*. These findings are consistent with those of [56] who found significant reduction in the growth of different *Candida* species has been investigated by well diffusion method. Similar anticandidal activity of green synthesized ZnO NPs against clinical isolates of *Candida* species has also been reported [57,58].

**Table (2): Antifungal susceptibility patterns among *Candida* species towards antifungal agents in comparison with zinc oxide nanoparticles synthesized using lemon peels' extract**

Yeast	Mean diameter of inhibition zone (mm)				Zinc oxide nanoparticles
	Fluconazole	Miconazole	Itraconazole	Voriconazole	
<i>Candida albicans</i> 1	0 R	0 R	0 R	7 R	19.5±1.5
<i>Candida albicans</i> 2	12 R	15 M	0 R	13 R	19.5±0.5
<i>Candida glabrata</i>	0 R	0 R	7 R	7 R	24.5±0.5
<i>Candida krusei</i>	0 R	12 M	0 R	0 R	16.0±0.0

R: Resistant M: Intermediate S: Susceptible

Note: Positive control (plant extract) = 7 mm & the tested five antifungal agents (table 2) Negative control (DEMSED) = 0 mm

The anticandidal activity of ZnO NPs has been assessed using broth dilution method to determine the MICs and MFCs. The obtained MIC values revealed that ZnO NPs inhibited the growth of all the tested *Candida* species. The recorded MIC for all *Candida* species was 0.25 mg/ml while their MFC was 0.5 mg/ml (**Table 3**). [56] reported that the MICs for *Candida albicans*, *Candida tropicalis* and *Candida dubliniensis* were ranged from 0.25 to 0.5 mg/ml, while MFCs were ranged from 0.5 to 1.0 mg/ml, respectively. The obtained MICs of ZnO NPs

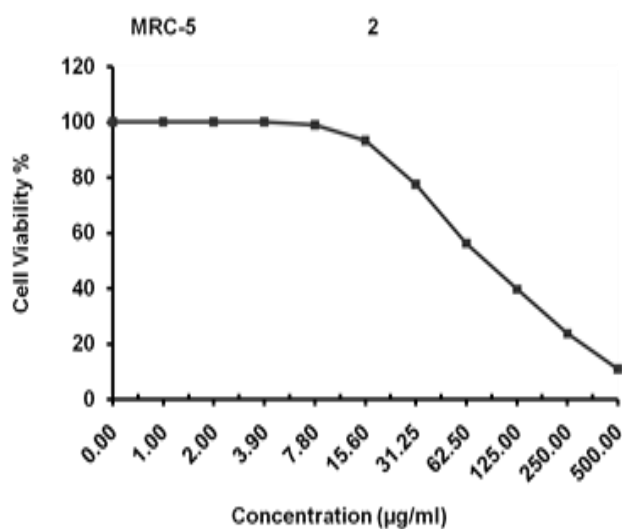
is in good agreement with the previous study of [58]. [12] reported that the MIC values of *C. albicans* and *C. glabrata* was 1.25 mg/ml. [59] found that ZnO NPs at 1.0 mg/ml killed 99.5 % of *C. albicans* cells and observed that smaller particles of ZnO NPs had better antifungal activity against *C. albicans* than larger particles. The possible mechanisms of antifungal action of nanoparticles are cell membrane disruption, cell division inhibition, and cell wall formation inhibition. Zinc oxide nanoparticles damage the cell membrane by inhibiting ergosterol synthesis or binding with sterol, creating pits and causing the membrane permeability to become leaky leading to cell death [60, 61]. Zinc oxide nanoparticles may affect the mitotic spindle cell (fungal cell) division by targeting the microtubule and also inhibit DNA transcription [62]. Zinc oxide nanoparticles are positively charged, will interact with negatively charged carboxylic groups and cell wall of mycelia to inhibit the growth of normal budding fungi [63].

**Table (3): Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (mg/ml) for *Candida* species treated with zinc oxide nanoparticles**

Yeast species	MIC	MFC
<i>Candida albicans</i> (1)	0.25	0.5
<i>Candida albicans</i> (2)	0.25	0.5
<i>Candida glabrata</i>	0.25	0.5
<i>Candida krusei</i>	0.25	0.5

Biosynthesized lemon ZnO-NPs have been detected for their cytotoxicity (**Fig. 5**). In the current study, ZnO NPs in the range of 13.58–30.7 nm were investigated via MTT cytotoxic assay against normal human lung fibroblast cell line (MRC5). Zinc oxide nanoparticles synthesized by lemon peels extract have half maximal inhibitory concentration (IC<sub>50</sub>) value =  $230.12 \pm 9.34$  µg/ml. Negative control without nanoparticles showed 100% cell viability. The toxic effects of ZnO NPs in normal cells depends greatly on their size, shape, and concentration, as well as culture time and cell type [64]. [65] investigated cytotoxic effects of ZnO NPs (55 nm) with doses of 10, 15, 30, and 100 µg/ml on different cell types including human epidermal keratinocytes (HaCaT), human gingival fibroblast (HGF-1), and human

gingival squamous carcinoma cell line (Ca9-22) and found that ZnO-NPs were less toxic to normal HaCaT and HGF-1 cells but showed severe toxicity to Ca9-22 cells at doses  $\geq 30 \mu\text{g/ml}$ . Thus, ZnO NPs exhibited selective toxicity to cancer cells due to the ROS generation, mitochondrial oxidative damage, and DNA fragmentation. As mentioned before, the toxic effects of ZnO NPs in normal cells depends greatly on their size, shape, and concentration, as well as culture time and cell type so further more investigations are required to evaluate the cytotoxic effects of ZnO NPs on different cell types under different conditions to be fair in judging their safety.



**Fig. (5):** Cytotoxic activity against normal human lung fibroblast cells (MRC5).

### Conclusion

The current study reports synthesis of ZnO nanoparticle by using aqueous extract of lemon peels and characterization of ZnO NPs using UV-Vis spectroscopy, HRTEM and DLS. The synthesized ZnO NPs have rod shape with sizes of 13.58 - 30.70 nm. Zinc oxide nanoparticles showed significant activity against important MDR fungal human pathogens (three different *Candida* species). Zinc oxide NPs are biocompatible and exert microbial inhibition by an array of mechanisms which make it a good candidate for multidrug resistant *Candida* species.

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## الملخص العربي

## باستخدام مستخلص قشور الليمون النشاط المضاد للكانديدا لجزيئات أكسيد الزنك النانوية المخلفة

ريهام سعيد متولى<sup>1</sup>، زينب محمد حسن خيرالله<sup>1</sup>، سناء محمد عاشور<sup>1</sup>، سناء صبحى زكى<sup>1</sup><sup>1</sup> قسم النبات، كلية البنات للاداب والعلوم والتربية، جامعة عين شمس، القاهرة، جمهورية مصر العربية

## الملخص العربي

ظهرت التكنولوجيا الحيوية النانوية كتقنية فعالة لتطوير الجزيئات المضادة للميكروبات من خلال نهج صديق للبيئة. في هذه الدراسة، تم وصف الجزيئات النانوية لأكسيد الزنك باستخدام المستخلص المائي لقشور الليمون بالتحليل الطيفي المرئي للأشعة فوق بنفسجية والميكروسكوب الإلكتروني عالي الدقة وتشتت الضوء الديناميكي (DLS). تم التحقق من النشاط المضاد للفطريات ضد أربع سلالات من الكانديدا متضمنة، سلالتين من *C. albicans* وسلالة واحدة من *C. glabrata* وسلالة واحدة من *C. krusi* باستخدام خمس مضادات فطرية قياسية بطريقة الانتشار القرصي. كما تم تقييم النشاط المضاد للفطريات لجزيئات الزنك النانوية بطريقة الانتشار القرصي وكذلك تحديد الحد الأدنى من التركيز المثبط للنمو والحد الأدنى من التركيز المبيد للفطريات. كشفت دراسات الوصف أن الجزيئات النانوية المخلفة لها شكل عصوي بأحجام تتراوح بين 13.58 و 30.70 نانومتر. كما كشفت معدلات مقاومة عالية (100%) مع المضادات الفطرية القياسية ممثلة في Voriconazole, Flucytosine, Itraconazole, Fluconazole ضد جميع سلالات الكانديدا المختبرة. إضافة إلى ذلك، أظهرت جزيئات الزنك النانوية نشاطا ملحوظا ضد سلالات الكانديدا المقاومة للعديد من مضادات الميكروبات مظهرة أكبر منطقة تثبيط  $24.5 \pm 0.5$  مم ضد *C. glabrata* يليها *C. albicans* ( $19.5 \pm 0.5$ ) و *C. krusi* ( $16.0 \pm 0.0$ ) على التوالي وكان الحد الأدنى من التركيز المثبط للنمو والحد الأدنى من التركيز المبيد للفطريات لجميع سلالات الكانديدا 0.25 و 0.5 مجم/ مل على التوالي. تشير نتائج السمية الخلوية إلى أن ZnO NPs لها نصف تركيز مثبط أقصى (IC50) =  $230.12 \pm 9.34$  ميكروجرام / مل على الخلايا الليفية الرئوية البشرية الطبيعية (MRC5). يتضح من الدراسة الحالية أن جزيئات أكسيد الزنك المخلفة باستخدام مستخلص قشور الليمون لها نشاط مضاد لسلالات الكانديدا المختلفة ولذلك يمكن تطويره كدواء جديد لعلاج الأمراض المرتبطة بالعدوى بالكانديدا في المستقبل القريب.