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Preparation and Characterization of Zinc Oxide Nanoparticles by Co-Precipitation Method and Evaluation of Theirs Antifungal Activity in Spore Germination of Dermatophytes

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ABSTRACT

This research study synthesized Prepared zinc oxide nanoparticles using the co-precipitation method. The antifungal activities of these nanoparticles (ZnO NPs) and their mode of action against *Trichophyton rubrum* and *Microsporum canis* were investigated using samples obtained from patients referred to consulting clinics in Kirkuk city. These nano oxides were identified through XRD diffraction analysis, FTIR measurement, and Scanning Electron Microscopy (SEM). The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values were recorded as 1.330 and 4.530 µg/mL for *Trichophyton rubrum*, and 0.333 and 10.67 µg/ml for *Microsporum canis*, respectively. The antifungal effect of (ZnO NPs) was similar to that of Griseofulvin, while it exhibited a higher effect than ketoconazole. Furthermore, (ZnO NPs) demonstrated a significant ($P < 0.05$) inhibition effect on spore germination for all tested dermatophytes, although the extent of this effect varied depending on the fungal isolates.

INTRODUCTION

Dermatophytes, a large group of fungi responsible for causing dermatophytosis or ringworm(Bouchara &Mignon, 2017). This group includes three main genera: *Trichophyton, Microsporum,* and *Epidermophyton* spp (Al-Janabi, 2014). Griseofulvin is considered one of the most widely used treatments for dermatophytosis(Gupta *et al.*, 2009) (Barros *et al.*, 2007), and therefore it was very important to increase its effectiveness through the development of nanoparticles(Zili Z *et al.*, 2005). Solid nanoparticles loaded with griseofulvin in the form of a gel showed very, very effective pharmacological capabilities against the pathogenic fungus(Aggarwal &Goind, 2013). *Microsporum canis* Zinc oxide nanoparticles (Zno-NPs) showed an effective effect against dermatophytes and show stronger inhibition compared to antifungals(Ahmadpour *et al.*,2021) (El-Diasty *et al.*, 2013) Many antifungals have shown resistance against skin fungi, which is considered a major challenge(Elad *et al.*, 1992) Therefore, it was important to prepare drugs for pathogenic fungi that replace current medications. Therefore, researchers have paid great attention to nanoparticles due to their high effectiveness due to their unique physical and chemical properties(Vatsha B *et al.*, 2013). Many researches have indicated There are many different types of nanomaterials, including silver nanoparticles(Abbood & A. H. S, 2020) and zinc nanoparticles(Liu *et al.*, 2009), which have shown antifungal and antibacterial activity.

Zinc oxide nanoparticles (ZnO NPs) are known for their ability to create large surface areas and unique crystalline structures. These metal oxides are highly ionic(Khaleel *et al.*,1999), unlike organic materials, possess exceptional durability, selectivity, and thermal resistance. Incorporating zinc oxide into daily zinc supplements is common due to its vital role in maintaining human health. Additionally, ZnO NPs have shown compatibility with human cells (Padmavathy &Vijayaraghavan, 2008). The antimicrobial properties of ZnO bulk powder have been extensively studied, demonstrating its effectiveness against bacteria and fungi. In the agricultural industry, zinc compounds are primarily used as fungicides (Bloom & Markson., 2001) .

MATERIALS AND METHODS

Isolation and Diagnosis of Fungi

Samples were obtained from patients who were referred to consulting clinics in the city of Kirkuk. The samples were collected either by scraping from the affected area or by cutting the affected part of the hair and nails. Afterward, the samples were treated with 10% KOH to analyze the keratinous material present. This analysis aimed to identify any highlights and spores. The isolated samples were then transferred to sterile Petri dishes containing Sabouraud's Dextrose Agar (SDA) medium, supplemented with 50 mg of the antibiotic Chloramphenicol. This was done to facilitate the growth of fungi without any interference from bacteria and yeasts. Subsequently, the isolated samples were sent to the Mycology Laboratory at the Alqalam of the university. They were placed in an incubator set at a temperature of 27-30°C for a duration of two weeks. Following the incubation period, the colonies were carefully tested (Figure 1), and additional tests were conducted to confirm the diagnosis (Table 1) (Khan *et al.*, 2021).

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Table 1: Shows species and fungal species isolated from spices

Mold type	Hair penetration test	Growth at a temperature of 37°C	Diagnosis of large conidia	Diagnosis of small conidia	Rice test	Characteristics of fungal culture
M.canis	+	-	Fusiform, thick, rough-walled, with a curved or hooked end	It is not found in abundance, and if it is found, it has a club shape	Yellow colony	tapering at both ends; spindle-shaped
T.rubrum	+	-	It takes the form of a cigar or a pen	Club-shaped to barrel-shaped, growing along the spindle filaments	-	The colony gives off a red dye. Below the plate, the conidia are club-shaped and the hyphae are tree-shaped

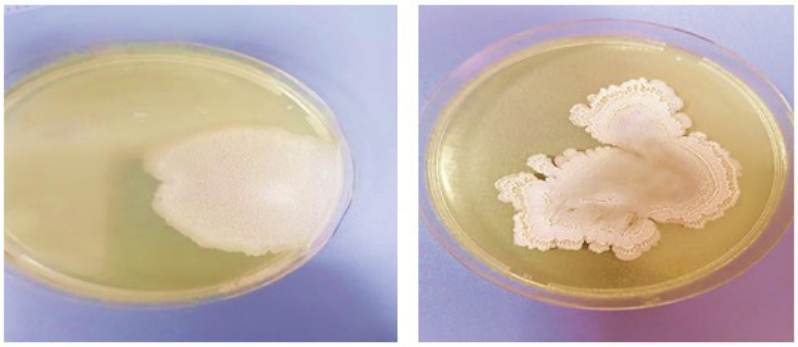

Figure 1: Colony morphology of the A. *T.rubrum* and B. *M.canis* isolate

Inoculum Preparation

In light of the need to obtain reproducible and comparable results in antifungal susceptibility testing, it is important to standardize the inoculum. Based on the CLSI guidelines, the tested dermatophyte inoculum suspensions were made (Wang *et al.*, 2018). To induce sporulation, colonies grown on PDA for 7-15 days were flooded with 10 ml of distilled water. The use of a sterile loop to mechanically break up the colonies yielded a suspension that contained some tiny fragments of hyphae and conidia. This mixture was then aliquoted into sterile tubes and allowed to settle at room temperature which facilitated sedimenting of larger particles. The suspension was then diluted to a final conidia concentration of 1-3 × 10^3 cells/ml using a haemocytometer according to CLSI M38-A2.

The Dilution and Preparation of Stock Solution of the Antifungal Agents

In performing the comparison with (ZnO NPs), commercially available antifungal reference powders of Griseofulvin and ketoconazole were used to prepare stock solutions. Another accurate way of measuring the antifungal powders was by using an analytical balance that measures up to 0. 0001g, according to the authors of the paper by (Wang *et al.*, 2018). Deionized distilled water was employed for dissolving Griseofulvin, ketoconazole, and (ZnO NPs) to prepare stock solutions. To reach the final strength as required, further dilutions were made on the broth medium, to attain twice the strength. Subsequently, a twofold serial dilution was performed to obtain the final concentration range of the drugs: It was found to be 0.04-19 µg/ml for ketoconazole and 0. 136-59 µg/ml for Griseofulvin and 0.171-75 µg/ml for (ZnO NPs).

Preparation of Zinc Oxide Nanoparticles by Co-Precipitation Method

A solution of 0.4 molar aqueous zinc acetate ($Zn(CH_3COO)_2\ 2H_2O$) was created by dissolving 9.2 g of the compound in 100 milliliters of non-ionic water and allowing it to dissolve completely on a magnetic stirrer for 30 minutes. Sodium hydroxide with a concentration of 1 molar was then added drop by drop until reaching a pH of 9. The solution was stirred at 70°C for 2 hours until a precipitate formed. The precipitate was filtered, washed with nonionic water and ethanol, and then dried at 80°C for 30 minutes. Then, the precipitate was burned at 500°C for 5 hours and the process was further continued for desiccation of the precipitate at room temperature for overnight (Khan *et al.*, 2015) .

$Zn(CH_3COO)_2 \cdot 2H_2O + 2NaOH \rightarrow ZnO + 2CH_3COONa + 3H_2O$

Nanoparticle Characterization Techniques Based on Zinc Oxide Nanoparticles are Being Prepared Fourier Transform Infrared Spectroscopy (FTIR)

Spectroscopy of infrared radiation was used to analyze FTIR samples and the presence of functional groups and various features of the nanocomposite was studied (Fadlelmoula *et al.*, 2022).

Scanning Electron Microscope (SEM) Analysis

It is applied for the average particle size analysis of the nanocomposite and the structure shape analysis. XRD was also employed to determine what elements are present in the sample. The results were taken and put down in pictures (Xiong *et al.*, 2020)

X-Ray Diffraction Analysis (XRD)

Analysis based on X-ray diffraction method is very popular technique for testing and determining the crystal structure of nanomaterials by using an XRD tool and it is a non-destructive way to characterize the physical properties, chemical composition and crystal structure, In addition atomic arrangement and thickness could be determine (Zhang *et al.*, 2023).

Determination of MIC Values for Dermatophytes

The MIC (ZnO-NPs) and other antifungal drugs considered were assessed against the fungal strain by employing broth microdilution method. The method followed Clinical Laboratory Standards Institute (CLSI) guidelines wherever possible, as described in Filamentous Fungi Document M38-A2 (Badiee *et al.*, 2013).

The Minimum Fungicidal Concentration (MFC)

When testing MIC, the minimum fungicidal concentration (MFC) of ZnO NPs against the dermatophytes studied was determined. For comparison, the bactericidal efficacy of griseofulvin and ketoconazole was also examined. Samples from wells with no visible growth were then transferred to SDA plates. Additionally, positive controls (from growth control wells) and negative controls (from sterile control wells) were included in the experiment.

The Impact of Biosynthesized Zinc Oxide Nanoparticles (Zno Nps) on the Germination of Dermatophyte Spores Was Assessed Using as Microscopic Method

To generate the conidia, the isolates were cultured on potato dextrose agar (PDA) for 10-14 days, following the specific requirements of each dermatophyte genus. The resulting suspension contained both conidial and mycelial fragments. The spore count for each dermatophyte isolate was maintained at 40-60 spores per microscopic field. In order to interact with the ZnO NPs, the spore suspension for each isolate was prepared, resulting in a concentration of 20-30 spores per microscopic field. The tubes containing the culture mixture were then placed in a shaking incubator at 35ºC for *Trichophyton* spp. or at 30ºC for *Microsporum* spp. to ensure even distribution of the ZnO NPs.

The germination of spores was studied through the daily microscope examination of the sample. The spore was deemed germinated once the length of the germ tube reached 1.5 times the diameter of the spore (Plascencia-Jatomea *et al.* ,2003).

The antifungal drugs and sterile broth were used as substitutes also to the (ZnO NPs) in the positive and negative controls. All the treatments were performed in three replications. The percentage of spore germination inhibition was determined with the help of the formula:

% spore germination inhibition = SC - CT/ SC × 100

*SC: average of spores germinated in control.

*CT: average of spores germinated in treatment.

RESULTS AND DISCUSSION

Diagnosis of Nano-Zinc Oxide by XRD

The X-ray diffraction analysis was conducted to characterize the crystal structure and phase purity of the synthesized ZnO nanoparticles. Figure (2) illustrates that the X-ray diffraction spectrum of the zinc oxide sample aligns with the standard spectrum of Zinc oxide as per the database (ZnO JCPDS 36-1451) (Wirunchit *et al.*, 2021).

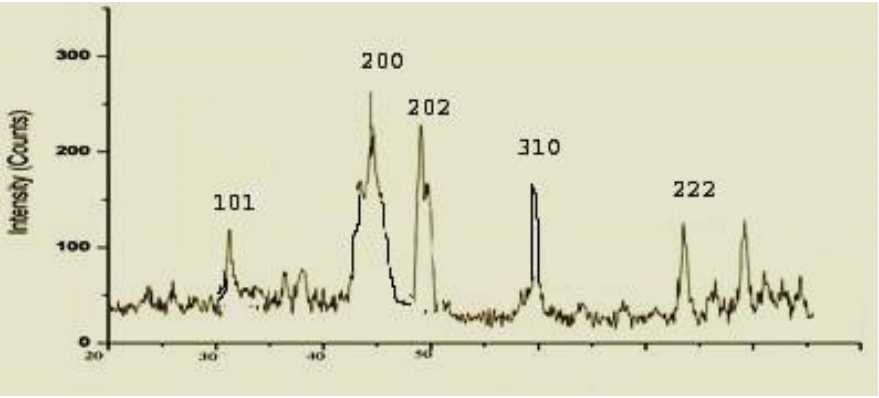

Figure 2: X-ray diffraction (XRD) of silver nanoparticles (ZnO NPs)

FTIR Measurement Results for the Prepared Oxides

The infrared spectra of $Zn(CH_3COO)_2.2H_2O$, which is the aqueous zinc acetate salt, are utilized in the preparation of nano-zinc oxide prior to conducting the reaction. This information is depicted in Figure (3). The results obtained from the experiment lead us to the conclusion that the protein present in the reaction medium serves as a reducing agent and forms a protective layer around the biosynthesized ZnO NPs, known as capping proteins. This capping protein plays a crucial role in preventing the undesirable agglomeration of particles in the medium, thereby ensuring the formation of highly stable ZnO NPs(Hadi&Kadhim, 2019). One significant advantage of using capping protein over polymer and surfactant, which are commonly used as capping agents in the preparation of ZnO NPs, is that it is cost-effective, safe, eco-friendly, and does not require any special conditions. Additionally, capping protein acts as an anchoring layer for the transportation of drugs or genetic materials into human cells (Hu *et al.*, 2011), surpassing the capabilities of surfactant and polymer. Moreover, the presence of a non-toxic protein cap enhances the uptake and retention of these materials inside human cells (Rodríguez *et al.*, 2013).

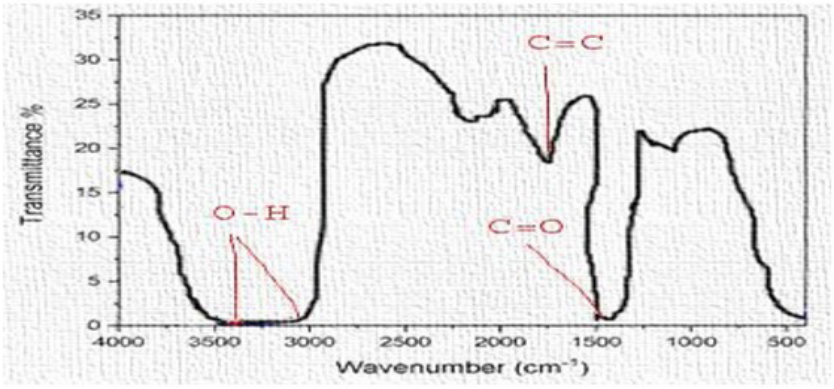

Figure 3: FTIR spectrum of ZnO NPs

Diagnosis of Nano-Zinc Oxide by SEM

The scanning electron microscope (SEM) was utilized to observe the morphological and structural properties of nanozinc oxide (ZnO). The nanometer range preparation of nanoparticles is evident in Figure (4). The SEM images revealed that certain nanoparticles were adequately dispersed, while the majority were found in agglomerated form. The agglomeration is attributed to both electrostatic effects and the presence of the aqueous suspension, which demonstrates the behavior of nanoparticle agglomeration. This finding aligns with previous studies on nanoparticle agglomeration (S Zain-Al-Abddeen *et al.*, 2017) (Gaikwad *et al.*, 2015).

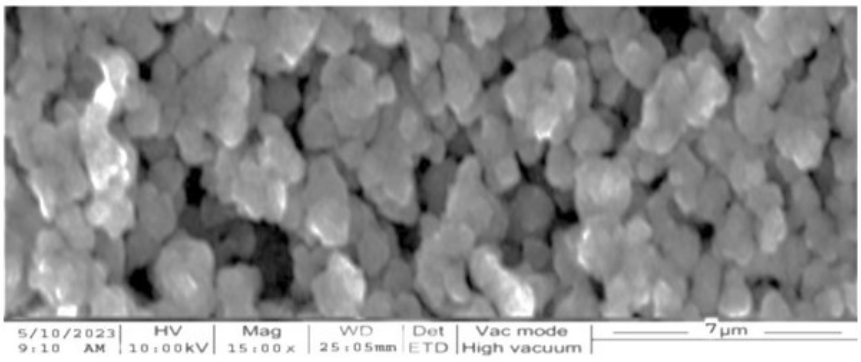

Figure 4: Scanning electron microscope (SEM) of ZnO NPs

Determination of MIC and MFC Value Against Dermatophytes

The antifungal efficacy of ZnO NPs was studied against *Trichophyton rubrum* and *Microsporum canis*. Griseofulvin and ketoconazole, commonly used antifungal medications, were employed as positive controls for comparison. The growth inhibition effect of ZnO NPs on the mentioned fungi was determined through the broth microdilution method to establish the MIC. The MIC values for the tested substances are detailed in Table 2. The findings indicated that ZnO NPs exhibited antifungal properties against all tested dermatophytes within the range of 0.171-75 µg/ml, with varying susceptibility levels based on the fungal species. Griseofulvin, with an MIC range

of 0.136-59 µg/ml, only demonstrated antifungal activity against M. canis and T. rubrum, with MIC values of approximately 10 µg/ml and 32 µg/ml, respectively. Ketoconazole, having an MIC ranging from 0.04 -19 µg/ml, exhibited a comparable antifungal effect to that of zinc oxide nanoparticles (ZnO NPs). Statistical analysis indicated no significant variance between the two. The most effective MIC of ketoconazole, at 0.6 µg/ml, was observed against M. canis. The biological activity of zinc oxide is attributed to the disruption of membrane lipids, which led to leakage of cell contents and ultimately cell death (Xie e*t al.*,2011) Zinc oxide can also generate hydrogen peroxide and Zn+2 ions, which cause cell death through a change in cellular metabolism. Preliminary studies indicate that the antimicrobial properties of ZnO NPs are due to the formation of free radicals on the nanoparticle surface. These free radicals are thought to damage lipids in the bacterial cell membrane, causing the membrane to leak and rupture (He *et al.*, 2017) (Reddy *et al.*, 2007).

Table 2: Comparative MIC value of biosynthesized ZnO NPs, Griseofulvin and Ketoconazole against *T.rubrum* and *M.canis*

Tested fungal strains	MIC/Mean(µg/ml)					
	ZnO NPs 0.171-75	**Griseofulvin (0.136-59)**	**pvalue**	**(ZnO NPs) 0.171-75**	**Ketoconazole (0.04-19)**	**pvalue**
T.rubrum	1.330	10	0.0439	1.330	1	>0.05
M.canis	0.333	32	<0.001	0.333	0.6	>0.05

The antifungal properties of ZnO NPs and standard antifungal medications were evaluated concurrently with their minimum inhibitory concentration against the dermatophytes being studied. Figures (5) and (6) show that the MFCs obtained are significantly higher than the MICs.

T.rubrum was found to be most susceptible to ZnO NPs, with a minimum fungicidal concentration (MFC) of 4.530 µg/ml, compared to an MFC of 35 µg/ml for Griseofulvin and 4 µg/ml for Ketoconazole. For *M.canis*, the MFC of ZnO NPs was 10.67 µg/ml, while the MFC of Ketoconazole was 3 µg/ml.

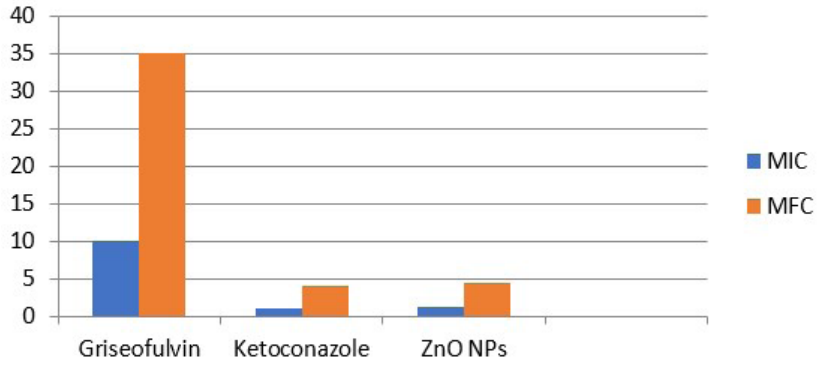

Figure 5: Sensitivity profile of *T.rubrum* to the antifungal agents

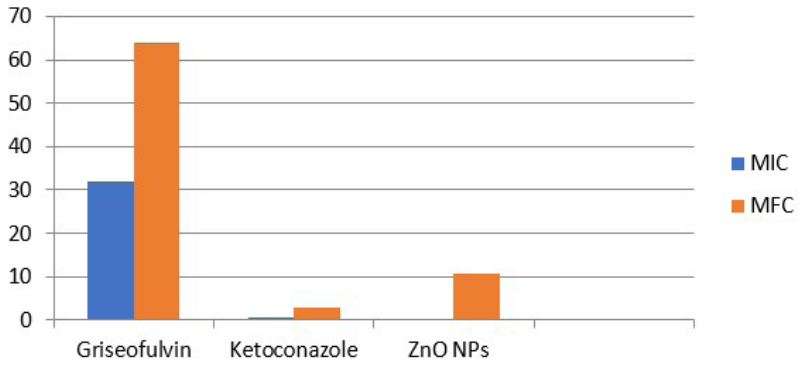

Figure 6: Sensitivity profile of *M.canis* to the antifungal agents

Table 3: Comparative MFC value of ZnO NPs, Griseofulvin and Ketoconazole against *T.rubrum* and *M.canis*

Tested fungal strains	MIC/Mean(µg/ml)					
	ZnO NPs 0.171-75	**Griseofulvin (0.136-59)**	**pvalue**	**(ZnO NPs) 0.171-75**	**Ketoconazole (0.04-19)**	**pvalue**
T.rubrum	4.530	35	<0.001	4.530	4	>0.05
M.canis	10.67	>64	<0.001	10.67	3	>0.05

Effect of ZnO NPs on Spore Germination of Dermatophytes

The impact of ZnO NPs on the germination of spores from various dermatophytes is outlined in Table (4)

The study demonstrated that ZnO NPs exhibited a notable and significant inhibitory effect on spore germination ($P < 0.05$) in all the dermatophytes tested. The impact of ZnO NPs on fungal strains differed in magnitude, as indicated in Table 4. Additionally, the majority of germinated spores exhibited morphological transformations, including spore enlargement. These alterations align closely with the fungistatic properties of ZnO NPs. Consequently, it is postulated that the mechanism behind the fungistatic effect involves modifications in the osmotic balance and permeability of the spores (Khalil *et al.*, 2013).

Table 4: Effects of ZnO NPs on spore germination in the tested dermatophytes, alongside the reference antifungal drugs Griseofulvin and Ketoconazole

Tested fungal strains	Mean percentage of spore germination inhibition					
	ZnO NPs	**Griseofulvin**	**pvalue**	**(ZnO NPs)**	**Ketoconazole**	**pvalue**
T.rubrum	98	0	<0.0001	98	0	<0.0001
M.canis	88	0	<0.0001	88	0	<0.0001

CONCLUSION

In the results obtained in this study and through the effectiveness of ZnO NPs on two types of fungi and comparing them with antifungals, we can conclude that ZnO NPs have a biological effectiveness more or similar to antifungals.

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REFERENCES

Abbood, A. H. S. H. A. (2020). The Role of Silver (Ag) Nanoparticles synthesis by Penicillium spp against the Toxicity of Echinococcus Granulosus in Adult Albino Male Rats. University of Kirkuk. *Medico-legal Update, 20*(1), 533. https://doi.org/10.37506/mlu.v20i1.414.

Abod, H. A. (2017). The effect of silver nanoparticles prepared using Aspergillus niger in some pathogenic bacteria. *University of Kirkuk, Kirkuk Journal of Science, 12*(1). https://doi.org/10.1166/jbmb.2008.401.

Aggarwal, N., & Goindi, S. (2013). Preparation and in vivo evaluation of solid lipid nanoparticles of griseofulvin for dermal use. *Journal of biomedical nanotechnology, 9*(4), 564-576. https://doi.org/10.1166/jbn.2013.1569.

Ahmadpour Kermani, S., Salari, S., & Ghasemi Nejad Almani, P. (2021). Comparison of antifungal and cytotoxicity activities of titanium dioxide and zinc oxide nanoparticles with amphotericin B against different Candida species: In vitro evaluation. *Journal of clinical laboratory analysis, 35*(1), e23577. https://doi.org/10.1002/jcla.23577.

Al-Janabi, A. A. (2014). Dermatophytosis: Causes, clinical features, signs and treatment. *J Symptoms Signs, 3*(3), 200-203. file:///C:/Users/h/Downloads/Dermatophytosiscausesclinical%20(1).

Badiee, P., Alborzi, A., Moeini, M., Haddadi, P., Farshad, S., Japoni, A., & Ziyaeyan, M. (2012). Antifungal susceptibility of the Aspergillus species by Etest and CLSI reference methods. *Archives of Iranian medicine, 15*(7), 0-0. https://pubmed.ncbi.nlm.nih.gov/22724880/.

Barros, M. E. D. S., Santos, D. D. A., & Hamdan, J. S. (2007). Evaluation of susceptibility of Trichophyton mentagrophytes and Trichophyton rubrum clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). *Journal of medical microbiology, 56*(4), 514-518. https://doi.org/10.1099/jmm.0.46542-0.

Bloom, P., & Markson, L. (2001). Are there principles that apply only to the acquisition of words? A reply to Waxman and Booth. *Cognition, 78*(1), 89-90. https://doi.org/10.1016/S0010-0277(00)00111-6.

Bouchara, J. P., Mignon, B., & Chaturvedi, V. (2017). Dermatophytes and dermatophytoses: a thematic overview of state of the art, and the directions for future research and developments. *Mycopathologia, 182,* 1-4. https://doi.org/ 10.1007/s11046-017-0114-z.

Elad, Y., Yunis, H., & Katan, T. (1992). Multiple fungicide resistance to benzimidazoles, dicarboximides and

diethofencarb in field isolates of Botrytis cinerea in Israel. *Plant Pathology, 41*(1), 41-46. https://doi.org/10.1111/j.1365-3059.1992.tb02314.x.

El-Diasty, E. M., Ahmed, M. A., Okasha, N. A. G. W. A., Mansour, S. F., El-Dek, S. I., El-Khalek, H. M. A., & Youssif, M. H. (2013). Antifungal activity of zinc oxide nanoparticles against dermatophytic lesions of cattle. *Rom J Biophys, 23*(3), 191-202. https://www.rjb.ro/articles/378/Ahmed-f.

Fadlelmoula, A., Pinho, D., Carvalho, V. H., Catarino, S. O., & Minas, G. (2022). Fourier transform infrared (FTIR) spectroscopy to analyse human blood over the last 20 years: a review towards lab-on-a-chip devices. *Micromachines, 13*(2), 187. https://doi.org/10.3390/mi13020187.

Gaikwad, S. S., Gandhi, A. C., Pandit, S. D., Pant, J., Chan, T. S., Cheng, C. L., ... & Wu, S. Y. (2014). Oxygen induced strained ZnO nanoparticles: an investigation of Raman scattering and visible photoluminescence. *Journal of Materials Chemistry C, 2*(35), 7264-7274. https://doi.org/10.1039/C4TC00566J.

Gupta, A. K., Williams, J. V., Zaman, M., & Singh, J. (2009). In vitro pharmacodynamic characteristics of griseofulvin against dermatophyte isolates of Trichophyton tonsurans from tinea capitis patients. *Medical mycology, 47*(8), 796-801. https://doi.org/10.3109/13693780802712523.

Hadi, F. A., & Kadhim, R. G. (2019, July). A study of the effect of nano zinc oxide on cure characteristics and mechanical properties of rubber composites. *Journal of Physics: Conference Series, 1234*(1), 012043. https://doi.org/10.1088/1742-6596/1234/1/012043

He, Q., Yuan, Z., Zhang, J., Zhang, S., Zhang, W., Zou, Z., & Wang, H. (2017). Insight into the impact of ZnO nanoparticles on aerobic granular sludge under shock loading. *Chemosphere, 173,* 411-416. https://doi.org/10.1016/j.chemosphere.2017.01.085.

Hu, L., Pan, H., Zhou, Y., & Zhang, M. (2011). Methods to improve lignin's reactivity as a phenol substitute and as replacement for other phenolic compounds: A brief review. *BioResources, 6*(3), 3515-3525. https://doi.org/10.15376/BIORES.6.3.3515-3525.

Khaleel, A., Kapoor, P. N., & Klabunde, K. J. (1999). Nanocrystalline metal oxides as new adsorbents for air purification. *Nanostructured Materials, 11*(4), 459-468. https://doi.org/10.1016/S0965-9773(99)00329-3.

Khalil, K. A., Fouad, H., Elsarnagawy, T., & Almajhdi, F. N. (2013). Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications. *International journal of electrochemical science, 8*(3), 3483-3493. https://doi.org/10.1016/S1452-3981(23)14406-3.

Khan, M. I., Akhtar, S., Zafar, S., Shaheen, A., Khan, M. A., Luque, R., & ur Rehman, A. (2015). Removal of Congo red from aqueous solution by anion exchange membrane (EBTAC): adsorption kinetics and themodynamics. *Materials, 8*(7), 4147-4161. https://doi.org/10.3390/ma8074147.

Khan, S. A., Shamsuzzaman, S. M., Rahman, A. K. M. S., Ashekin, N. A. K., Mahmud, R., Sharmin, R., ... & Haque, F. (2021). Isolation and Identification of Dermatophytes Causing Dermatophytosis at a Tertiary Care Hospital in Bangladesh. *Archives of Clinical and Biomedical Research, 5*(3), 437-451. https://doi.org/10.26502/acbr.50170178.

Liu, M., Sun, J., Sun, Y., Bock, C., & Chen, Q. (2009). Thickness-dependent mechanical properties of polydimethylsiloxane membranes. *Journal of micromechanics and microengineering, 19*(3), 035028. https://doi.org/10.1088/0960-1317/19/3/035028.

Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. *Science and Technology of Advanced Materials.* https://doi.org/10.1088/1468-6996/9/3/035004

Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M. M., & Shirai, K. (2003). Effect of chitosan and temperature on spore germination of Aspergillus niger. *Macromolecular Bioscience, 3*(10), 582-586. https://doi.org/10.1002/mabi.200350024.

Reddy, T. A., Maor, I., & Panjapornpon, C. (2007). Calibrating detailed building energy simulation programs with measured data—Part I: General methodology (RP-1051). *Hvac&R Research, 13*(2), 221-241. https://doi.org/10.1080/10789669.2007.10390952.

Rodríguez, R. L., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour, 85*(5), 1041-1047. https://doi.org/10.1016/j.anbehav.2013.01.006.

Vatsha, B., Tetyana, P., Shumbula, P. M., Ngila, J. C., Sikhwivhilu, L. M., & Moutloali, R. M. (2013). Effects of precipitation temperature on nanoparticle surface area and antibacterial behaviour of Mg (OH) 2 and MgO nanoparticles. *Journal of Biomaterials and Nanobiotechnology, 4*(04), 365. http://dx.doi.org/10.4236/jbnb.2013.44046.

Wang, H. C., Hsieh, M. I., Choi, P. C., & Wu, C. J. (2018). Comparison of the Sensititre YeastOne and CLSI M38-A2 microdilution methods in determining the activity of amphotericin B, itraconazole, voriconazole, and posaconazole against Aspergillus species. *Journal of Clinical Microbiology, 56*(10), 10-1128. https://doi.org/10.1128/jcm.00780-18.

Wirunchit, S., Gansa, P., & Koetniyom, W. (2021). Synthesis of ZnO nanoparticles by Ball-milling process for biological applications. *Materials Today: Proceedings, 47,* 3554-3559. https://doi.org/10.1016/j.matpr.2021.03.559.

Xie, Y., He, Y., Irwin, P. L., Jin, T., & Shi, X. (2011). Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. *Applied and environmental microbiology, 77*(7), 2325-2331. https://doi.org/10.1128/AEM.02149-10.

Xiong, J., Lipsitz, O., Nasri, F., Lui, L. M., Gill, H., Phan,

L., ... & McIntyre, R. S. (2020). Impact of COVID-19 pandemic on mental health in the general population: A systematic review. *Journal of affective disorders, 277,* 55-64. https://doi.org/10.1016/j.jad.2020.08.001.

Zhang, Y., Li, H. N., Li, C., Huang, C., Ali, H. M., Xu, X., ... & Said, Z. (2022). Nano-enhanced biolubricant in sustainable manufacturing: from processability to mechanisms. *Friction, 10*(6), 803-841. https://doi.org/10.1007/s40544-022-0674-x.

Zili, Z., Sfar, S., & Fessi, H. (2005). Preparation and characterization of poly-ε-caprolactone nanoparticles containing griseofulvin. *International journal of pharmaceutics, 294*(1-2), 261-267. https://doi.org/10.1016/j.ijpharm.2005.01.020.