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New Biogenic nanoparticles as natural products in medicine production and water pathogen elimination, Characterization, Cytotoxic Evaluation and Antimicrobial Resistivity of Zinc Oxide Nanoparticles from *Psidium guajava* leaves

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Abstract

Biogenic production of nanomaterials gained great importance in nanotechnology industry. Zinc Oxide is one of the most effective antimicrobial agents. Zinc oxide nanoparticles (ZnO-NPs) may be produced cheaply and sustainably for a variety of biomedical uses and water disinfection for removing water pathogens. An efficient reducing and capping agent, *Psidium guajava* leaves aqueous extract and zinc sulphate ($ZnSO_4$), as precursors were used in this study to biosynthesizeZnO-NPs. Several methods were used to characterize PgZnONPs (*Psidium guajava* zinc oxide nanoparticles) including X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy(TEM) and UV-Vis Spectroscopy. Disc Diffusion was applied to evaluate Antimicrobial Resistivity of PgZnONPs (*Psidium guajava* zinc oxide nanoparticles). The cytotoxicity test preformed against African Green Monkey Kidney cell lines (Vero) using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]assay by applying different concentrations. Results indicate resistivity of most tested bacterial and fungal strains with varying degrees. A dose-dependent cytotoxic effect was observed, where 50% cytotoxic concentration (CC50) for $ZnSO_4$ and PgZnONPs (*Psidium guajava* zinc oxide nanoparticles) were (13.46µg/ml) and (785.20µg/ml) respectively.

Keywords: Antimicrobial Activity - Disc Diffusion - Medicine Industry- Green synthesis of Nanomaterial – Zinc oxide Nonmaterial- guava –*Psidium guajava* leaves- Water Purification- water disinfection-MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]assay - Vero Cell Line- Cytotoxicity

Introduction

In order to tackle the problems of hazardous waste and energy, biogenic synthesis of NPs has been suggested and successfully applied. Plants are the best biological resource together with bacteria, algae and fungi for the production of NPs. Although the biogenic synthesis of ZnO-NPs has already been proven, there is little information on the variety of their biological advantages. For material scientists, the development of environmentally sustainable methods for the production of nanoscale materials has recently played a central role. In this perspective, the rapid, and safe plant-based synthesis of NPs is an emerging trend in green chemistry [1].

Zn is one of the essential micro minerals known as antioxidants since it has been shown to have a key function as a cofactor for more than 300 enzymes, including the crucial ones, in the metabolic system. Plant secondary metabolites, such as phytogenic substances derived from plant extracts that are converted into bio-reducers and bio-stabilizers during the creation of nanoparticles, can support metabolic processes in certain concentrations [2]. Zinc oxide is a semiconductor metal with extensive diversity in nano-sized structures and have an important antimicrobial activity. Zinc oxide at the nanoscale has demonstrated various antibacterial properties and can be used in food preservation for prolonged periods of time. ZnONPs have been embedded in polymeric matrices to aid in packing and provide antibacterial properties to the components of the packaging [3]. ZnONPs have established an effective function against food-borne pathogens, for example, B. subtilis, S. aureus, L. plantarum, and E. coli . ZnONPs show a variety of promising bactericidal potentials against both Gram-positive and Gram-negative bacteria, as well as other diverse features such as barrier characteristics, mechanical strength, and stability. Zinc oxide has the benefit of containing mineral components essential to humans and has more potential even at low concentrations as an antibacterial agent. A combination of frequently

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used chemicals and other physical techniques could be employed for the synthesis of zinc oxide nanoparticles [4,5].

An environmentally favorable method is the phytogenic synthesis using plant extract. Plant components as terpenoids, alkaloids, flavonoids, and total phenols were the main participant in nanoparticles operation. Phytogenic components from plant extracts are used as stabilizer's and reductants in production processes to produce nanoparticles. Guava leaves, or (*Psidium guajava*) has been widely reported to have high quantities of several different phenolic compounds that enhancing biosynthesis of nanoparticles [6].

Nanoparticles have various properties because of their distinctive characteristics, such as size, dispersion, and shape. A nanoparticle, also known as a nanopowder, nanocluster, or nanocrystal, is a microscopic particle with at least one dimension less than 100 nm. Nano remediation has been a creative and effective response to green cleaning, and it currently plays a significant role in preventing, detecting, observing, and resolving pollution. It may be able to resolve some cleanup-related problems and increase the overall efficiency of remediation efforts. The toxicants that are dangerous for both chemicals and catalysts can be reduced by these nanoparticles. Nanomaterials provide a wide range of predicted properties for on-site applications [7].

Several investigations on the antibacterial properties of zinc oxide nanoparticles demonstrated that they have effective antimicrobial activity against a variety of bacteria and fungi. The antimicrobial activity of zinc oxide nanoparticles against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Candida albicans*, *Candida tropicalis*, *Fusarium oxysporum*, and *Aspergillus niger* was identified [8,9] .

Many synthetic substances have antibacterial properties, but only a small number of them can be utilised as biocides for use in medications or coatings. Their toxicity in comparison to their bactericidal action is the main barrier to their usage; some of them are so poisonous to eukaryotic cells that they cannot be recommended as antibiotics. ZnO and nano-ZnO compounds stand out among these materials as powerful antibacterial agents. These inorganic oxides have the benefit of containing environmentally acceptable mineral components that are necessary for humans and exhibiting high activity even when delivered in a little amount. In our study *Psidium guajava* leaves extract was used as a reducing and capping agent during the synthesis of zinc oxide nanoparticles PgZnONPs (*Psidium guajava* zinc oxide nanoparticles) from zinc sulphate ($ZnSO_4$), which served as the precursor material. This was done in consideration of the benefits of green nanoparticle synthesis and social economic problems brought on by microbes, determining how well it inhibits bacterial and fungal development. Many characterizations were carried out with antimicrobial activity and cytotoxicity evaluation to investigate *Psidium guajava* zinc oxide nanoparticles to further save ecological applications as water purification, soil remediation, food and medical industry.

1. 2. Materials and Methods

2.1 Materials

For biogenic synthesis of the PgZnONPs analytical-grade compounds were used, zinc sulphates ($ZnSO_4$) as a precursor, sodium hydroxide pellets were purchased from Merck. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, Saint Louis, MO, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich, Saint Louis, MO, USA), dimethyl sulfoxide (DMSO) ≥ 99.5% *Psidium guajava* leaves used as a reducing and capping agent were gathered from a farm in Giza, Egypt, Fig (1).

2.2 Preparation of *Psidium guajava* leaves Extract & Green Synthesis of Zinc Oxide Nanoparticles (PgZnONPs).

30 g of *Psidium guajava* leaves was cleaned, washed with water, then chopped into small pieces, peeled, and dried in the air. They were then added to 150 ml of distilled water and boiled for 15 minutes at 60°C. The Whatman (No. 1) filter paper was used to filter the cooled aqueous extract before it was kept at 4 °C and used without any further purification (*Menna Zayed , 2021, 1). After dissolving 11.5 g of $ZnSO_4$ in 40 ml of distilled water producing 1M mixture, then10 ml of the produced PgLE was added progressively to the mixture for 10 minutes with continuous shaking. Finally, adding sodium hydroxide solution (2 M) to the mixture dropwise. The mixture was periodically washed with distilled water and centrifuged at 3000 rpm for 15 minutes before being dried at 150 °C in a hot air oven. PgZnONPs, a completely dried nanopowder, was carefully obtained, Fig (1) [10, 11].

$$ZnSO_4 + 2NaOH \rightarrow Zn(OH)_2 + Na_2SO_4 \quad \text{Eq.1}$$

$$Zn(OH)_2 \rightarrow ZnO + H2O \quad \text{Eq.2}$$

Oxide Nanoparticles PgZnONPs

Synthesized PgZnONPs were examined quantitatively using UV-Vis spectroscopy (Shimadzu, Kyoto, Japan) at a wavelength range of 200-800 nm. Their shape was then confirmed using SEM (Hitachi-Regulus, Tokyo, Japan) by applying samples directly to a carbon tape without any coatings. A collection of SEM pictures was used to calculate the particle diameters. The obtained

dimensions were approximated using average measurements. PgZnONPs were sonicated, deposited onto a copper grid, and suspended in distilled water to analyze their structure. After drying, TEM examination was performed on them by (Zeiss, Oberkochen, Germany). Additionally, XRD (Empyrean, Malvern Panalytical, Malvern, UK) analysis was carried out to confirm the crystallite size and the phase composition of PgZnONPs using Co-K1 radiation (= 1.54056 A), and strains. Using Cu-K1 radiation, a current of 40 kV/30 mA was utilized to test the powder, and the Williamson-Hall method was employed to determine the crystallite size and lattice strain of the synthesized PgZnONPs [12].

Figure (1): Represents steps for biosynthesis of PgZnONPs from the collection of leaves to obtaining PgZnONPs.

2.3 Characterization of Green Synthesized Zinc

2.4 Susceptibility Test for determination of Antimicrobial activity

We evaluated the inhibitory activity of PgZnONPs against several bacterial and fungal strains using the disc dilution method. The Clinical and Laboratory Standards Institute (CLSI) was used to conduct the assay (M02-A11). This was the process that was as followed: First, sterile Sabourdad Dextrose Agar and Nutrient Agar (20 ml each) for fungi and bacteria respectively were poured into Petri dishes. After being prepared about24 hours, the test cultures of inoculums (certified bacterial and fungal strains) were swabbed onto the solidified media and given 10 minutes to dry. On separate bacterial and fungal aseptically prepared sensitized plates, discs were impregnated with previously generated PgZnONPs (20 µg/disc), PgLE (20 µg/disc), ZnSO4 (20 µg/disc), and antibiotic disc. To allow compound diffusion, the loaded discs were placed on the surface of the medium and left there for 30 min. at room temperature. The plates were then subsequently incubated for a further 24 hours at 37°C for bacteria and at 25-28°C from 5-7 d for fungus. The antibacterial activity was measured in millimeters by measuring the clear zone of growth inhibition on the agar surface surrounding the discs [13, 14, and 15].

2.4.1. Microorganisms

The disc diffusion method was used to evaluate the antibacterial and antifungal activity of PgZnONPs against different certified (bacterial and fungal) strains in order to assess its efficacy.

2.4.2. Bacterial Strains

All Bacterial certified strains were purchased from the Regional Centre for Mycology & Biotechnology(RCMB), Al-Azhar University, Cairo, Egypt and Sigma-Aldrich Co., St. Louis, Missouri, USA, Namely, *Pseudomonas aeruginosa* RCMB005B001(7), *Proteus vulgaris* RCMB004B001(2), *Klebsiella pneumonia* RCMB003B001(2), *Escherichia coli* RCMB002B001(4), *Staphylococcus aureus* RCMB008B001(5), *Salmonella typhimurium* RCMB006B001(1), *Bacillus subtilis* NCTC 10400/ATCC6633.

2.4.3. Fungal Strains

All certified fungal strains were purchased from the Regional Centre for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Namely, Fungal certified strains: *Saccharomyces Cerevisiae* RCMB002Y001(2), *Candida albicans* RCMB001Y003(4), *Candida glabrata* RCMB001Y009(9), *Penicillium pinophilum* RCMB001F042(1)), *Fusarium Oxysporum* RCMB005F002(4), *Aspergillus fumigatues* RCMB 002009(9), *Aspergillus flavus*RCMB 002F002(2). *Aspergillus niger* RCMB002F008(4).

2.4.4. Antibiotic Standard Disc

(A) For Bacteria: tetracycline TE 30mcg/disc (B), for fungi ketoconazole 10 mcg/disc. The generic antibiotic discs were acquired from Sigma Aldrich Co., St. Louis, Missouri, USA.

2.5. Cytotoxicity.

The cytotoxicity of the synthesized PgZnONPs, $ZnSO_4$ in the African Green Monkey Vero Cell Line was evaluated separately using an MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay. The cells were cultivated at a density of ($1.2 * 10^6$ cells/ml) in 96-well microplates and then kept in an incubator at 37 °C with 5% CO_2 for 24 hr. PgZnONPs were introduced to fresh DMEM media in the wells at final concentrations ranging from 6.25 to 800 µg/ml.100 µL fresh medium was added to 10-µL MTT (5 mg/ml). Additionally, 4 h later, the medium was discarded again and another 100-µL DMSO was added to dissolve the formazan crystals.The cells were left to mature for 24 or 48 hours [10]. A microplate reader was used to calculate the optical density at 570 nm (BioTek, USA). The formula used

to determine cell viability (%) was calculated using the formula:

Cell viability (%) = OD of sample/OD of control × 100%, Eq.2

Cytotoxicity % = 100 – Viability%, Eq.3

Where OD of control is the absorbance of the untreated cells and OD of sample is the sample absorbance of the treated cells.

3. Results and Discussion

3.1. Characterization of synthesized PgZnONPs

Using Transmission Electron Microscope (TEM) and the Scanning Electron Microscope (SEM) images, it was possible to determine the nanofabrication of PgZnONPs, as shown in Fig (2a&2b). NPs with a light oval form were found, according to SEM scans. SEM measurements revealed that the PgZnONPs had a diameter of 35.82 ±10.17 nm. The TEM images showed that PgZnONPs also had spherical morphologies, with a diameter of 44.68± 11.60 nm (Figure 2b).

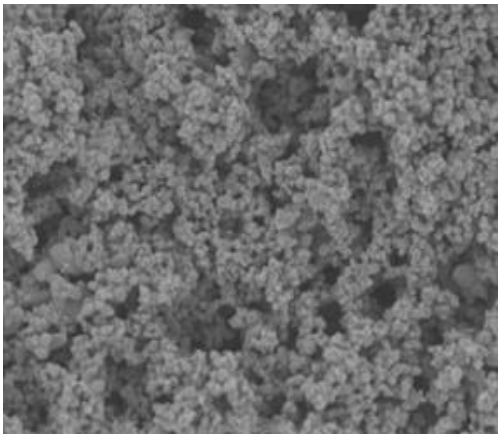

Figure 2a

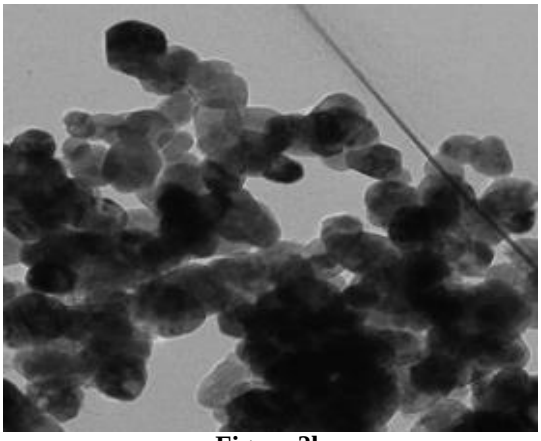

Figure 2b

Figure (2) revealed (a): SEM (b): TEM image of biogenic syntheses PgZnONPs

The flavonoid-mediated ZnO NPs were found to be fiber-shaped, single-crystalline, and used as a reducing agent for a zinc precursor (zinc sulphates), with an average diameter of 31.24 nm. A number of kinetic factors, such as the originating plant species, reactant concentration, and pH, affect the shape and size of ZnO NPs. In this investigation, we used a one-pot green reaction with PgLE as the reducing agent. PgLE 's capacity to bioaccumulate metal ions, as well as their bioreduction and stabilization during the process, can be used to fabricate PgZnONPs. PgLE 's hydroxyl (OH) groups cause the formation of a $[Zn(OH)_4]_2$ complex, which is essential for PgZnONPs ' abilityfor confirm. Then, by adjusting the zinc-base ratio, one can induce preferential growth along a single crystalline plane that influences both the nucleation and subsequent growth of ZnO nanostructures [17].

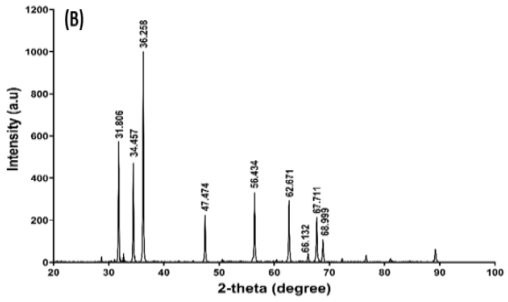

Figure (3) indicate XRD pattern for PgZnONPs

An X-Ray diffraction analysis (XRD) pattern was used to physically characterize the synthesized nanoformulation, as shown in Figure 3. All of the diffraction peaks were located at the following coordinates: 31.806, 34.457, 36.258, 47.474, 56.434, 62.671, 67.711 and 68.999, which correspond to the (110), (002), (101), (102), (110), (103), (200), and (112) orientation planes. This indicates a typical ZnO structure diffraction with hexagonal phase [16].

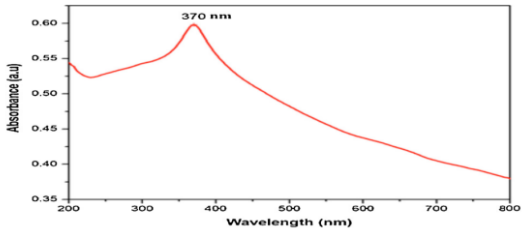

Figure (4) Figure (4): The absorption spectrum of PgZnONPs at UV-Visible region

PgZnONPs in Fig(4) exhibits a distinctive absorption peak in the spectrum at a wavelength of 370 nm, which can be attributed to the material's inherent band-gap absorption caused by electron transitions from the valence band to the conduction band. [18].

Chemical preparation is time-consuming, costly, and involves hazardous reducing agents, all of which have a negative impact on the environment. Whereas green synthesis was determined to Pseudomonas aeruginosa RCMB005B001 (7)e a cheap and cost-effective technology. As efficient reducing agents, plant extracts were utilized to cause physiochemical changes in nanoparticles [17].

3.2. Antimicrobial (Antibacterial &Antifungal) Activity

3.2.1. Antibacterial Activity

The antibacterial activity of the produced PgZnONPs was evaluated using the disc diffusion Susceptibility Test method Bauer et al., 1966 [19]. The zone of inhibition that PgZnONPs create for bacteria was used to detect antibacterial activity. The sterilized cotton swabs were used to spread the bacterial culture on nutrient agar. The agar plate was divided into four sections, one of them included impregnated disc with C1: tetracycline TE 30 mcg, C2:$ZnSO_4$, PgZnONPs, and PgLE were incubated at 37 ◦C for 24 hours. For each microbial strain separately, the inhibitory zones that formed were measured and evaluated [14] & [20].

Table (1): The Disc Diffusion assay of The piogenic synthesis PgZnONPs with PgLE, C1:tetracycline TE 30mcg and C2:ZnSo4with for evaluating antibacterial resistivity.

Certified Bacterial Strains	Inhibition Zones in milimiters			
	tetracycline TE 30mcg:C1	$ZnSO_4$: C2	PgZnONPs	PLE
Pseudomonas aeruginosa	50± 0.25	90± 0.09	98± 0.16	10± 0.10
Proteus vulgaris	5± 0.02	40± 0.13	35± 0.38	15± 0.10
Klebsiella pneomoniae	50± 0.11	28± 0.44	15 ± 0.27	5± 0.01
Escherichia coli	10± 0.14	40± 0.27	25± 0.31	10± 0.10
Staphylococcus aureus	20± 0.18	38± 0.38	10± 0.21	2± 0.08
Salmonella typhimurium)	5± 0.01	20± 0.17	42± 0.20	5± 0.27
Bacillus subtilis	50± 0.46	30± 0.14	63± 0.76	10± 0.12

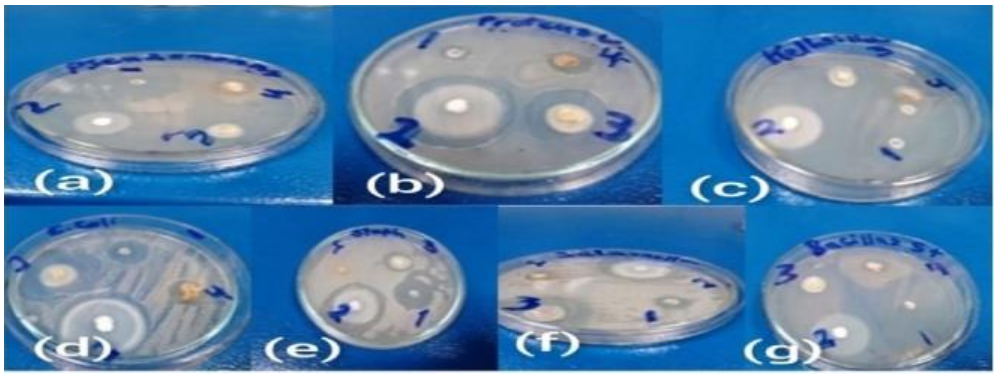

Figure (4) The Disc Diffusion assay of Biogenic synthesis PgZnONPs with PgLE, tetracycline TE 30mcg:C1 and ZnSO4: C2 for evaluating antibacterial activity for (a) *Bacillus subtilis, (*b) *Escherichia coli* (c) *Salmonella typhimurium* (d) *Staphylococcus aureus* (e)*Pseudomonas aeruginosa* (f) *Klebsiella pneomoniae* (g) *Proteus vulgaris*.

The findings of the PgZnONPs disc diffusion test are summarized in Table (1), Fig. 5. According to the results, *Pseudomonas aeruginosa* RCMB005B001(7), *Bacillus subtilis* NCTC 10400/ATCC6633, and *Salmonella typhimurium* RCMB006B001(1) had the strongest antibacterial activity, with inhibition zones of 98± 0.16, 63± 0.76 and 42± 0.20 respectively, compared to C1:50± 0.25, 50± 0.46 and 5± 0.01. Staphylococcus aureus RCMB008B001 (5) had the lowest antibacterial score with 10± 0.21. The disc diffusion test revealed that PgZnONPs exhibited antibacterial activity that might prevent the growth of water bacterial pathogens because a clean zone appeared around the PgZnONPs disc. Our outcome displays the apparent clear zone produced by PgZnONPs against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus vulgaris*, *Klebsiella pneomoniae*, and *Escherichia coli* are some examples of different types of Gram negative bacteria that are in agreement with [18,19]. *Proteus vulgaris* RCMB004B001(2) had the greatest values for PgLE's antibacterial activity when compared to PgZnONPs, with an inhibition zone of 15± 0.10mm [20, 21].

Changes in shape, size, surface area and the surface charge of PgZnONPs enhance the antibacterial effect by rupturing the microbe's cell membrane and resulting in cell death. Many different types of pharmaceutical formulations contain plant leaves as Herbal remedies for chronic bronchitis, whooping cough, fever, cold, cough, jaundice, asthma, diarrhea, and excruciating rheumatic inflammatory swellings are mostly made from plant leaves because they

contain secondary metabolites and phytochemicals that have biological effects [22, 23].

3.2.b. Antifungal Activity

The antifungal activity of the produced PgZnONPs was evaluated using the disc diffusion Susceptibility Test method Bauer et al., 1966. The zone of inhibition that PgZnONPs create for testing fungi was used to detect antifungal activity. The sterilized cotton swabs were used to spread the fungal culture on Sabroud dextrose agar. The agar plate was divided into four sections, one of them included impregnated disc with C1: ketoconazole 10 mcg/disc, C2:$ZnSO_4$, PgZnONPs, and PgLE were incubated at 28 ◦C for 5-7d. For each microbial strain separately, the inhibitory zones that formed were measured and evaluated [14] & [20].

Table (2): The Disc Diffusion assay of biogenic synthesis PgZnONPs with PgLE, ketoconazole 10 mcg (vl/50) D.:C1 and ZnSO4: C2 for evaluating antifungal activity.

Certified Bacterial Strains	Inhibition Zones in milimiters			
	ketoconazole 10 mcg (vl/50) D.:C1	$ZnSO_4$: C2	PgZnONPs	PLE
Saccharomyces Cerevisiae RCMB002Y001(2)	15± 0.44	50± 0.11	35± 0.28	2± 0.24
Candida albicans RCMB001Y003(4)	10± 0.09	40± 0.10	25± 0.08	0± 0.0
Candida glabrata RCMB001Y009(9)	30± 0.27	67± 0.14	19± 0.21	0± 0.0
Penicillium pinophilum RCMB001F042(1)	15± 0.13	60± 0.25	45± ± 0.38	5± 0.07
FusariumOxysporum RCMB005F002(4)	5± 0.16	20± 0.20	10± 0.06	0± 0.0
Aspergillus fumigatues RCMB 002009(9)	5± 0.18	28± 0.18	12± 0.13	0± 0.0
Aspergillus flavus RCMB 002F002(2)	5± 0.12	15± 0.10	19± 0.21	0± 0.0
*Aspergillus niger*RCMB002F008(4)	2± 0.2	46± 0.25	18± 0.2	0± 0.0

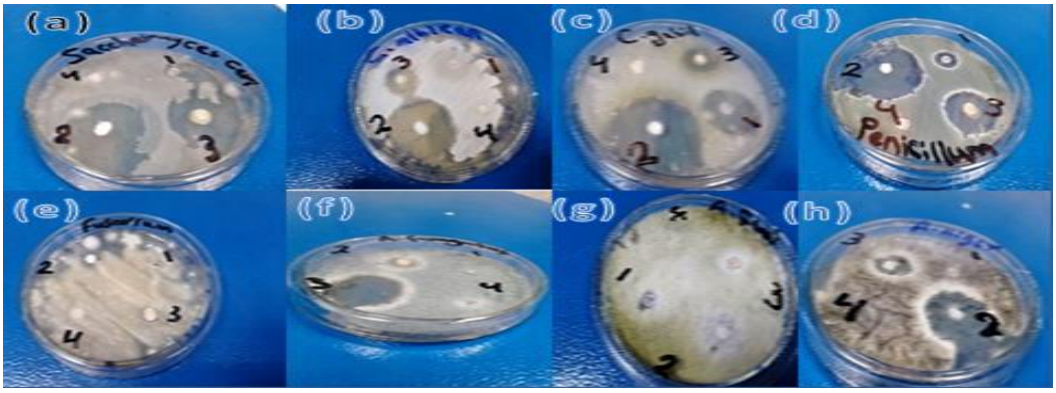

Figure (5) The Disc Diffusion assay of Biogenic synthesis PgZnONPs with PgLE, tetracycline TE 30mcg:C1 and $ZnSO_4$: C2 for evaluating antifungal activity for (a) *Saccharomyces Cerevisiae (b) Candida albicans* (c) *Candida glabrata*(d) *Penicillium pinophilum (e) Fusarium Oxysporum* (f) *Aspergillus fumigatues (g) Aspergillus flavus* (f) *Aspergillus niger* .

From the results shown in table (2), Figures (5) from (a:h) it can be concluded that the following organisms have antifungal activity with different degrees against PgZnONPs and PgLE compared with C1: ketoconazole 10 mcg/disc D&C2:$ZnSO_4$.

It is clear that PgZnONPs have strong antifungal activity against Fig (5d):*Penicillium pinophilum* RCMB001F042(1) 45± 0.38 mm, Fig (5a): *Saccharomyces Cerevisiae* RCMB002Y001(2) 35± 0.28 mm and Fig(5b): *Candida albicans* RCMB001Y003(4) 25± 0.08mm recoded highest antifungal values respectively.

On the other hand the lowest antifungal activity obtained at Fig (5e) :*Fusarium Oxysporum* RCMB005F002(4) with inhibition zone 10± 0.06 mm compared with 5± 0.16 mm for ketoconazole 10 mcg/disc and 20± 0.20 mm for $ZnSO_4$ respectively with no inhibition zone , while inhibition zones for *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* were recorded as 19± 0.21, 18± 0.2 and 12± 0.13 respectively.

The rapid rate of reproduction of bacteria and fungi is one of the main causes of their infectivity. Given that PgZnONPs were effective in inhibiting and killing bacteria and fungi in a dose- and time-dependent manner, limiting the microorganism's ability to reproduce hence it would be a good way to avoid a viable infection. Smaller PgZnONPs may be more toxic to bacteria and fungi due to their increased surface area, which makes them preferable to larger particles in terms of bactericidal or fungicidal properties, according to [24].

The inhibitory effect of ZnO particles is usually inversely related to their size. Therefore, ZnO nanostructures should exhibit greater antibacterial activity than bulk ZnO. In general, Gram-negative bacterial species are less sensitive to ZnO than Gram-positive bacterial strains. This could result from the differences in the nature and organization of microbial cell walls, which confirm our results [25,26].

Due to fungi's resistance to several common fungicides, including benzimidazoles and dicarboximides, it is difficult to control the growth of fungi. Investigating innovative antifungal drugs that could take the place of present control methods is crucial to overcoming this resistance. Due to their distinct physical and chemical characteristics, which differ greatly from those of their conventional counterparts, nanoparticle (NP) materials have attracted more interest recently. Silver, copper, titanium dioxide, and zinc oxide have all been shown in recent studies to exhibit antibacterial and antifungal properties [27,28].

Different growth morphologies of these fungi could, however, be the cause of the various antifungal effects. P. expansum has a tendency to grow more densely on the surface of agar plates than other strains, giving it more exposure to ZnONPs. In our study, it was evident that P. expansum had an inhibition zone of 10± 0.06 mm, which is the lowest value when compared to other strains. The inherent tolerance of each fungus to ZnONPs may also be a factor in the differences. Due to their distinctive qualities, namely their huge surface area, ZnO NPs in our study exhibit a significant improvement in the antimicrobial activity. However, [28] discovered that nano and bulk ZnO were equally hazardous to S. cerevisiae, which is consistent with our findings of 35± 0.28 mm was the second higher values for the inhibition zone obtained by applying PgZnONPs.

3.3. Cytotoxicity

3.3.a. cytotoxicity of PgZnONPs

Vero cell line was tested at various concentrations (6.25-800 µg/ml) to see whether the biosynthesized PgZnONPs had any cytotoxic effects. When compared to untreated cells, as shown in Fig (6&7) the produced PgZnONPs showed a significant increase in cytotoxicity the results revealed that the 50% cytotoxic concentration (CC50) was found up to (785.20µg/ml), with viability % 59.11 which indicated that higher cell mortality occurs at increasing concentrations of the utilized nanoparticles for the investigated cell lines ,whereas the highest inhibition % obtained at 800 µg/ml with value 40.81%. Additionally, the reaction was retained and incubated for 24 hours to check the effect of the inhibition.The variability in the level of inhibition valuable in medication. Figure (7): indicated the cytotoxic effect of different concentrations of PgZnONPs on Vero Cell Line variability where a: Control, non-treated b: treated with PgZnONPs 6.25 µg/ml C: treated with PgZnONPs 50 µg/ml d: treated with PgZnONPs 600 µg/ml, which revealed that PgZnONPs could be useful in many applications as water purification, food perseverate and medical applicationsafely.To assess the toxicity of the obtained nanoparticles, the dose-dependent approach (MTT assay) was applied. The observed mortality data demonstrated the potential antimicrobial activity of the biosynthesized PgZnONPs [29].

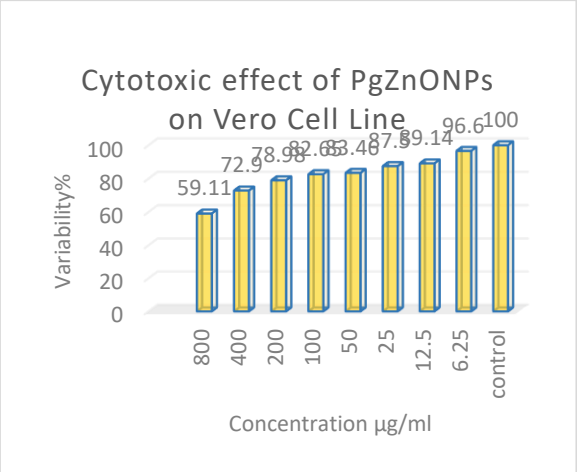

Figure (6): The cytotoxicity for different concentration of Biogenic PgZnONPs on Vero Cell Line viability.

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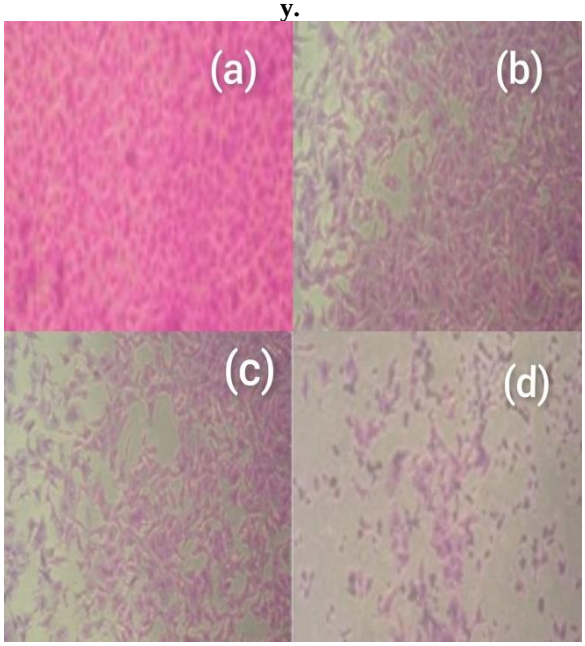

Figure (7): The cytotoxic effect of different concentrations of PgZnONPs on Vero Cell Line variability where a: Control, non-treated b: treated with PgZnONPs 6.25 µg/ml C: treated with PgZnONPs 50 µg/ml d: treated with PgZnONPs 600 µg/ml

3.3.2. Cytotoxicity of $ZnSO_4$

Vero cell line was tested at various concentrations (6.25-800 µg/ml) to see $ZnSO_4$ cytotoxic effects. When compared to untreated cells, as shown in Fig (8) & (9) the produced $ZnSO_4$ showed a significant increase in cytotoxicity, the results revealed that the CC50 was found up to (13.54µg/ml), with viability % 27.11 which indicated that higher cell mortality occurs at low concentrations, as extremely toxic,as the highest inhibition % 72.89% [30,33].

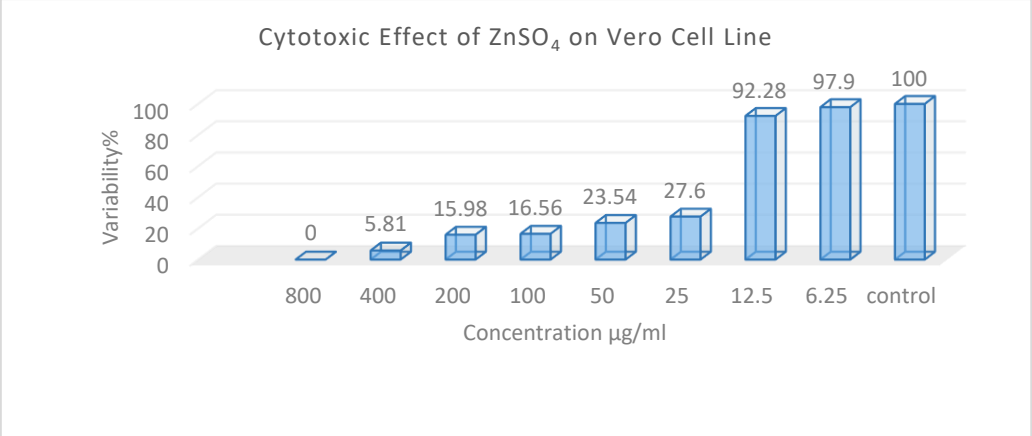

Figure (8): The cytotoxic effect for different concentration $ZnSO_4$ on Vero Cell Line variability%

The primary cause of cytotoxicity in vitro is the solubility of ZnO NPs. $Zn^{+2}$is required for maintaining cellular functions and metabolism at low concentrations, but at excessive concentrations, it can be harmful. Through the intrinsic mitochondrial route, ZnO NPs can cause apoptosis in cells. It has been demonstrated that ZnO NPs treatment lowers the potential of the mitochondrial membrane in vitro. Furthermore, the mitochondrion was found to adsorb excessive Zn^{+2}releases. The mitochondrion's generation of ROS and ATP is controlled by the presence of cations. In contrast, the quick Zn^{+2} influx causes a quick drop in mitochondrial membrane potential, which then triggers caspase-dependent apoptosis and the release of LDH. The results of this investigation support those found in [10], which indicated that which demonstrated that ZnO nanoparticles can be lethal by ER stress, cytotoxicity, and genotoxicity, which are strongly related to ROS formation and invariably result in cell death in vitro [25,34].

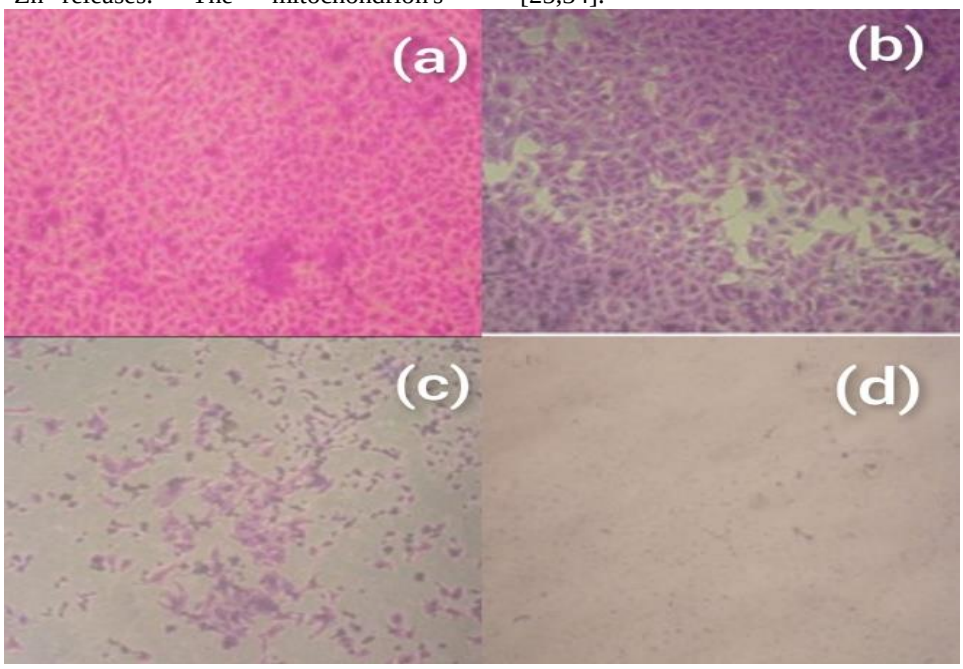

Figure (9): The cytotoxic effect of different concentrations of $ZnSO_4$ on Vero Cell Line variability% where a: Control, non-treated b: treated with 6.25 µg/ml $ZnSO_4$ C: treated with 25 µg/ml $ZnSO_4$ d: treated with 400 µg/ml $ZnSO_4$

Table (3): A Comparison between our study and other studies:

Antibacterial activity							
Zone of inhibition							Ref.
P aeruginosa	*E. coli*	*Staph. aureus*	*S. typhimurium*	*P. vulgaris*	*K. pneomoniae*	*B. subtilis*	
-	*24*	*22*	-	14	16	24	[23]
11	*9*	-	-	-	9	9	[26]
13	*11*	-	-	-	-	61	[27]
30	*17*	*21*	-	-	13	-	[25]
98	*25*	*10*	*42*	35	15	63	This study

Antifungal activity								
S. Cerevisiae	*C. albican*	*C. glabrata*	*P. pinophilum*	*F. Oxysporum*	*A. fumigatues*	*A. flavus*	*A niger.*	Ref.
-	18	-	-	-	-	-	[23]	[23]
-	-	-	-	13	11	11	13	[26]
-	10	-	-	-	-	-	-	[27]
35	25	19	45	10	12	19	18	This study

cytotoxicity			
Cell line Type	Concentration (µg/ml)	CC 50%	Ref.
Normal lung epithelial cell (WI38)	1000	902.83	[33]
Human leukemia monocytic cell (THP-1)	*200*	107	[35]
Vero cell (monkey kidney)	100	16.42	[34]
Vero cell (monkey kidney)	800	785.20	This study

4. Conclusion

1. Biogenic PgZnONPs were effectively created utilizing Psidium guajava L extract (PgLE) and ZnSO4 synthesizing NPs can be done in an uncostly, because the reducing agent can also be derived from trees , Ecologically friendly, and non-toxic manner.
2. UV spectroscopy revealed photocatalytic activity, suggesting it might be used in water treatment. The generation of biogenic ZnONPs is confirmed by XRD, TEM, and SEM.
3. Studies on antifungal and antibacterial resistance against produced PgZnONPs were conducted using the agar disc diffusion method with a variety of certified bacterial and fungal strains (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Bacillus subtilis, and Proteus vulgaris) (Saccharomyces Cerevisiae , Aspergillus niger , Candida albicans , Candida glabrata , Penicillium pinophilum , Fusarium Oxysporum, Aspergillus fumigatudes). The results support the high level of activity against numerous tested strains.
4. Biogenic PgZnONPs have powerful antifungal capabilities against the majority of the studied fungal strains, and the inhibitory effects are stronger as PgZnONP concentrations increased.
5. Through disruption of cell function and deformation of fungal hyphae, PgZnONPs could prevent the growth of fungus. higher ZnO NP concentrations result in a full inhibition of fungal growth by blocking the growth of conidiophores and conidia. These findings imply that ZnONPs nanoparticles could be utilized as a potent fungicide in agricultural and food safety applications.
6. The present finding clearly revealed that the prepared PgZnONPs using Pg L could be applied as a coating against that enhances the effect of antibacterial activity in paints, cosmetics, food preservatives, flavoring agents, as well as for fabrics, pipe manufacture and water treatment purification.
7. PgZnONPs can be applying to process and detoxify pollutants in nanoremediation.
8. Nanotechnology can also offer successful solutions to contamination-related problems such heavy metal pollution, the negative impacts of chemical poisons, and oil contamination and used as a tools for reducing it.
9. Due to its many advantageous properties, including as its superior thermal conductivity, ZnO is commonly used in a range of applications, including sensors, thin film electronics, and solar cells. In terms of their chemical and physical properties, ZnONPs have a relationship between their shape and crystallinity, are excellent

photocatalysts, and are useful for reducing environmental contaminants. The photocatalysis process is used to drive electrons from the valence to the conduction band and can form a hole in the surface of ZnO by the use of solar light irradiation. The radical that is created can then be used to eliminate pollutants.

10. The cytotoxicity study was conducted using PgZnONPs revealed a significantly lower CC_{50} values. However, it demonstrated no significant toxicity towards Vero Cell line. These results highlight the biogenic ZnO NPs and their possible medicine and water purification potential.

5. References

[1].Jan, H.; Shah ,M.; Usman, H.; Khan, M.A.; Zia ,M.; Hano, C. and Abbasi , B .H., (2020): Biogenic Synthesis and Characterization of Antimicrobial and Antiparasitic Zinc Oxide (ZnO) Nanoparticles Using Aqueous Extracts of the Himalayan Columbine (Aquilegia pubiflora). Frontier in Materials. 7:249. doi: 10.3389/fmats.2020.00249.

[2] Hidayat; Sumiat, E. ; Wina ;and Jayanegara , A.,(2021): Characteristics of Nano Zn-Fitogenik (NZF) made by green synthesis process using guava leaves (Psidiumguajava) for feed additives .Earth and Environmental Science ,888 - 012056 .IOP Publishing doi:10.1088/1755-1315/888/1/012056.

[3] Guleria, A.; Sachdeva, H.; Saini, K.; Gupta, K.; Mathur, J. Recent trends and advancements in synthesis and applications of plant-based green metal nanoparticles (2022): A critical review. Applied Organometallic Chemistry, 36, e6778, https://doi.org/10.1002/aoc.6778.

[4] Bukhari, A.; Ijaz, I.; Gilani, E.; Nazir, A.; Zain, H.; Saeed, R.; Alarfaji, S.S.; Hussain, S.; Aftab, R.; Naseer Y.(2021): Green synthesis of metal and metal oxide nanoparticles using different plants' parts for antimicrobial activity and anticancer activity: a review article. Vol.11, 1374, https://doi.org/10.3390/coatings11111374.

[5] Rohit Kumar Verma, Varad Nagar , Anuj Sharma, Badal Mavry ,, Poonam Kumari, Sneha Lohar, Ashrut Singhal , Monika Kumari Prajapati , Apoorva Singh , Kumud Kant Awasthi , Mahipal Singh Sankhla,(2023): Green Synthesized Nanoparticles Targeting Antimicrobial Activities, biointerfaceresearch, Volume 13, Issue 5, 469, https://doi.org/10.33263/BRIAC135.469

[6] Menna, Z. ;Hanan ,O. ;Heba, G. ; Ahmed, G. ;Hassabo, (2021): Psidium Guajava Leave Extract as Reducing Agent for Synthesis of Zinc Oxide Nanoparticles and its Application to Impart Multifunctional Properties for Cellulosic Fabrics . Biointerface Research in applied chemisitry- Platinum open acessJournal(iSsN:2069-5837), Volume 11, Issue 5, 2021, 13535 – 13556 https://doi.org/10.33263/BRIAC115.135351355 6.

[7] Alazaiza, M.Y. ; Albahnasawi, A. ; Ali, G.A. ; Bashir, M.J. ; Copty, N.K. ; Amr, S.S.A. ; Al Maskari, T., (2021): Recent advances of nanoremediation technologies for soil and groundwater remediation: a review. Water 13 (16), 2186.

[8] Mohammad hassan Gholami Shabani, Fattah Sotoodehne jadnem talahi, Masoomeh Shams Ghahfarokhi, Ali Eslamifar, Mehdi Razzaghi Abyaneh, (2022): Physicochemical properties, anticancer and antimicrobial activities of metallic nanoparticles green synthesized by Aspergillus kambarensis, IET Nanobiotechnol. Vol. 16:1–13, https://doi.org/10.1049/nbt2.12070.

[9] Mohamed S. Hasanin, Mona Nassar, Youssef R. Hassan , Łukasz Piszczyk , Mohammad Reza Saeb , Agata Kot-Wasik. (2023): Sustainable multifunctional zinc oxide quantum dots-aided double-layers security paper sheets, Heliyon 9, e14695, https://doi.org/10.1016/j.heliyon.2023.e14695.

[10] Hajiashrafi, S .and Kazemi, N.M. (2018): Green synthesis of zinc oxide nanoparticles using Parsley extract. Nanomed Res J 2018; 3(1): 44-50.

[11] onzález, C.A.; Anaya-Esparza, L.M.; Romero-Toledo, R.; Rico, J.L.; González-Vargas, O.A.; PérezLarios, A.(2022): Effect of the Precursor on the Synthesis of ZnO and Its Photocatalytic Activity. Inorganics 10, 16.

[12] Jabir, S.A.-A.; Harbbi, K.H. (2020): A comparative study of Williamson-Hall method and size-strain method through X-ray diffraction pattern of cadmium oxide nanoparticle. In AIP Conference Proceedings; AIP Publishing LLC.: Melville, NY, USA, p. 020015.

[13] Antibiotic Resistance, Fact Sheet, World Health Organization, Geneva, 2020: www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

[14]. Samah, A. Mohamed and Lameas, A. Mohamed, (2022): Eco-friendly, Non-Toxic, And Highly Antimicrobial Biosynthesized of Silver Nanoparticles (PAgNPs) From Pomegranate (PUNICA GRANATUM) PEEL (Waste) Extract (PPE) With Many Application in Nano Industries and Pathogen Elimination from Water. Egyptian Journal of Chemistry, accepted 4/10/2022. https://doi.org/10.21608/ejchem.2022.156513.67 82. Volume 65, Issue 13, December 2022.

[15]Chiu, H.I.; Che Mood, C.N.A.; MohamadZain, N.N.; Ramachandran, M.R.; Yahaya, N.; Nik Mohamed Kamal, N.N.S.; Tung, W.H.; Yong, Y.K.; Lee, C.K.; Lim, V. ,(2021):Biogenic Silver Nanoparticles of Clinacanthusnutans as Antioxidant with Antimicrobial and Cytotoxic Effects. Bioinorg. Chem. Appl., 9920890. [CrossRef].

[16] Saleemi, M.A.; Alallam, B.; Yong, Y.K.; Lim, V. Synthesis of Zinc Oxide Nanoparticles with Bioflavonoid Rutin, (2022): Characterisation, Antioxidant and Antimicrobial Activities and In Vivo Cytotoxic Effects on Artemia Nauplii. Antioxidants, 11, 1853. https:// doi.org/10.3390/antiox11101853.

[17] Jeyaleela, G.D.; Vimala, J.R.; Sheela, S.M.; Agila, A.; Bharathy, M.S.; Divya, M.(2020): Bio-fabrication of Zinc Oxide Nanoparticles using the Isolated Flavonoid from Combretumovalifolium and its Anti-oxidative Ability and Catalytic degradation of Methylene blue Dye. Orient. J. Chem., 36, 655–664. [CrossRef].

[18] Salehiabar, M.; Nosrati, H.; Davaran, S.; Danafar, H.; Manjili, H.K.,(2018): Facile synthesis and characterization of l-aspartic acid coated iron oxide magnetic nanoparticles (IONPs) for biomedical applications. Drug Res., 68, 280–285.

[19] Bauer A. W., W M Kirby, J C Sherris, M Turck, (1966): Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45, 493-496.

[20] SamahAbdelrazik Mohamed, (2022): Eco-Friendly Green Biosynthesis of Silver Nanoparticles (Or-AgNPs) Using Orange Peel (Citrus sinensis) Waste and Evaluation of their Antibacterial and Cytotoxic ActivitiesNano Hybrids and Composites Submitted: 2022-03-03 ISSN: 2297-3400, Vol. 36, pp 57-68 Revised: 2022-03-09 © 2022 Trans Tech Publications Ltd, Switzerland Accepted: 2022-03-14 Online: 2022-06-20.

[21] Shahid, H.; Shahzad ,A.; Shahid ,C.;Abdullah ,I.; Hussain, R.; Hussain, M.; Yasir ,M.; Shabbir ,M.; Zaheer ,A., and Khurshid, A. ,(2020): Zinc-Doped Boron Phosphide Nanocluster as Efficient Sensor for SO2. Hindawi Journal of Chemistry Volume 2020, Article ID 2629596, 12 pages https://doi.org/10.1155/2020/2629596.

[22] ShriniwasP. ,(2020):Psidiumguajava leaves assisted green synthesis of metallic nanoparticles: a review Beni-Suef University Journal of Basic and Applied Sciences (2020) 9:60 https://doi.org/10.1186/s43088-020-00088-2.

[23] Jehad M. Yousef, Enas N. Danial Journal of Health Sciences 2012, 2(4): 38-42 DOI: 10.5923/j.health.20120204.04 In Vitro Antibacterial Activity and Minimum Inhibitory Concentration of Zinc Oxide and Nano-particle Zinc oxide Against Pathogenic Strains

[24] Menna Zayed 1 , Hanan Othman 1 , Heba Ghazal 1 , Ahmed G. Hassabo. Psidium Guajava Leave Extract as Reducing Agent for Synthesis of Zinc Oxide Nanoparticles and its Application to Impart Multifunctional Properties for Cellulosic Fabrics, Biointerface Research in Applied Chemistry Volume 11, Issue 5, 2021, 13535 – 13556, https://doi.org/10.33263/BRIAC115.135351355 6

[25] Michael John Klink * , Neelan Laloo, Anny Leudjo Taka, Vusumzi Emmanuel Pakade , Mzimkhulu Ephraim Monapathi and Johannes Sekomeng Modise, 2022, Synthesis, Characterization and Antimicrobial Activity of Zinc Oxide Nanoparticles against Selected Waterborne Bacterial and Yeast Pathogens, Molecules 2022, 27(11), 3532, https://doi.org/10.3390/molecules27113532

[26]Hasnain Jan , Muzamil Shah1 , Hazrat Usman1 , Muhammad Aslam Khan1,2 , Muhammad Zia1 , Christophe Hano3,4 * and Bilal Haider Abbasi , (2020),Biogenic Synthesis and Characterization of Antimicrobial and Antiparasitic Zinc Oxide (ZnO) Nanoparticles Using Aqueous Extracts of the Himalayan Columbine (Aquilegia pubiflora), vol. 7 ,249 ,Frontiers materials.

[27]Tugba ˘ Gur a, Ismet Meydan , Hamdullah Seckin , Muhammed Bekmezci b , Fatih Sen, (2022): Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles,204, 111897 Enviromental research, https://doi.org/10.1016/j.envres.2021.111897

[28] Zhang, X.-F.; Liu, Z.-G.; Shen, W.; and Gurunathan, S., (2016): Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int. J. Mol. Sci. 17:E1534. doi: 10.3390/ijms17091534 3.3.2. For fungal strains.

[29] Lili ,He. ; Yang, Liu.;Azlin, Mustapha. andMengshi, Lin.,(2011): Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicilliumexpansum, Microbiological research, V.166, issue 3, 207:215.

[30]Ong, C.B.; Ng, L.Y.; Mohammad, A.W. (2018): A review of ZnO nanoparticles as solar photocatalysts: Synthesis, mechanisms and applications. Renew. Sustain. Energy Rev, 81, 536–551. [CrossRef]

[31] Bai, X.; Sun, C.; Liu, D.; Luo, X.; Li, D.; Wang, J.; Wang, N.; Chang, X.; Zong, R.; Zhu, Y.(2017): Photocatalytic degradation of deoxynivalenol using graphene/ZnO hybrids in aqueous suspension. Appl. Catal. B Environ., 204, 11–20. [CrossRef].

[32] Ng, C.T., Yong LQ, Hande MP, Ong CN, Yu LE, Bay BH, Baeg GH.(2017): Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and Drosophila melanogaster. Int J Nanomedicine. vol.12:1621-1637. doi: 10.2147/IJN.S124403. PMID: 28280330; PMCID: PMC5339013.

[33]Yasser A. Selim1*, MahaA.Azb2, Islam Ragab3 & Mohamed H. M.Abd El-Azim4, (2020), Green Synthesis of Zinc Oxide Nanoparticles Using Aqueous Extract of Deverra tortuosa and their Cytotoxic Activities ,YasserA. Selim1*, MahaA.Azb2, Islam Ragab3 & Mohamed H. M.Abd El-Azim4, 10:3445, https://doi.org/10.1038/s41598-020-60541-1

[34]Afraa Ali Kadhim1 · Nahida R. Abbas2 · Hussam H. Kadhum3 · Salim Albukhaty4,5 · Majid S. Jabir6 · Amel Muhson Naji7 · Sawsan S. Hamzah8 · Mustafa K. A. Mohammed9 · Hassan Al-Karagoly, (2023), Investigating the Efects of Biogenic Zinc Oxide Nanoparticles Produced Using Papaver somniferum Extract on Oxidative Stress, Cytotoxicity, and the Induction of Apoptosis in the THP-1 Cell Line, Biological Trace Element Research, https://doi.org/10.1007/s12011-023-03574-7