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# Biosynthesis of ZnO nanoparticles from plant extracts and using them to remove wastewater bacteria

**Hala Faez Al-Jawahery**
College of Science, University of Babylon, 51001, Babylon, Iraq. E-mail: sci.hala.ali@uobabylon.edu.iq

**Noor S Naji**
College of Science, University of Babylon, 51001, Babylon, Iraq. E-mail: sci.noor.saadallah@uobabylon.edu.iq

**Atheer SN Al-Azawey**
* Corresponding author. Environmental sciences, Al-Qasim Green University, 51001, Babylon, Iraq. E-mail: atheersaieb@environ.uoqasim.edu.iq

## *Abstract*

**Objective**

The aim of this study was to investigate the antibacterial efficacy of greenly synthesized zinc oxide nanoparticles (ZnO NPs). These nanoparticles were prepared using eucalyptus and green tea extracts for wastewater treatment applications. Another aim was to compare the antibacterial performance of plant extracts alone with ZnO nanoparticles used at different concentrations to determine their practical potential for wastewater disinfection.

**Materials and Methods**

Plant extracts were first evaluated as control treatments. aqueous extracts of *Eucalyptus* leaves and green tea as reducing and stabilizing agents were used to synthesize ZnO nanoparticles. The synthesized nanoparticles were applied to wastewater samples at two concentrations (0.5 g and 1 g per 100 mL). Controlled laboratory conditions were used to incubate treated samples before analysis. Antibacterial activity was assessed through total bacterial count reduction. Scanning electron microscopy (SEM) was used to observe surface morphology and structural damage of bacterial cells. Fourier transform infrared spectroscopy (FTIR) was performed to confirm nanoparticle formation and detect functional group changes.

**Results**

Compared to herbal extracts alone, we observed a significant reduction in total bacterial counts after treatment with ZnO nanoparticles. The removal efficiency increased in the following order: plant extract < 0.5 g ZnO nanoparticles < 1 g ZnO nanoparticles, indicating a clear dose-dependent response. SEM images demonstrated cell wall disruption, deformation, and reduced bacterial attachment on treated samples, especially at the higher nanoparticle concentration. FTIR analysis revealed a decrease in the intensity of organic and bacterial-related functional groups after treatment. This observation was along with the appearance of characteristic ZnO absorption bands. It confirmed successful nanoparticle synthesis and structural stability.

**Conclusion**

The results of this study showed that compared to plant extracts alone, ZnO nanoparticles synthesized by green method have better antibacterial activity. The antibacterial performance increased with concentration, demonstrating a strong concentration-effect relationship. According to the results of this study, it can be concluded that greenly synthesized ZnO nanoparticles can be proposed as an efficient, sustainable and environmentally friendly material for wastewater treatment and microbial control.

**Keywords:** bacterial removal, FTIR, SEM, wastewater treatment, ZnO nanoparticles

**Paper Type:** Research Paper.

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## Introduction

Nanotechnology is a multidisciplinary scientific field that uses a set of tools and techniques derived from engineering, physics, chemistry and biology (Heidarpour et al. 2011). Advances in nanoscience and nanotechnology have routinely enabled the fabrication and identification of submicron bioactive carriers. The delivery of bioactive substances to target sites in the body and their release behavior are directly affected by particle size (Mortazavi et al., 2005). Compared to micrometer-sized carriers, nanocarriers provide more surface area and have the potential to increase solubility, increase bioavailability, improve controlled release, and enable precise targeting of entrapped substances (Mohammadabadi and Mozafari, 2019). Green synthesis of zinc oxide nanoparticles (ZnO NPs) has become particularly popular over the past few years as one of the ways to address the principle of sustainability, cost-effectiveness, and environmental safety. Conventional synthesis of ZnO NP through traditional physical and chemical processes can be associated with the use of dangerous substances and consume a lot of energy, which does not allow making them large-scale and environmentally friendly. Conversely, biogenic/plant-based synthesis implements the naturally available phytochemicals as reducing, capping, and stabilizing reagents and avoids toxic chemicals, increasing the biocompatibility of the obtained nanomaterials (Al-Darwesh et al., 2024). ZnO NPs produced by plants have been also identified to possess special optical, structural, and antibacterial functions owing to their nanoscale size and great surface area, which are applicable across a variety of applications, such as environmental cleanup and wastewater treatment. Cassia fistula, Melia azadarach, and various algal extracts are examples of many medicinal plants and agricultural wastes that have been successfully used to produce ZnO NP by green synthesis methods, expanding the breadth of green synthesis methods (Naseer et al., 2020; Alprol et al., 2024). The formation of plant-mediated ZnO NP usually occurs through the reduction of zinc precursor salts by flavonoids, phenolics, and other secondary metabolites in the extract and then, nucleation and growth of nanoparticles. Not only do these compounds enable a reduction process, but they also offer the form of capping that leads to the improved stability of the particles and inhibition of their aggregation (Nguyen et al., 2024). In addition, UV-vis spectroscopy, SEM, and FTIR are typical methods of characterization to ensure the formation of nanoparticles and their morphology and interaction with functional groups respectively to ensure a reproducible and well-defined nanostructure (Abdelbaky, 2022; Rani et al., 2025; Farhan and Al-Azawey, 2024). Green synthesized ZnO NPs have shown high antibacterial action against various pathogenic bacteria. The augmented antibacterial effectiveness has been ascribed to conglomeration of processes such as augmented reactive oxygen species (ROS) production, Zn 2 + ion liberation, and direct contact with microbial cell walls resulting in disruption of the membrane and cellular harm (Sirelkhatim et al., 2015; Ahmad and Kalra, 2020). Green-synthesized ZnO NPs were also demonstrated to be more effective in antimicrobial activity when compared to plant extract alone in a number of comparative studies, indicating the synergistic effect of bioorganic capping agents and nanoscale ZnO (Ali and Risan, 2024). The use of ZnO NPs is not limited to antibacterial purposes, and it has been considered in areas of photocatalytic degradation of dyes and pollutants, adsorption of contaminants of industrial effluents, and even biomedicine (anticancer activity) (Alprol et al., 2023; Hussien et al., 2025). Moreover, the type of plant extract, and the change in the synthesis parameters (e.g., pH, temperature) have been found to have a significant effect on the nanoparticle size, morphology, and functional performance (Xu et al., 2021). Due to these versatile applications and the current necessity to rely on sustainable water treatment technologies, studies on plant-based ZnO NP synthesis are constantly growing, providing a potential way to remove the wastewater sustainably and helping to solve the issue of microbial contamination related to the population health.

## Materials and methods

**Chemicals and reagents:** Chemicals and reagents that have been employed in this research were of analytical quality and were not purified further. The precursor that was used in the biosynthesis of zinc oxide nanoparticles was zinc acetate dihydrate used as the source of zinc ions. All the procedures in the experiment were done using distilled and deionized water.

**Plant materials:** Leaves of fresh eucalyptus (Eucalyptus sp.) and green tea (Camellia sinensis) in Babylon Governorate in Iraq were brought. Plant materials were taxonomically verified and authenticated using Herbarium in the College of science, University of Babylon. To eliminate dust and surface impurities on the collected leaves, the leaves were after that fully washed using tap water and then distilled water before additional processing.

**Wastewater samples:** Sampling of wastewater was done in Al-Muaymira wastewater treatment station, Babylon Governorate, Iraq. The samples underwent sterile containers, were taken to the laboratory and kept at 4 °C, and their bacteriological properties were analyzed within 24 hours (APHA, 2017).

**Plant extracts preparation**: Plant extracts are prepared by dissolving plant materials in the right solvents with subsequent incubation of the mixture to a set time until the desired level of solubility is attained.

**The eucalyptus extract was prepared according to the following steps:** The Eucalyptus leaves were dried in an oven at 40 °C and then finely ground and sieved using a sieve of 100 mesh. In the case of extract preparation, the mixture of 6 g of eucalyptus powder and 100 mL of deionized water was heated at 60 °C over 1 hour. It was then filtered, and the extract stored at 4 °C to be used further. This extraction process was implemented based on the reported in the past synthesis techniques of using plants with minor adjustments (Shakeel et al., 2016).

**The green tea extract was prepared according to the following steps:** The green tea leaves were washed with the tap water and rinsed using the deionized distilled water. Approximately 24 hours were allowed to make the leaves air-dried and dried in the oven at 50 °C, followed by grind into fine powder. The powder of 10 grams was dissolved in 100 mL of distilled water and stirreded under magnetic stirring at 80 °C over a period of 2 hours. The sample was centrifuged at 3000 rpm, filtered using Whatman No.1 paper and later using a membrane filter with a pore size of 0.22 µm. The extract was kept at 4 °C and utilized in the next 1 month in accordance with the standard procedures of green synthesis (Ramesh et al., 2015).

**Biological synthesis of zinc oxide nanoparticles (ZnO NPs)-Preparation by eucalyptus extract:** The process involves the biosynthesis of a product using Eucalyptus Extract. ZnO nanoparticles were biosynthesized in the presence of the eucalyptus extract based on the reported green methods of synthesis with slight modifications (Sangeetha et al., 2011). Adding 50 mL of 0.2 M zinc acetate dihydrate solution, 50 ml of eucalyptus extract was stirred 350rpm and 60 °C until a brown precipitate was obtained. 2.0 M KOH was used to adjust the pH to 12 and centrifuge the mixture at 4000 rpm, 20 minutes. To obtain crystalline ZnO nanoparticles, the product was precipitated using deionized water, dried at 60 °C overnight, and calcinated at 400 o C after 2 hours.

**Preparation by green tea extract:** In case of green tea-mediated synthesis, the 25 mL of green tea extract was slowly put into 70 mL of 0.2 M zinc acetate dihydrate solution under constant mixing at 60 °C over a period of about 2 hours. The mixture became slowly pale white, which is evidence of the formation of nanoparticles. The mixture was centrifuged thrice and subjected to heating at a temperature of 100 °C in an hour. The product obtained was kept to be characterized and antibacterial further, using the already reported approaches (Nagarajan and Kuppusamy, 2013).

**Experimental design:** The experimental design entailed the application of six treatment groups on 100 mL of wastewater under the same experimental condition:

Eucalyptus-based treatments: 1- E1:1g eucalyptus leaves / 100 mL wastewater (plant control), 2- E2: 0.5 g biosynthesized ZnO nanoparticles / 100 mL wastewater, 3- E3: 1 g biosynthesized ZnO nanoparticles / 100 mL wastewater, 4- Green tea-based treatments, 5- T1: green tea leaves/ 100 mL wastewater (plant control), 6- T2: 0.5 g of ZnO nanoparticles in 100 mL wastewater were biosynthesized, and 7- T3: 1 g biosynthesized ZnO nanoparticle/ 100 mL wastewater.

**Wastewater treatment procedure:** The treatments were obsessed on 100 mL of wastewater using sterile containers. A bacteriological test was done on samples after treatment to measure the efficiency of antibacterial.

**Biosynthesized ZnO nanoparticles are characterized-UV-Visible spectroscopy:** Presumably, ZnO nanoparticles were biosynthesized, which was mostly validated by UV-Visible spectroscopy. The spectra of absorption obtained were taken in the range of 200-800 nm. The emergence of typical absorption peaks in the ultraviolet region served as the indication of the formation of ZnO nanoparticle, as indicated in the prior cases of green synthesis (Kołodziejczak-Radzimska and Jesionowski, 2014).

**Characterization techniques in Support:** The supporting techniques were Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM), which were performed to compare the results. FTIR was required to identify functional groups used in biosynthesis whereas SEM was required to observe the changes in surface morphology before and after treatment. They were used to corroborate and compare the findings as opposed to being used as the main confirmation techniques (El-Saadony et al., 2024).

**Bacteriological analysis of wastewater:** The standard culture-based methods were used to perform bacteriological examination (APHA, 2017). The total bacteria count was counted by using of nutrient agar incubated at 37 °C in 24 hours. Coliform bacteria were identified by incubating MacConkey agar at 37 °C and fecal coliforms by incubating the agar at 44 - 44.5 °C to select thermotolerant, fecally related coliforms (APHA, 2017). The isolation of the Pseudomonas spp. (Cetrimide agar) and Salmonella spp. and Shigella spp. (SS agar) was carried out selectively. They were treated and the bacteria counts were determined in CFU/mL.

**Removal efficiency determination:** The following equation, which is usually used in the literature on wastewater treatment, was used to compute removal efficiency (Metcalf and Eddy, 2014):

$$Removal\, efficiency\,(\%) = \left(\frac{(A - B)}{A}\right) \times 100$$

Where A represents the number of bacteria that are present before treatment and B represents the number of bacteria present after treatment.

**Statistical analysis:** All the experiments were performed in replicates, and the results were obtained in the form of mean values.

## Results

**The methods of UV-Vis analysis of the zinc oxide nanoparticles by Green Tea:** The UV-Vis absorption spectrum of the zinc oxide nanoparticles biosynthesized with the green tea extract showed a sharp peak of absorption in the ultraviolet region with a wavelength of about 300-330 nm (Figure 1). Such a characteristic absorption band is associated with the intrinsic band-gap absorption of ZnO nanoparticles and it proves the successful formation of ZnO nanostructures. The behavior of absorption was observed, which is in line with the UV-Vis spectra of ZnO nanoparticles synthesized through the green methods in the past, showing the development of nanoscale ZnO with good optical characteristics.

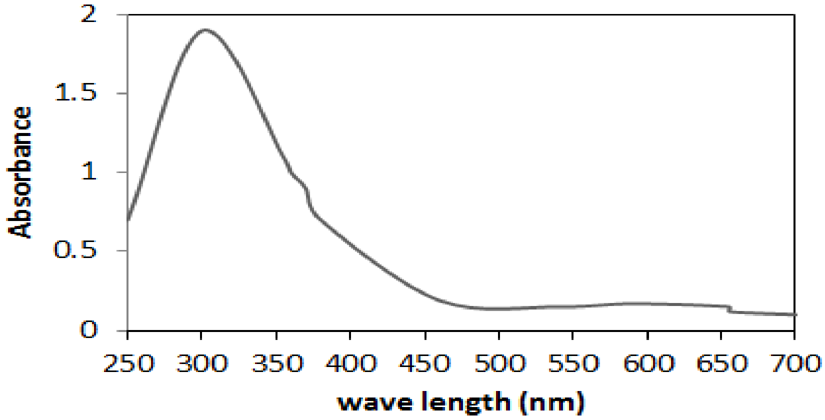


**Figure 1. UV- Vis absorption spectrum of biosynthesized zinc oxide (ZnO) nanoparticles using green tea extract**

**UV Vis characterization of zinc oxide nanoparticles by Eucalyptus:** The UV Vis absorption spectroscopy of the zinc oxide nanoparticles biosynthesized with the help of eucalyptus extract indicated the absorption peak in the ultraviolet region at about 340-360 nm (Figure 2). This absorption band is typical of intrinsic band-gap absorption of ZnO nanoparticles and it enough proves the successful formation of ZnO nanostructures. The occurrence of a high absorption peak in this wavelength range shows that nanoscale ZnO particles were formed through a green method.

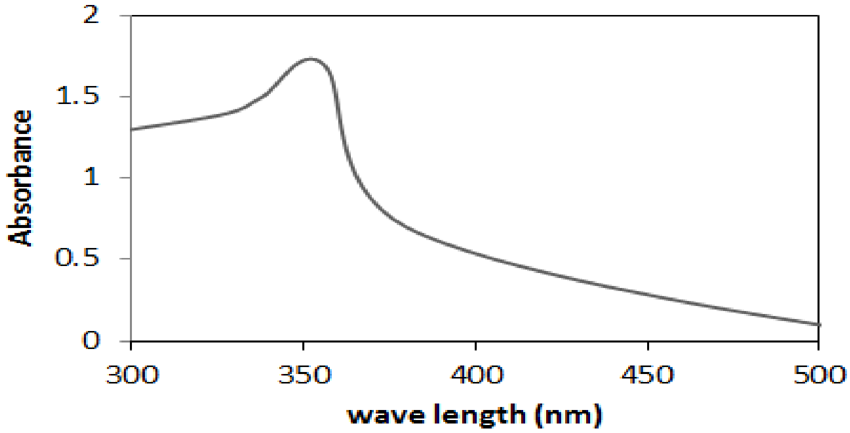


**Figure 2. UV Vis absorption spectrum of zinc oxide (ZnO) nanoparticles biosynthesized using eucalyptus extract**

**Comparison between the antibacterial efficiency of eucalyptus and green tea extracts and ZnO nanoparticles biosynthesized:** The antibacterial effect of green tea and Eucalyptus extracts and biosynthesized zinc oxide nanoparticles (ZnO NPs) was compared based on the measurement of the decrease in bacterial indicators of wastewater. The outcomes were in terms of bacterial counts (CFU) and efficacy of removal (percent) compared to the untreated wastewater.

**Green tea treatments (T1-T3):** Green tea extracts by (T1) demonstrated greater antibacterial activity in relation to the eucalyptus extract by itself. The total bacterial count was reduced to 1100CFU as compared to the initial number of 3700 CFU and a removal efficiency of 70.3 was achieved. Fecal coliforms and E. coli had a removal efficiency of 74.2% and 74.2% respectively. There was a reduction in Pseudomonas spp., Salmonella spp., and Shigella spp. of 70.0, 76.5, and 83.7, respectively. The introduction of the biosynthesized ZnO nanoparticles at 0.5 g/100 mL (T2) had a significant effect on antibacterial activity. The overall number of bacteria decreased by 97.6 percent, fecal coliforms and E. coli had 99.1 percent removal efficiencies. This concentration yielded complete (100) removal of Pseudomonas spp., Salmonella spp. and Shigella spp. The antibacterial activity was even enhanced at the maximum concentration of ZnO nanoparticle (1 g/100 mL, T3). Efficiencies of total bacterial count and fecal coliforms and E. coli were found to be 98.9 and 99.5 percent and 100 percent of Pseudomonas spp., Salmonella spp., and Shigella spp., respectively. In general, these findings indicate that ZnO nanoparticles produced with the help of bio-synthesis have a concentration-dependent antibacterial effect. ZnO nanoparticles mediated by both eucalyptus and green tea demonstrated a high level of removal efficiency, with a few more pretreatments made by green tea displaying a better level of antibacterial activity using similar concentration levels (Table 1) and (Figure 3).

**Table 1. Removal efficiency (%) and bacterial counts using green tea extract and biosynthesized ZnO nanoparticles**

| Bacterial type | Before treatment (CFU) | After treatment (CFU) | | | | | |
|---|---|---|---|---|---|---|---|
| | | T1 | Removal % | T2 | Removal % | T3 | Removal % |
| Total bacterial count | 3700 | 1100 | 70.3% | 88 | 97.6% | 39 | 98.9% |
| Fecal coliform bacteria | 1900 | 490 | 74.2% | 17 | 99.1% | 9 | 99.5% |
| *Escherichia coli* | 1900 | 490 | 74.2% | 17 | 99.1% | 9 | 99.5% |
| *Pseudomonas* spp. | 220 | 66 | 70.0% | 0 | 100% | 0 | 100% |
| *Salmonella* spp. | 310 | 73 | 76.5% | 0 | 100% | 0 | 100% |
| *Shigella* spp. | 98 | 16 | 83.7% | 0 | 100% | 0 | 100% |

T1: 1 g green tea extracts/ 100 mL wastewater, T2: 0.5 g ZnO NPS/ 100 mL wastewater, T3: 1 g ZnO NPS / 100 mL wastewater.

**Eucalyptus treatments (E1-E3):** The antibacterial activity of biosynthesized zinc oxide nanoparticles (ZnO NPs), and eucalyptus extract was examined by determining changes in bacterial indicators in wastewater. The findings were in form of bacterial counts (CFU) and removal efficiency percentage, which were measured against untreated wastewater. The experiment using eucalyptus extract alone (E1) led to a significant alteration in bacterial load whereby the number of total bacteria reduced to 1400 CFU, and the removal efficiency was 62.2. The counts of fecal coliform bacteria and Escherichia coli were minimized down to 190 CFU to

610 CFU which had a removal efficiency of 67.9. The recorded removal efficiencies of Pseudomonas spp., Salmonella spp., or Shigella spp. were 65.9, 67.7 and 67.3, respectively.

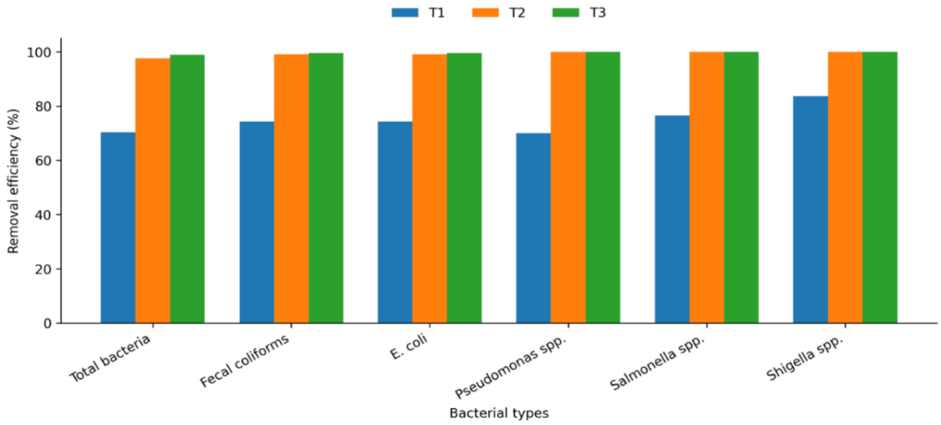


**Figure 3. Degradation of total and pathogenic bacteria in wastewater sampled at 0.5 g/100 mL concentration of green tea extract (T1), biosynthesized ZnO nanoparticles at 0.5 g/100 mL (T2) and 1 g/100 mL (T3) concentration**

Using biosynthesized ZnO nanoparticles at the concentration of 0.5 g/100 mL (E2) elevated the antibacterial activity to a significant level and the total counts of bacteria decreased to 110 CFU which is a removal efficiency of 97.0. Fecal coliforms and E. coli had removal efficiencies of 98.9 and high removal efficiencies of Pseudomonas spp. (99.1) and Salmonella spp. (99.0) and 100% removal of Shigella spp. The antibacterial activity was further enhanced by the highest concentration of ZnO nanoparticles (1 g/100 mL, E3) whose removal efficiencies were 98.6% total bacterial count, 99.4% fecal coliforms and E. coli. and, at this concentration, total removal (100% removal) of Pseudomonas spp., Salmonella spp., and Shigella spp. was attained. On balance, the findings indicate that there is a direct correlation between the concentration of the antibacterial effect by means of ZnO nanoparticles and eucalyptus, and significantly higher removal efficiencies are observed at higher concentrations of nanoparticles in comparison to the solution of eucalyptus alone (Table 2, Figure 4).

**Table 2. Removal efficiency (%) and bacterial counts with eucalyptus extract and biosynthesized ZnO nanoparticles**

| Bacterial type | Before treatment (CFU) | After treatment (CFU) | | | | | |
|---|---|---|---|---|---|---|---|
| | | E1 | Removal % | E2 | Removal % | E3 | Removal % |
| Total bacterial count | 3700 | 1400 | 62.2% | 110 | 97.0% | 52 | 98.6% |
| Fecal coliform bacteria | 1900 | 610 | 67.9% | 20 | 98.9% | 12 | 99.4% |
| *Escherichia coli* | 1900 | 610 | 67.9% | 20 | 98.9% | 12 | 99.4% |
| *Pseudomonas* spp. | 220 | 75 | 65.9% | 2 | 99.1% | 0 | 100% |
| *Salmonella* spp. | 310 | 100 | 67.7% | 3 | 99.0% | 0 | 100% |
| *Shigella* spp. | 98 | 32 | 67.3% | 0 | 100% | 0 | 100% |

E1: 1 g Eucalyptus extract/ 100 mL wastewater, E2: 0.5 g ZnO NPS / 100 mL wastewater, E3: 1 g ZnO NPS / 100 mL wastewater.

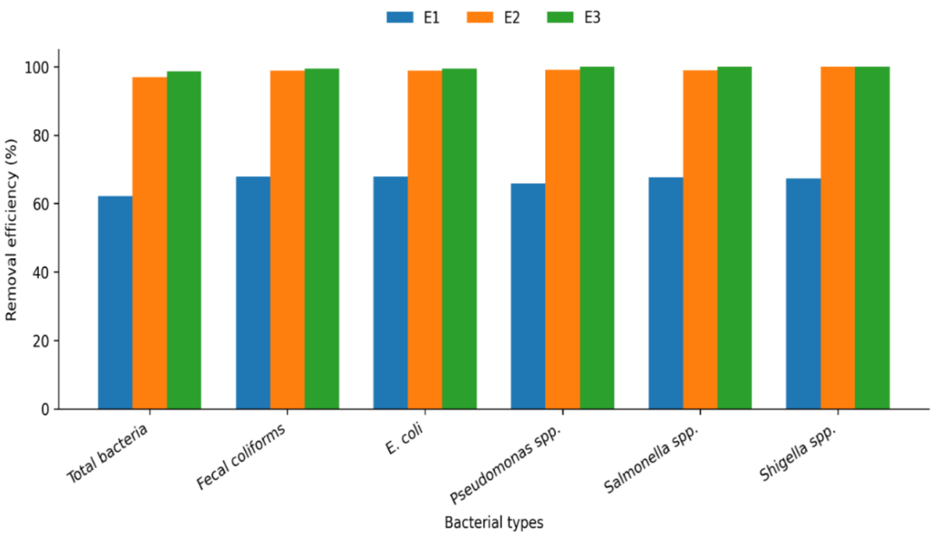


**Figure 4. Total and pathogenic bacteria present in wastewater have been eliminated by the use of eucalyptus extract (E1), biosynthesized ZnO nanoparticles at 0.5 g/100 mL (E2) and 1 g/100 mL (E3)**

**Comparison of the results of eucalyptus and green tea treatment:** A comparative analysis of the treatments made of eucalyptus and green tea showed that there are discernible disparities in antibacterial performance. Plant extracts by themselves were moderately antibacterial, with the most removal efficiencies (70.3-83.7) observed in green tea extract (T1), and less removal efficiencies (62.2-67.9) in eucalyptus extract (E1). When both the systems of plants were subjected to the effect of biosynthesized ZnO nanoparticles a high level of antibacterial activity was observed in both. ZnO nanoparticles mediated by green tea (T2) at a concentration of 0.5g /100mL showed a little higher removal efficiency (97.6 %) than those of eucalyptus-mediated ZnO nanoparticles (E2) which was between 97.0 and 100%. With a maximum nanoparticle concentration of (1 g/100 mL), both eucalyptus-mediated and green tea-mediated ZnO nanoparticles exhibited almost 100% or complete bacterial removal with removal efficiencies of more than 98.6% total bacteria and 100% Pseudomonas spp., Salmonella spp., and Shigella spp. All in all, treatments made using green tea demonstrated slightly better antibacterial behavior at lower concentrations and similar and highly effective results with higher concentration of ZnO nanoparticles.

**Description of SEM results (green tea control):** The SEM picture of untreated leaves of green tea depicts the natural surface morphology of the surface which has smooth and compact areas that are irregularly shaped and have a layered structure. The surface is heterogeneous, and it is covered with layers of dense organics and does not show any modification caused by treatment. This picture will be the baseline morphology of the green tea leaf before wastewater or any treatment process is applied to it and serve as a reference point to compare it with the treated samples (T1, T2, and T3) (Figure 5).

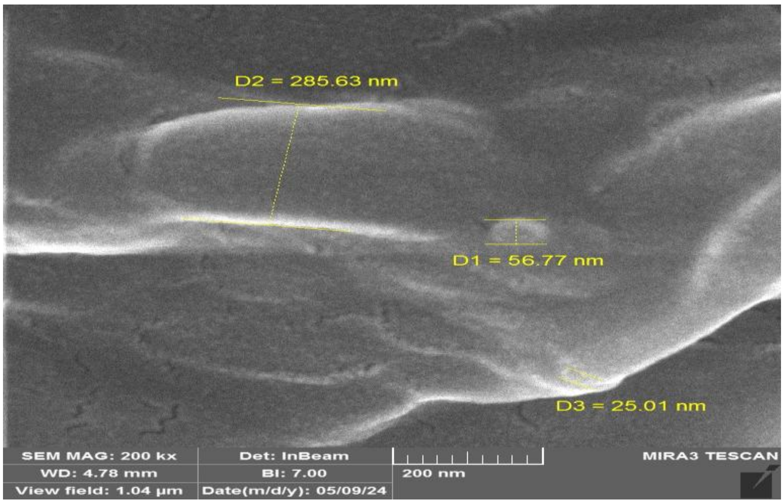


**Figure 5. SEM photograph of untreated green tea leaves of the natural surface morphology before any form of treatment**

**Description of SEM results (T1 - treated green tea):** SEM image of the T1-treated sample indicates the presence of obvious surface morphological alteration in the T1-treated leaves of green tea relative to the untreated ones. Whereas the untreated sample had a compact and comparatively smooth surface with thick organic layers, the surface of T1-treated one is less compact with some fragmentation of layers on the surface. The existence of fragmented and uneven surface characteristics points out to the elimination or decrease of surface-related bacterial cells and contaminants after dealing with green tea extract. T1 is a plant-based control without the inclusion of ZnO nanoparticles; thus, the changes observed are moderate bacterial removal but not formation of nanoparticles (Figure 6).

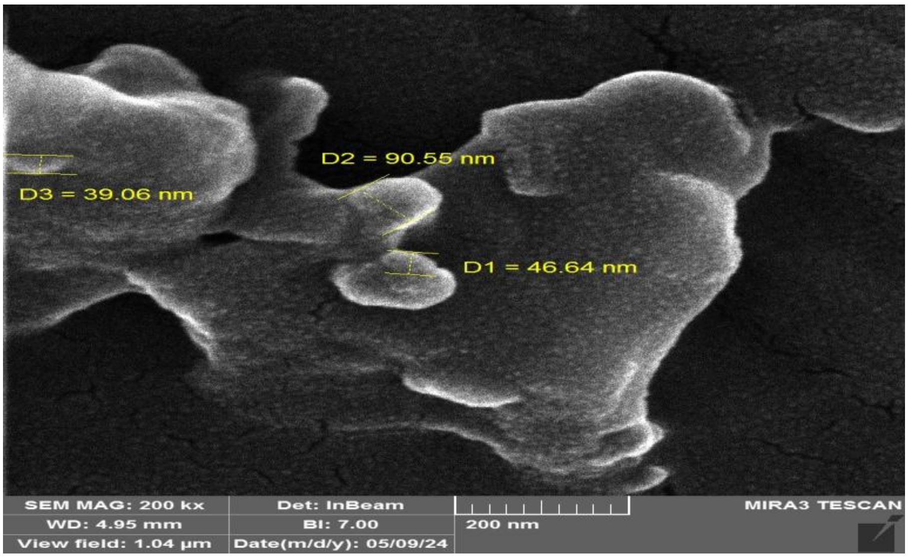


**Figure 6. SEM image of wastewater that has been treated by using green tea extract alone (T1), which shows the surface morphology after the treatment**

**SEM results description (T2):** The SEM image of the T2 treated sample indicates a significant surface morphological changes relative to green tea leaves that are not treated and T1.

The unextraction sample had a compact surface with thick organic layers whereas the T2-treated sample has less compact surface with broken surface structures and less deposits attached to the surface. The decreased aggregation of features and emergence of cleaner reaction zones signifies efficient eradication of bacteria subsequent to treatment with biosynthesized ZnO nanoparticles that were formed with the help of green tea extract. They are in agreement with the increased antibacterial activity observed in T2 among the bacteriological outcomes (Figure 7).

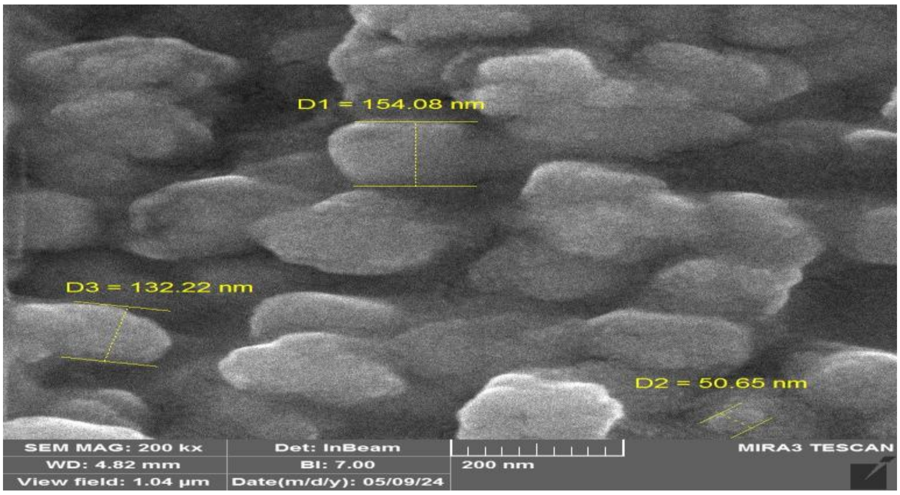


**Figure 7. SEM image of wastewater, which was treated with biosynthesized ZnO nanoparticles made using green tea extract at a concentration of 0.5 g/100 mL (T2) with surface morphology after the removal of bacteria**

**SEM results description (T3):** The S.E.M image of the T3 treated sample depicts the strongest morphological surface variations in comparison with the untreated green tea leaf and the T1 and T2. Whereas the untreated sample has a dense and compact surface with large surface-related deposits, the T3 treated surface only shows a much cleaner surface with less roughness and few aggregated structures. The observed reduction in the surface coverage and the existence of smooth areas depicts that there is a high efficiency in removing bacteria in the highest concentration of biosynthesized ZnO nanoparticles. The observations are in line with the findings of the bacteriological analysis which showed nearly full or total removal of bacteria in (T3 Figure 8).

**Relationship between SEM and bacteriological results (green tea):** The SEM analysis of the treatment based on green tea indicated a high level of agreement with the bacteriological findings obtained with the samples T1, T2 and T3. The untreated green tea leaves had high surface morphology in terms of surface coverage and a dense and compact surface morphology, which is similar to the high levels of bacteria detected before the treatment. It is a confirmation that there is a high level of bacterial contamination in the untreated samples. After treatment with green tea extract alone (T1), the SEM images were shown to depict moderate surface morphological alteration which is characterized by partial surface layer disruption and diminished surface compactness. These findings are in line with the bacteriological data, which revealed the moderate bacteria removal efficiencies of T1. The fact that the surface-associated structures are still present in the SEM images demonstrates the unfinished elimination of bacteria that was observed in the

CFU data. The T2-treated samples showed a tremendous increase in surface morphology with biosynthesized ZnO nanoparticles produced by using green tea extract with a concentration of 0.5 g/100 mL.

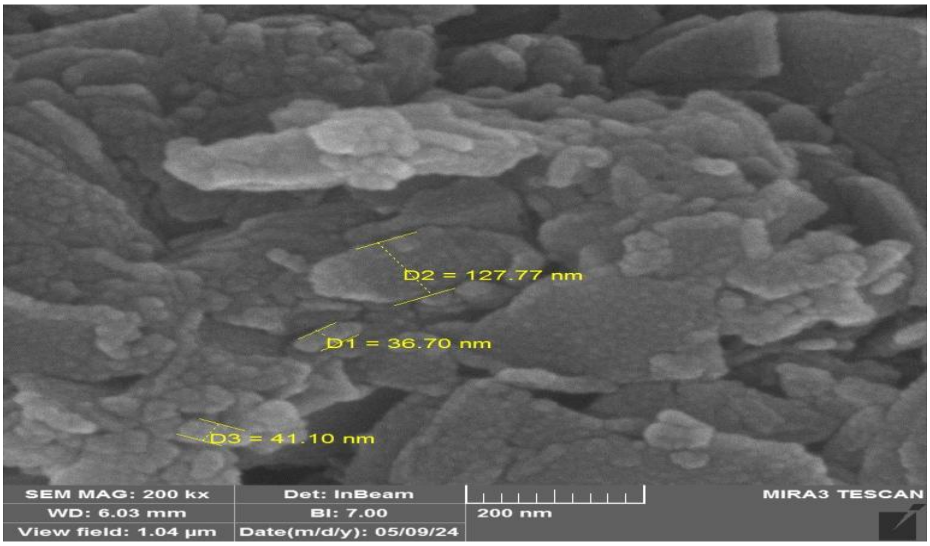


**Figure 8. SEM image of the wastewater that was treated with biosynthesized ZnO nanoparticles prepared with green tea extract at the concentration of 1 g/100 mL (T3), with the surface morphology observed after removing bacteria**

The surface of the SESM images showed less surface deposits and disrupted aggregates when compared to the surface of the SEM. These morphological changes are consistent with high bacterial removability of the bacteriological outcomes, such as the virtually complete removal of a number of bacterial indicators. The strongest surface and intensity of the changes were seen in the samples that were treated with T3 and in which the highest level of biosynthesized ZnO nanoparticles (1 g/100 mL) was realized. The cleanest and least compact surface morphology of T3 SEM images presented the least compact surface morphology together with the least number of surface-associated aggregates of all green tea samples. These were consistent with the findings of the bacteriological results which indicated that it had the highest bacterial removal efficiencies and total eradication of certain pathogenic bacteria. All in all, SEM observations are very supportive of the bacteriological data showing a definite concentration-dependent correlation between the application of the ZnO nanoparticles in biosynthesis and bacteria removal efficiency. The data obtained through the quantitative determination of bacterial in surface morphology give visual validation of the progressive increase in surface morphology between untreated green tea leaves and T3-treated samples. SEM analysis revealed that the bacteriological evidence is supported by the visual evidence, in which higher bacterial removal efficiencies of treatments based on green tea were linked to more and more clean surface morpholo gies.

**Description of SEM results (untreated eucalyptus):** SEM image of untreated eucalyptus leaves reveals the natural and rough surface morphology with irregular forms, folds of surfaces and organic stratifications. The surface of the leaf is found to be heterogeneous and devoid of being treated. This picture will be the baseline morphology of the leaves of eucalyptus before wastewater or any form of treatment is applied and used as the reference to compare with samples treated (Figure9).

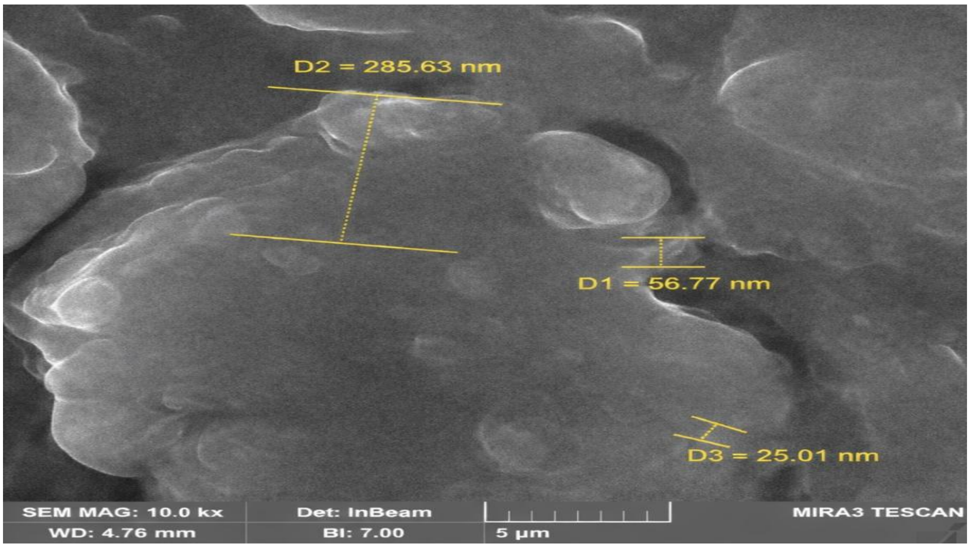


**Figure 9. SEM image of untreated eucalyptus leaves showing the natural surface morphology prior to any treatment**

**SEM comparative results (untreated vs. E1):** The SEM image of untreated eucalyptus leaves shows a rough and heterogeneous surface morphology characterized by irregular layers, folds, and dense organic structures. The surface appears compact with the presence of natural plant tissue features and no signs of treatment-induced modification. In contrast, the SEM image of the eucalyptus-treated sample (E1) reveals clear morphological changes after treatment. The surface appears less compact and more fragmented, with visible reduction in dense surface layers. Elongated and irregular structures are observed on the surface, indicating the removal of bacterial cells and surface-associated contaminants following treatment. These changes confirm the effectiveness of eucalyptus extract in reducing bacterial load and modifying the surface morphology of the treated sample. Since E1 represents a plant-based control without ZnO nanoparticles, the observed features are attributed to bacterial removal rather than nanoparticle formation (Figure 10).

**SEM results description (E2):** The SEM image of E2 sample reveals that there are strong variations of the morphology of the surface of the leaves that are not treated (un-treated eucalyptus leaves). Although in the untreated sample there is a dense and irregular surface with aggregated organic layers, in the E2-treated sample, the surface is cleaner and less compact with less deposits on the surface. The loss of thick surface cover and bacterial-like objects are signs of efficient bacterial cleaning after treatment. The coarse granular characteristics on the surface can be said to be a result of the treatment procedure but the key observation in E2 is that the contaminants found on the surface have been greatly reduced compared to the untreated leaves. These morphologic variations authenticate the increased bacterial elimination power by the mutual influence of the eucalyptus extract and ZnO therapy (Figure 11).

**SEM results description (E3):** The SEM image of E3 sample reveals clear surface morphological variations compared to leaves of eucalyptus treated with no treatment. The unmodified sample has a rough, heterogeneous and dense surface with a massive coverage of the surface. Conversely, the E3 treated sample has a significantly clean surface with a lesser amount of compactness and fragmented layers on its surface.

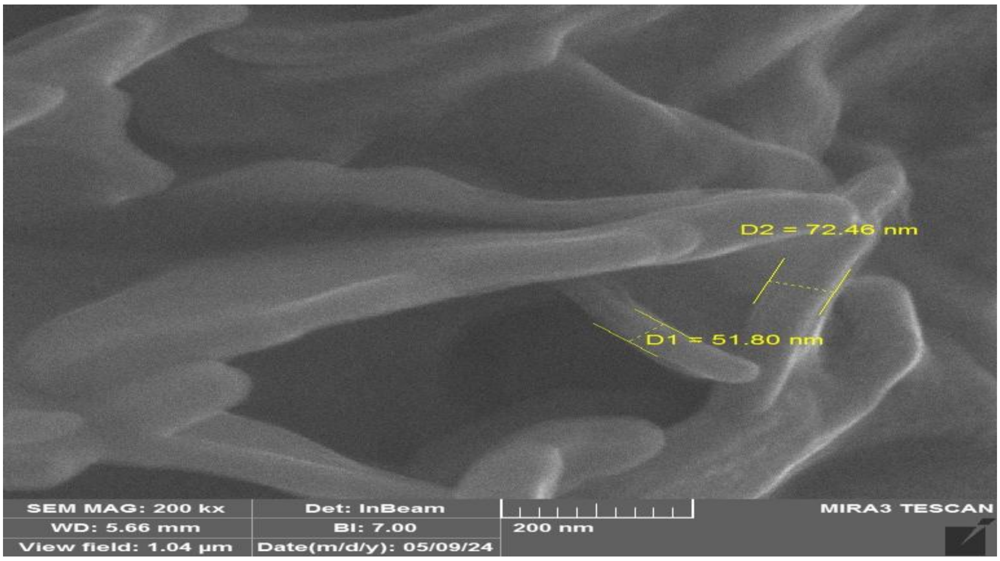


**Figure 10. SEM image of untreated eucalyptus leaf surface and the morphology of the surface after treatment with wastewater using eucalyptus extract only (E1)**

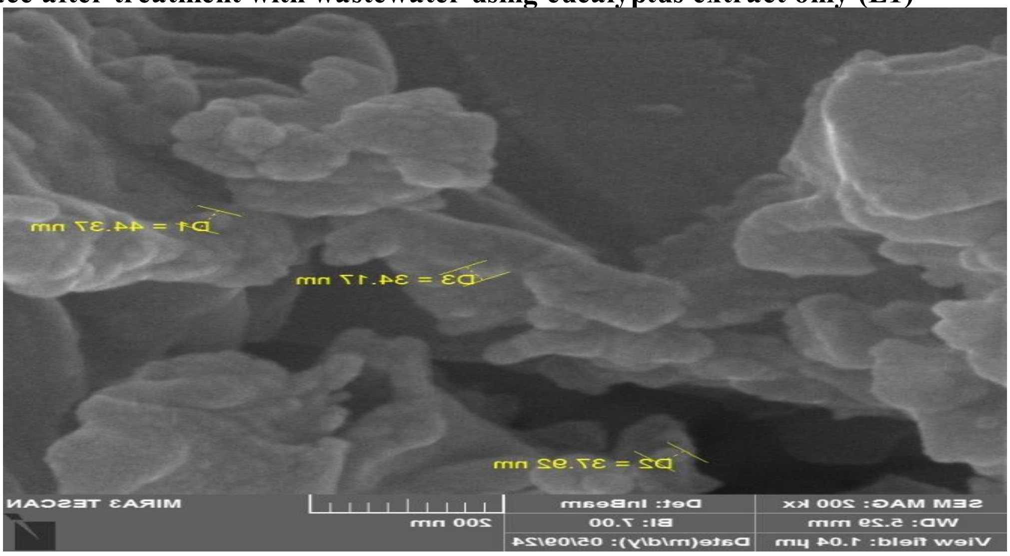

**Figure 11. SEM image of eucalyptus leaves that were added with wastewater with the help of eucalyptus extract and ZnO in the concentration of 0.5 g / 100 mL (E2), where the surface morphology was seen after removing bacteria**

This means that there is a tremendous reduction in the number of aggregates attached to the surfaces and this means that there is an increase in the elimination of bacterial cells and surface contaminants. The morphological characteristics that are observed indicate that as the concentration of the biosynthesized ZnO nanoparticles increases, the ability to clean the surface and remove bacteria also increases as compared to the lower concentration of ZnO nanoparticles (Figure 12).

**Correlation of SEM with bacteriological results - eucalyptus:** The results of the bacteriological analysis were visually confirmed by SEM analysis, where the higher the ability to remove bacteria, the more clean and less compact the surface morphologies. Scanning electron microscopy (SEM) analysis was highly correlated to the bacteriological measurements that were achieved in the treatments based on eucalyptus.

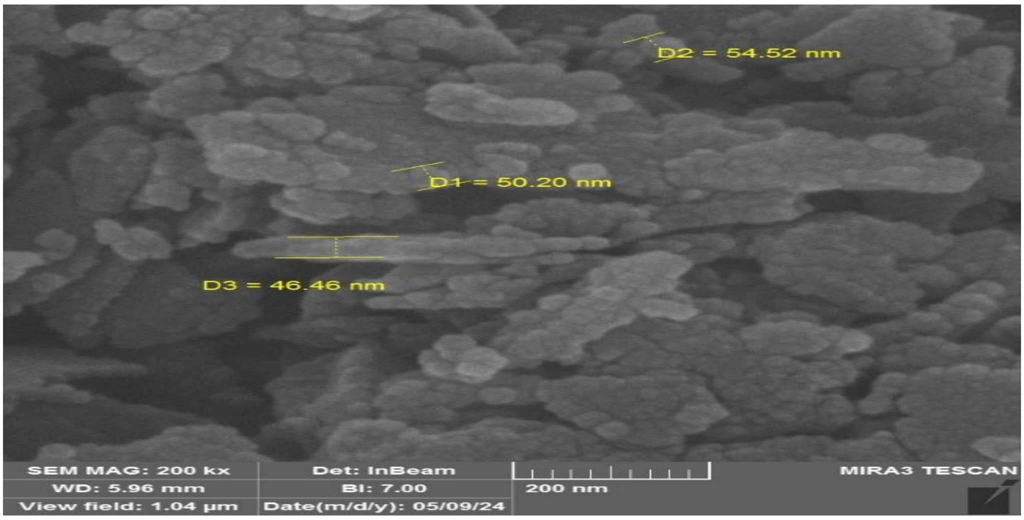


**Figure 12. SEM image of wastewater that was treated with biosynthesized ZnO nanoparticles prepared using eucalyptus extract with the concentration of 1 g/100 mL (E3) representing the surface morphology of wastewater following the elimination of bacteria**

The uncultivated specimen had thick, coarse, and discontinuous surface morphology, which is in line with high bacterial counts, which were observed before the treatment. This establishes the occurrence of large amounts of bacterial contamination and surface-bound organic matter in the untreated wastewater samples. In the second experiment, the surface morphology of the SEM images showed moderate variations in the treatment with eucalyptus extract alone (E1) with partial disruption of surface layers and a decrease in aggregated structures. These observations are correlated with the bacteriological data, that showed moderate efficiencies of bacterial removal. The fact that irregular surface features appear in the SEM images even with the fact that the bacteria are not fully removed as shown in the CFU counts indicate that the bacterium have not been fully removed. Significant change in the morphology of the surface was recorded in the samples treated with E2, in which the bio synthesized ZnO nanoparticles prepared with eucalyptus extract were applied at concentration of 0.5 g/100 mL. The SEM photographs depicted a much cleaner and less compact surface with a significant decrease in aggregates on the surface. This morphological change is directly associated with the high bacterial clearance efficiencies in the bacteriological tests such as near-total removal of a number of bacterial indicators. The greatest surface changes were noted in the E3 treated samples which were subjected to majority of the biosynthesized ZnO nanoparticles (1 g/100 mL). SEM image of E3 showed the best surface morphology as compared to all the samples with low compactness, surface layers broken and surface deposits limited. These characteristics are in concurrence with the greatest bacterial removal efficiencies that have been reported in the bacteriological findings including total elimination of pathogenic bacteria. All in all, the SEM results can be compared to the bacteriological ones, indicating that there is a definite concentration-related correlation between the utilization of biosynthesized ZnO nanoparticles and the ability of the nanoparticles to eliminate bacteria. The surface morphology of untreated samples to E3-treated samples is progressively enhanced by the visual analysis of the samples which substantiates the quantitative data of CFU and validates the applicability of the eucalyptus-mediated ZnO nanoparticles in the wastewater treatment.

**FTIR results of green tea-based samples:** Figure (13) shows the FTIR spectra of green tea extract, ZnO nanoparticles, and the samples treated by wastewater (A, B, T1, T2, and T3) under various dosage ratios between plant extract and ZnO nanoparticle on the ability of both to remove organic and bacterial contaminants in wastewater. Sample A: Green Tea extract (Control): Green tea extract diluted to form an extract. The FTIR spectrum of sample A exhibits typical absorption bands that have been linked to organic compounds that are plant-derived. A wide band at approximately 3400 cm -1 is ascribed to O-H stretching vibrations of phenolic compounds and the hydroxyl groups. The C -H stretch of the aliphatic structures can be seen in the C -H stretching at the peaks of about 2920 cm -1, and several bands in the fingerprint region (1500-1000 cm -1) are associated with C -O, C -C and C -N vibrations, indicating the presence of complex organic structure in the green tea extract.

**Sample B: nanoparticles of ZnO (control):** The FTIR spectrum of Sample B is much cleaner than that of the plant extract. The majority of organic related absorption bands are highly suppressed or nonexistent. A typical band at the low wavenumber range (c. 500 - 600 cm -1) is attributed to Zn -O vibrations, which confirms the successful synthesis and purity of ZnO nanoparticles.

**Sample T1: 1 g green tea extract / 100 mL wastewater:** FTIR spectrum of T1 still shows the key functional groups that were present in the sample A but with less intensity of the bands especially in the OH and fingerprint regions. The decrease shows some level of interaction with and elimination of organic and bacterial pollutants in wastewater by the green tea extract. Nonetheless, the multitude of the organic bands would indicate poor efficiency of treatment.

**Sample T2: 0.5 g zno nanoparticles/100 mL wastewater:** In sample T2, another reduction in the intensity of organic related absorption bands is observed. To show that the removal efficiency is higher than T1, attenuation of the peaks in the fingerprint region is observed. The residual organic characteristics are still visible, which means that 0.5 g ZnO nanoparticles offer intermediate removability of contaminants.

**Sample T3: 1g ZnO nanoparticles/100mL wastewater:** The overall band intensities and the smoothest FTIR profile of sample T3 can be observed as the lowest and the smoothest in the entire series of treated samples. The majority of bands associated with organic and fingerprint are effectively suppressed, whereas the Zn-O vibration is clear. This tendency implies the highest removal efficiency, which is explained by the high dosage of ZnO nanoparticles, which has a demonstrative dose-dependent trend. The FTIR analysis indicates that there was a gradual decline in organic and bacterial absorption bands after treatment of the wastewater. Green tea extract is not that effective in terms of removal ability, but ZnO nanoparticles add considerably to the ability to treat. The maximum contaminant removal was done with the amount of 1 g of ZnO nanoparticles (T3), so ZnO nanoparticles is superior to the performance of plant extract.

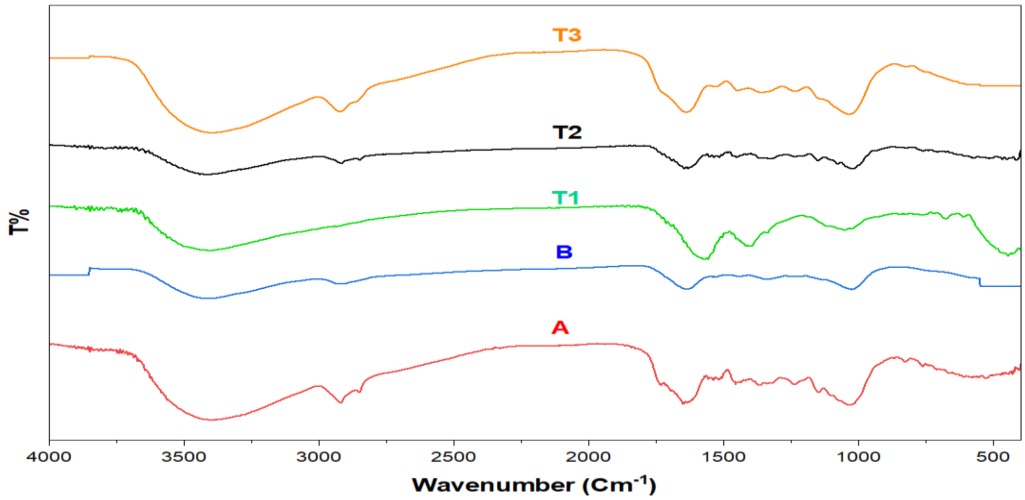


**Figure 13. FTIR spectra of green tea extract, ZnO nanoparticles and wastewater-treated samples (A) green tea extract (control), (B) ZnO nanoparticles (control), (T1) 1 g green tea extract per 100 mL wastewater, (T2) 0.5 g ZnO nanoparticles per 100 mL wastewater and (T3) 1 g ZnO nanoparticles per 100 mL wastewater**

**FTIR results eucalyptus-based samples:** Figure (14) presents the FTIR spectra of eucalyptus extract, ZnO nanoparticles and wastewater-treated samples under various conditions (A, B, E1, E2 and E3), which could demonstrate the influence of the type of the treatment and ZnO nanoparticles concentration on the removal of organic and bacterial contaminants in the wastewater.

**Sample A: eucalyptus extract (control):** The FTIR spectrum of sample A has typical absorption bands of plant-derived organic compounds. The presence of a wide band at approximately 3400 cm -1 is explained by the presence of O -H vibrations of phenolic compounds and alcohol groups. Peaks at the position of -2920 cm -1 were C -H stretching of aliphatic groups, the fingerprint region (between 1500 -1000 cm -1) has several peaks at -1 which were attributed to C -O, C-C and C-N vibrations which justified the complex organic nature of the eucalyptus extract.

**Sample B: nanoparticles of ZnO (control):** Sample B FTIR spectrum indicates a much cleaner profile of sample B than of the plant extract. Majority of the organic related bands are either non-existent or heavily diminished. The presence and purity of ZnO nanoparticles are confirmed by a typical absorption band at the low wavenumber regime that is attributed to ZnO stretching vibrations at around 500 600 -1.

**Sample E1: 1 g eucalyptus extract/ 100 mL wastewater:** The spectrum of sample E1 has a similar functional group as sample A, which reveals the occurrence of plant-based organic compounds. Nonetheless, a significant decrease in band intensities is seen, especially in the O -H and fingerprint regions. It implies that the extract of eucalyptus played a role in a partial elimination or reaction with organic and bacterial pollutants in the wastewater, but it has a lower removal capacity than ZnO nanoparticles.

**Sample E2: 0.5 g ZnO nanoparticles/ 100 mL wastewater:** In sample E2, additional attenuation of the FTIR bands is seen. The fact that the intensity of organic-related peaks was reduced shows that more organic and bacterial components were removed than in case of E1. Organic residual features are also not eliminated, which indicates that 0.5 g ZnO nanoparticles have moderate contaminant removal capacity.

**Sample E3: 1 g ZnO nanoparticles/ 100 mL wastewater:** Sample E3 has the most intense FTIR spectrum and the smoothest band intensities of the overall bands when compared to the treated samples. The majority of organic and fingerprint-related maxima are highly suppressed, and the ZnO vibration can be traced. It signifies the greatest removal efficiency which is explained by the fact that the concentration of ZnO nanoparticle is increased, and it increases adsorption and interaction with organic and bacterial contaminants in wastewater. The FTIR results have clearly shown the presence of concentration-dependent removal effect. Though there are limited treatment capabilities with eucalyptus extract, ZnO nanoparticles enhance treatment ability greatly with 1 g ZnO nanoparticles (E3) having the highest purification efficiency. The maintenance of the Zn-O bands and the decrease in the number of organic functional groups supports the effective treatment without the introduction of other contaminants.

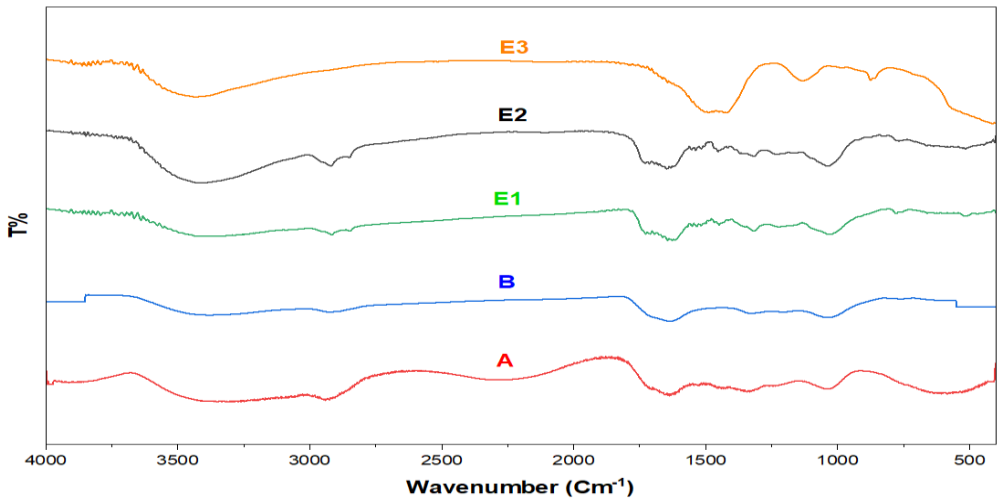


**Figure 14. FTIR spectra of eucalyptus extract, ZnO nanoparticles, and wastewater-treated at various treatment conditions: (A) eucalyptus extract (control), (B) ZnO nanoparticles (control), (E1) 1 g eucalyptus extract/100 mL wastewater, (E2) 0.5 g ZnO nanoparticles /100 mL wastewater, and (E3) 1 g ZnO nanoparticles/100 mL wastewater**

## Discussion

**Removal efficiency of bacteria and comparison with other previous studies:** The results of the bacteriological tests conducted in the research reveal clearly that the implemented treatment can be applied only to eliminate bacterial loads. The un-treated waste water samples had large bacterial loads which verified that there is high contamination by microbes. On treatment, a gradual decrease in bacterial counts was noted, in the following order: plant extract < 0.5 g ZnO nanoparticles < 1 g ZnO nanoparticles. This is a trend that is observed in both systems based on eucalyptus and green tea and this suggests that there is a dose-dependent antibacterial action of the ZnO nanoparticles. The same concentration-dependent pattern of bacterial elimination has been commonly observed. El-Saadony et al. (2024) established that the bacterial inactivation is boosted remarkably with a rise in the concentration of ZnO nanoparticle owing to an increase in the contact between the nanoparticle and the cell. Similarly, Ali and Risan (2024) affirmed that ZnO nanoparticles have also been observed to be more effective in antibacterial activity compared to plant extracts on their own even when synthesized through green pathways. These results are very much similar to the CFU counts in the current research, which prove the credibility of the antibacterial performance in this study. Extracts of the plants only had weak antibacterial activity and this can be explained by the fact that they contained phenolic compounds and flavonoids which have weak bacteriostatic effects. Nevertheless, such an activity was not enough to attain high bacterial removal, a shortcoming that has already been mentioned in plant-based treatments of wastewater systems (Jain et al., 2021; Al-Darwesh et al., 2024).

**SEM supportive evidence of bacterial cell damage:** SEM examination offers first hand morphological data in support of the bacteriological information. Untreated samples had intact bacterial cells that had smooth and continuous surfaces, which are characteristic of viable bacteria. The aggregation was also shown to be less and some surface abnormalities were observed after the treatment with plant extracts which implies less bacterial stress but does not necessarily imply full inactivation. This was not the case as samples after ZnO nanoparticle treatment exhibited severe morphological damage, i.e. rupture of cell wall, surface pitting, deformation and collapse especially at higher dosage of nanoparticle (1 g/100 mL). These findings are typical signs of bacterial death by metal oxide nanoparticles and have widely been documented in the literature. Gupta et al. (2018) and Abdelbaky (2022) noted the similarity of SEM in bacteria treated with ZnO nanoparticles synthesized with the green method and attribute the results to membrane destabilization and intracellular content leakage. Other more recent publications with green-synthesized ZnO nanoparticles also indicate similar SEM results. Sabah Khadim et al. (2025) and Naiel et al. (2022) have reported severe bacteria membrane disruption at high concentrations when ZnO nanoparticles were used, and the SEM morphology was highly correlated with a CFU reduction. The bacteriological counts and SEM observations were very similar in the current study and this supports the finding that bacterial removal is the predominant treatment effect.

**FTIR interpretation with regards to bacterial removals:** The FTIR also proves the antibacterial character of the treatment. The control plant extracts displayed typical bands that are related to hydroxyl, aliphatic and finger print zones. A progressive decrease in the amplitude of bands that corresponded to bacterial surface biomolecules e.g. amide and polysaccharide-related vibrations after bacterial treatment was observed. Notably, the changes are not the indication of the removal of organic matter but the signs of the disruption of bacteria cells and their detachment. FTIR analyses of ZnO nanoparticles were used by Umavathi et al. (2021) and Hameed et al. (2023) to show that membrane damage causes a reduction in the intensity of protein and carbohydrate-related bands, but does not cause a change in the overall chemical composition of the surrounding medium. The fact that the Zn-O stretching vibrations persisted in all the samples treated with ZnO proves that the nanoparticles were structurally stable during the treatment. Rani et al. (2025) and Jayachandran et al. (2021) have demonstrated similar FTIR behaviour and stressed that the variation of FTIR intensity in studies of antibacterial bioremediation should be regarded as an indication of microbial interaction, rather than chemical degradation. In this way, SEM and CFU data are entirely consistent with FTIR results obtained in this study and prove bacterial removal as the main process.

**Antibacterial action mechanisms: Literature interpretation:** Well-established mechanisms of ZnO nanoparticles can explain the presence of the antibacterial activity. They involve the production of reactive oxygen species (ROS), the release of Zn 2+ ions, and direct contact of nanoparticles with cells, all of which cause an oxidative stress and cell membrane damage in bacterial cells (Abdo et al., 2021; Swain et al., 2025). The said mechanisms are selective to bacterial cells and do not suggest the degradation of organic pollutants, which is consistent with the findings of the current research. The improvement of these mechanisms with higher nanoparticle concentrations due to increase in surface area and contact probability is the reason behind the high performance of the 1 g ZnO nanoparticle treatment. This is a dose-dependent effect that has been maintained through antibacterial ZnO research (Ali and Risan, 2024; Kumie et al., 2025).

**General agreement with the published research:** The combination of bacteriological, SEM, and FTIR findings indicates high levels of internal consistency and good concordance with research results on green-synthesized ZnO nanoparticles. Almost the same antibacterial pattern and morphological findings have been observed by studies that use Camellia sinensis and Eucalyptus, algae, and other plant extracts (Abdo et al., 2021; Naiel et al., 2022; Truong et al., 2023; El-Saadony et al., 2024). The current results are thus strong experimental evidence on the application of green ZnO nanoparticles as a good antibacterial agent in wastewater treatment.

**Conclusion:** In this paper, it is clear that ZnO nanoparticles synthesized in green synthesis utilizing Eucalyptus and Green tea extracts are very effective in bacteria reduction in wastewater. Although plant extracts showed little antibacterial capability when used alone, ZnO nanoparticles showed a high level of removal especially at increased concentrations. The uniformity of the bacteriological counts, SEM observation, and the spectral variation of FTIR is a validation of the soundness of the treatment process. In general, the results contribute to the potential of the green ZnO nanoparticles as affordable, numerous available, and efficient substances in the advancement of wastewater treatment.

## Author contributions

All authors contributed to the study design, experimental work, data analysis, and manuscript preparation and approved the final version.

## Data availability statement

All data are included in the article.

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**Ethical considerations**

This study did not involve human or animal subjects; therefore, ethical approval was not required.

**Conflict of interest**

The authors declare no conflict of interest.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۲۲۲۸-۶۷۰۵ شاپا الکترونیکی: ۲۲۲۸-۶۵۰۰

# سنتز زیستی نانوذرات اکسید روی (ZnO) با استفاده از عصاره‌های گیاهی و کاربرد آن‌ها در حذف باکتری‌های فاضلاب

**هاله فائز الجواهری**

دانشکده علوم، دانشگاه بابل، ۵۱۰۰۱، بابل، عراق. ایمیل: sci.hala.ali@uobabylon.edu.iq

**نور س. ناجی**

دانشکده علوم، دانشگاه بابل، ۵۱۰۰۱، بابل، عراق. ایمیل: sci.noor.saadallah@uobabylon.edu.iq

**اثیر س. ن. العزاوی**

نویسنده مسئول. علوم محیط‌زیست، دانشگاه القاسم سبز، ۵۱۰۰۱، بابل، عراق. ایمیل: atheersaieb@environ.uoqasim.edu.iq

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## چکیده

**هدف:** هدف از این پژوهش، بررسی کارایی ضدباکتریایی نانوذرات اکسید روی (ZnO) سنتزشده به روش سبز بود. این نانوذرات با استفاده از عصاره‌های اکالیپتوس و چای سبز برای کاربرد در تصفیه فاضلاب تهیه شدند. همچنین، مقایسه عملکرد ضدباکتریایی عصاره‌های گیاهی به‌تنهایی با نانوذرات ZnO در غلظت‌های مختلف، به‌منظور ارزیابی پتانسیل عملی آن‌ها در گندزدایی فاضلاب، از دیگر اهداف این مطالعه بود.

**مواد و روش‌ها:** ابتدا عصاره‌های گیاهی به‌عنوان تیمارهای شاهد ارزیابی شدند. عصاره‌های آبی برگ اکالیپتوس و چای سبز به‌عنوان عوامل کاهنده و پایدارکننده برای سنتز نانوذرات ZnO مورد استفاده قرار گرفتند. نانوذرات سنتزشده در دو غلظت (۰/۵ گرم و ۱ گرم در ۱۰۰ میلی‌لیتر) به نمونه‌های فاضلاب افزوده شدند. نمونه‌های تیمارشده تحت شرایط کنترل‌شده آزمایشگاهی انکوبه شدند و سپس مورد تجزیه‌وتحلیل قرار گرفتند. فعالیت ضدباکتریایی از طریق کاهش شمارش کل باکتری‌ها ارزیابی شد. برای بررسی مورفولوژی سطح و آسیب ساختاری سلول‌های باکتریایی از میکروسکوپ الکترونی روبشی (SEM) استفاده گردید. همچنین طیف‌سنجی تبدیل فوریه مادون قرمز (FTIR) برای تأیید تشکیل نانوذرات و شناسایی تغییرات گروه‌های عاملی انجام شد.

**نتایج:** در مقایسه با عصاره‌های گیاهی به‌تنهایی، پس از تیمار با نانوذرات ZnO کاهش معنی‌داری در شمارش کل باکتری‌ها مشاهده شد. کارایی حذف به ترتیب زیر افزایش یافت: عصاره گیاهی< ۰/۵ گرم نانوذرات ZnO < ۱ گرم نانوذرات ZnO، که نشان‌دهنده پاسخ وابسته به دوز بود. تصاویر SEM تخریب دیواره سلولی، تغییر شکل و کاهش اتصال باکتری‌ها را در نمونه‌های تیمارشده، به‌ویژه در غلظت بالاتر نانوذرات، نشان دادند. نتایج FTIR کاهش شدت گروه‌های عاملی آلی و مرتبط با باکتری‌ها را پس از تیمار، همراه با ظهور باندهای جذبی مشخصه ZnO که بیانگر سنتز موفق نانوذرات و پایداری ساختاری آن‌ها بود نشان داد.

**نتیجه‌گیری:** نتایج این پژوهش نشان داد که نانوذرات ZnO سنتزشده به روش سبز در مقایسه با عصاره‌های گیاهی به‌تنهایی، فعالیت ضدباکتریایی بالاتری دارند. عملکرد ضدباکتریایی با افزایش غلظت نانوذرات تقویت شد که بیانگر رابطه قوی غلظت-اثر است. بر اساس یافته‌های این مطالعه، نانوذرات ZnO سنتزشده به روش سبز می‌توانند به‌عنوان ماده‌ای کارآمد، پایدار و دوستدار محیط‌زیست برای تصفیه فاضلاب و کنترل میکروبی پیشنهاد شوند.

**کلمات کلیدی:** تصفیه فاضلاب، حذف باکتری، نانوذرات اکسید روی (ZnO)، FTIR، SEM

**نوع مقاله:** پژوهشی

**استناد:** هاله فائز الجواهری، نور س. ناجی، اثیر س. ن. العزاوی (۱۴۰۵). سنتز زیستی نانوذرات اکسید روی (ZnO) با استفاده از عصاره‌های گیاهی و کاربرد آن‌ها در حذف باکتری‌های فاضلاب. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۴)، ۲۰۸-۲۲۸.

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