

INSIGHT ON ANTIBACTERIAL EFFECT OF ZINC OXIDE NANOPARTICLES SYNTHESIZED USING ARTEMISIA LEAF AGAINST VARIOUS BACTERIA AND ITS OUTCOME ON WATER REMEDIATION PROCESS

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Abstract

*Nowadays, water resources are suffering from the release of waste products from commercial and industrial sectors besides untreated domestic sewage and chemical pollutants those leaked into fresh water resources which considered as a horribly detrimental thing to both the human community and the ecosystem, including animals and plants. In this regard, the major water contaminants are heavy metal ions, organics (e.g., dyes), and oils which can disqualify any water stream for drinking. In that context, we have done studies to evaluate the effectiveness of the anti-bacterial action that mediated by ZnO nanoparticles which have been extracted using a green synthesis method which involves utilization of artemisia plant. The activity has been evaluated against a diversity of bacteria which exist in the water resources. Our research has been conducted against gram-positive bacteria, such as *S. aureus* and gram-negative bacteria, such as *E.coli* and *Klebsiella pneumoniae*. The bacterial strains have been isolated and identified by means of many biochemical tests. The effectiveness of ZnO nanoparticles has been determined through measurement of mean inhibition zone value against each type of bacteria, which appeared to be 20 mm against *S. aureus*, 22 mm against *Klebsiella pneumoniae* and 24 mm against *E.coli*, therefore, those values indicate effectiveness of ZnO nanoparticles as an anti-bacterial.*

Keywords: Zinc oxide, Artemisia, Water remediation, Nanoparticles

1. INTRODUCTION

Water contamination is a serious global concern triggered by industrial, domestic and environmental factors. In addition to that, the global population is ramping up so fast and the human pressure exerted on our water supplies is expected to magnify with potentially greater probability of pollution. For example, The United Nations states that over 300 million tons of heavy metals, solvents and other waste are released into the world's water supplies annually as a harmful side product of industrial activity [1]. Water can hold a diversity of microorganisms as water availability is a crucial requirement for microbial growth resulting in offering permission for a variety of gram-negative and gram-positive bacteria. For instance, *E. coli* can cross contaminate a variety of sources including drinking water through poor handling of foods or farm animals. Humans and farm animals can get *E. coli* infection by drinking water from the contaminated sources [2]. *E. coli* normally live in the intestines of human infants within a few hours after birth. In fact, *E. coli* and its human host coexist in good health and

with mutual benefit for each side. It is very uncommon for these commensal *E. coli* strains to cause a disease except in immunocompromised hosts [3]. Another gram-negative bacterium is *Klebsiella pneumoniae* which has been known as a major cause of community-acquired pneumonia in individuals with impaired pulmonary defenses and is a main pathogen in nosocomial pneumonia [4, 5]. Moreover, *Klebsiella pneumoniae* is identified not only in clinical but also in different aquatic settings all around the world [6-8]. In concerning with gram-positive bacteria which have a probability to own an existence in contaminated water, *S. aureus*, comes to the mind as it has the ability to survive in drinking water distributed by public devices. *S. aureus* is widely recognized as the major leading community based bacterial agent in the world. It is worth the efforts to display its importance as a human pathogen, due to its ability to cause infections as well as its capacity to adapt to diverse environmental conditions and having a resistance to different antimicrobial agents [9]. Additionally, *S. aureus* has a special feature which is the formation of biofilms, which enhances its survival in water systems [10]. In the last few years Nano-technology has been tremendously evaluated as a potential replacement for common water treatment methods in order to deliver clean water at a reduced cost while meeting increasingly rigorous water quality standards [11]. The European Commission has identified 'Nano-material' as a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm – 100 nm [12]. The ZnO nanoparticles have been confirmed to be the future, economical disinfecting agent, playing a great role in different nanotechnology applications [13] such as electronics [14, 15], chemical sensing devices, solar-cells [16], anti-microbes [17–19], water medication automations [20] and have the capability to be used in sun screens and cosmetics, by blocking UV rays [21]. ZnO displays unique anti-microbial properties due to its efficacious photo-catalytic activity in Ultra-Violet light [22–24]. ZnO nanoparticles have a wide range of biological applications [25] due to the tiny size of nanoparticles which exhibit high surface area to volume ratio [26–31], longer shelf time, robustness, non-toxicity and bio-compatible nature [32]. Synthesis of nanoparticles using biological means, particularly plants is biocompatible as they produce functional biomolecules which actively reduce metal ions [33, 34]. Furthermore, plants as a biological agent are eco-friendly and so are the reducing and capping agents involved in the synthesis process [35, 36]. Artemisia is one of the largest genera of the family Asteraceae and is privileged to have a wide range of substances those have a diversity of anti-inflammatory, anti-tumor, antioxidant, and anti-proliferative actions [37, 38]. In this regard, the objective of our study is to investigate the effect of ZnO nanoparticles which are extracted from Artemisia plant as antimicrobial against different gram-negative and gram-positive bacteria and evaluate the outcome of this study on the process of water remediation.

2. MATERIALS AND METHODS

2.1. Collection of Sample

The collection of sample has taken place at Taif city, Saudi Arabia. The water samples were collected monthly from April 2022 to April 2023 and a total of 125 samples were gathered from different water sources which include, tap water (n=35), bottled water (n=45), rain water (n=20), and water from the drinking troughs of poultry (n=25). Water samples were collected according to Standard Methods for the Examination of Water and Wastewater [39] and examined within a 24 h period.

2.2 Isolation and Identification of the Bacterial Strains

2.2.1 Isolation of the Bacterial Strains

About 0.1 mL of each water sample was flooded on sterilized nutrient agar plates and was incubated for 24 h at 37°C. The isolated colonies were observed after incubation and a few colonies were selected

randomly. They were inoculated after that on a fresh nutrient agar plate followed by quadrant streaking and the plates with streaked colonies was maintained as pure cultures. The pure cultures were again inoculated in Nutrient broth and were used for further characterization tests.

2.2.2. Identification of the Bacterial Strains

The bacterial biochemical tests were performed to determine the ability of microbes for the production of certain enzymes which aids them in utilizing complex nutritional substance for their nutrition. Thus, the changes caused in the media such as gas production, change in pH and end products formed can be determined by using some chemicals and indicators. This makes easier to characterize the microbes. Various biochemical tests like Indole, Methyl red, citrate utilization test, catalase, coagulase, urease, oxidase, and starch utilization test were performed. Along with this the cellular characterization, gram's staining was done, and the results were tabulated accordingly [40].

2.3. Plant Collection and Extraction

The plant was collected from Alshafa region, Taif city, Saudi Arabia. The samples were initially washed with tap water and then washed several times using distilled water as shown in (Figure 1a). The leaves of the plant samples have left to be dried for 48 hours in room conditions (Figure 1b). A total of 50 g of the dried plant leaves have been placed into a 500 ml beaker, then 250 ml of distilled water was added and the mixture has been boiled (Figure 1c). To create the desired reaction, the mixture boiled for 5 minutes. The extract left to cool in room temperature. Filtering was carried out using coarse filter paper and what man filter paper No. 1. The obtained extract stored at +4 °C until the experiments conducted [41].

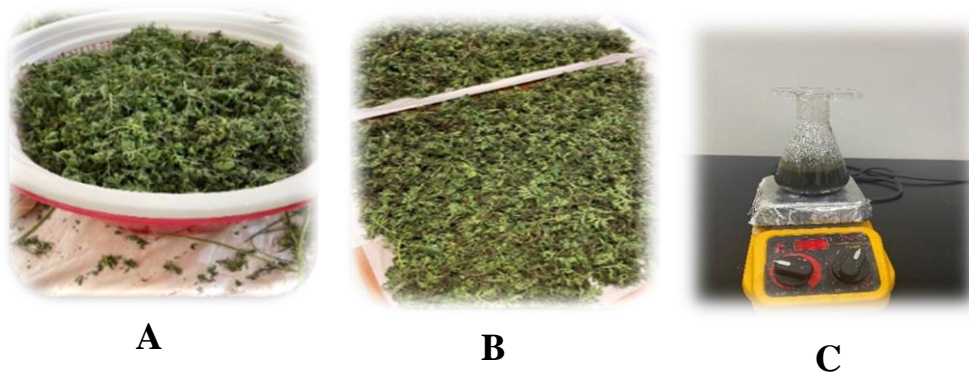


Figure (1a), (1b) and (1c): Are Showing the Flow of the Sample Collection and Extraction Processes

2.4. Synthesis of ZnO Nanoparticles

50 ml of the previous extract has been taken from filtered artemisia leaves and mixed with 3 g Zn (SO₄)₂ 7H₂O. The salt was heated at 60 ° C and stirred continuously in a stirrer at 500 rpm for two hours (Figure 2a).The solution obtained by centrifugation at 12,000 rpm / 30 min (Figure 2b). The granules containing ZnO nanoparticles have been carefully washed 3-4 times with double-distilled water. The obtained powder as ZnO nanoparticles placed in an oven at 100 °C for 3 h. Furthermore, the ZnO nanoparticles have undergone through calcination process in an oven to obtain crystalline ZnO nanoparticles (Figure 2c) [42, 43].

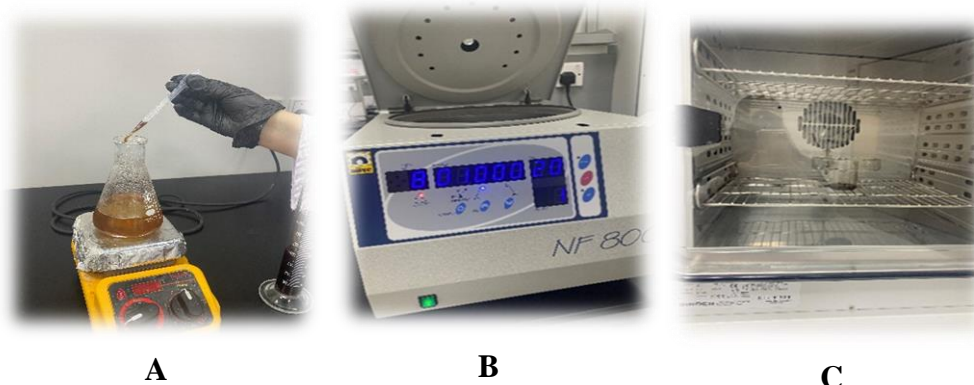


Figure (2a), (2b) and (2c): Reveal the Procedures of the ZnO Nanoparticles Synthesis

2.5. The anti-bacterial effect of ZnO nanoparticles on different bacterial strains

The +ve antibacterial activity of ZnO nanoparticles (calcined and uncalcined) on Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *Klebsiella pneumonia*) bacteria has been tested by the agar well diffusion method [44, 45]. Microbial strains have grown aerobically in nutrient broth for 24 hrs. At 37°C until the turbidity of bacterial suspensions achieve 1.5×10^8 CFU/mL by comparison with the 0.5 McFarland standards. Bacterial cultures were maintained on nutrient Muller–Hinton agar at 37°C. The dimethyl sulfoxide (DMSO) solvent used as a negative control (NC). The +ve plates then turned upside down and incubated at 37°C for 24 hrs. in an incubator. The +ve plates were shaken gently to allow equal mixing of bacteria [46].

3. RESULTS

3.1. Isolation and Identification of Bacterial Strains

3.1.1. Number of bacterial strains isolated and identified

Table 1: Number of bacterial strains isolated and identified from different water sources

Total number of samples	125
Number of <i>S. aureus</i> strains isolated and identified	33
Number of <i>Klebsiella pneumonia</i> strains isolated and identified	27
Number of <i>E. coli</i> strains isolated and identified	42
Other bacterial strains	23

3.1.2. Biochemical identification of the bacterial strains

Table 2: Biochemical Tests which have been done to Identify *Klebsiella Pneumonia*

Biochemical tests	Results
Catalase	+
Voges- Proskuer test (VP)	+
Citrate	+
Urease	+
Indole	-
Oxidase	-
Methyl red	-

Table 3: Biochemical tests which have been done to identify *E. coli*

Biochemical tests	Results
1-Indole test	Positive (production of red ring colour)
2-Methyl red test	Positive (bright red)
3-Voges- Proskuer test (VP)	Negative
4-Citrate utilization test	Negative
5-Urease test	Negative
6-Triple sugar iron test (H ₂ S production)	Acid/ Acid with gas
7-Catalase test	Positive (production of gas bubbles)
8-Oxidase test	Negative
9-Sugar fermentation test	Positive
10- Nitrate reduction test	Positive

Table 4: Biochemical Tests which have been done to Identify *S. Aureus*

Biochemical tests	Results
Catalase	+
Oxidase	-
Coagulase	+

3.2. Characterization of ZnO Nanoparticles

3.2.1. FE-SEM Analysis

The FE-SEM technique is commonly used to determine surface morphology. The FE-SEM image shown in (Figure3) indicates the formation of ZnO NPs with hexagonal wurtzite structure. All FE-SEM images show some agglomeration of particles .The morphology and topology of the surface of zinc oxide nanoparticles mixture with Artemisia were determined using scanning electron microscopy (SEM). As shown in (Figure 3a), the SEM image illustrated the Smooth surface and homogenous topology of zinc oxide nanoparticles mixture with Artemisia, (which emphasized that the formation of zinc oxide nanoparticles association with Artemisia, homogenously and without any aggregation as displayed in (Figure3)).

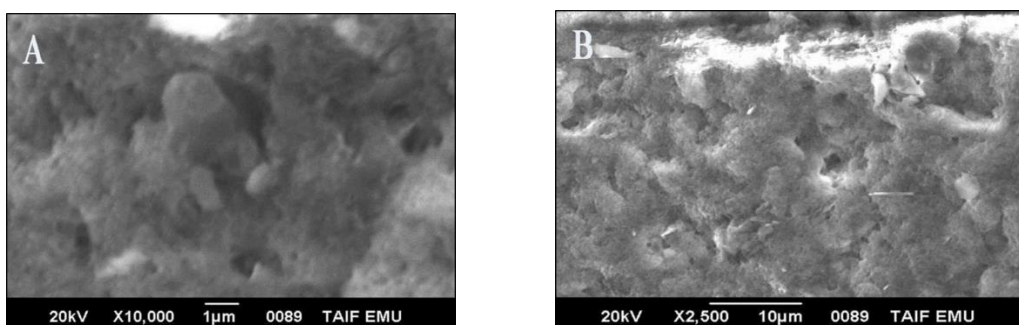


Figure (3a) and (3b): Are Showing FE-SEM images and Histograms of the As-Prepared ZnO Nanoparticles

3.2.2. Physiochemical report using UV-Visible spectroscopy

The UV-Visible spectrum displayed a plasmon absorption peak (λ_{max}) around 390 nm. This indicates that zinc oxide nanoparticles are prepared as shown in (Figure 4) Also, as(Figure 4b) showed a strong peak at 390 nm with high absorption that refer to preparation a homogenous mixture zinc oxide nanoparticles mixture with Artemisia

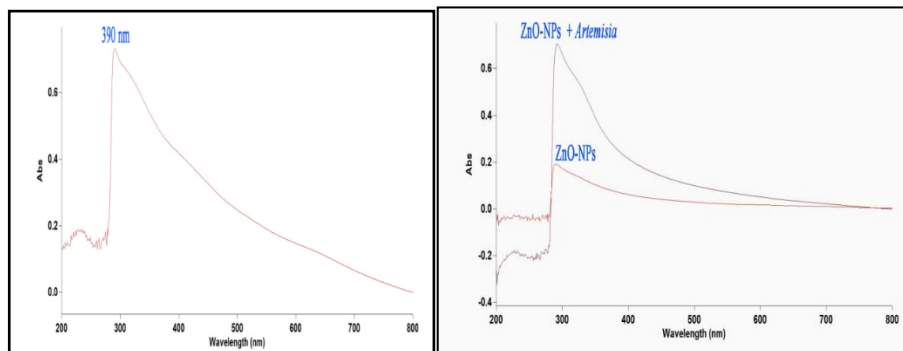


Figure (4a) and (4b): Are Displaying the UV-Visible Spectrum of ZnO Nanoparticles

3.3. Evaluation of the anti-bacterial activity of ZnO nanoparticles

The activity of ZnO nanoparticles and its effect as antibacterial was studied using three types of bacteria, the first was *Klebsiella pneumoniae* which is negative of the chromium dye, and the second was *E.coli* which is negative of the chromium dye and the third bacteria was *S. aureus* which is positive of the chromium dye. Considering to the measurement of the anti-bacterial effect of ZnO nanoparticles the inhibition zone assay was carried out by the agar well diffusion test to evaluate the anti-bacterial action of ZnO nanoparticles and the test was done using the diffusion agar test. The sample was tested at 50 mg/ml concentration.

Table 5: The measurement of mean inhibition zone value for ZnO nanoparticles against bacteria

Tested bacteria	Zone (mm)
<i>Klebsiella pneumoniae</i>	22
<i>E.coli</i>	24
<i>S. aureus</i>	20

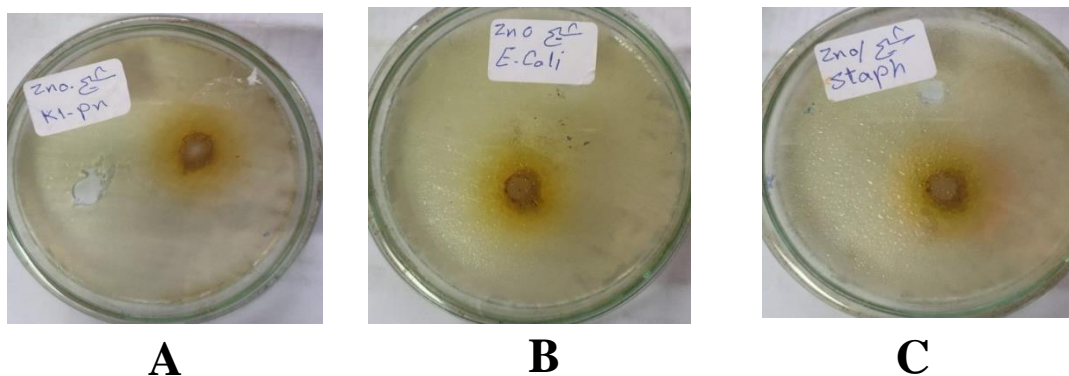


Figure (5a: The mean inhibition zone of ZnO nanoparticles mixture with Artemisia against *Klebsiella pneumoniae*), (5b: The mean inhibition zone of ZnO nanoparticles mixture with Artemisia against *E.coli*) and (5c: The mean inhibition zone of ZnO nanoparticles mixture with Artemisia against *S. aureus*)

3.4. The Suspected mechanism for the anti-bacterial activity of ZnO nanoparticles

The potential toxicity mechanism of ZnO nanoparticles is consistent with the interaction of the ZnO with the cell wall, which resulted in a loss of bacterial integrity. The toxicological effect of ZnO nanoparticles against *E. coli* was studied. The results showed that the bacteria cells were damaged;

therefore, there was an increase in membrane permeability, which leads to the accumulation of ZnO nanoparticles in the bacterial cell membrane as well as the internalization of the nanoparticles. After contacting with ZnO nanoparticles, the cells develop holes in the membrane. The toxicity of ZnO nanoparticles does not always depend on its uptake into the bacterial cell. ZnO nanoparticles can cause changes in the environment around the bacteria, i.e. reactive oxygen species production or increased solubility of ZnO nanoparticles, which can induce cellular damage [47].

4. DISCUSSION

Water was considered in the past as plentiful, free resource that has existed around the world without bearing any problem in regard to being available, but it has become a rare and costly object over recent decades. Currently, water shortage is going to be a challenge for sustainable development of the human community [48, 49]. This crisis is dramatically expanding and is regarded as a global systemic risk, mainly resulting from urban, agricultural, and industrial pollution. In those areas, water consumption has ramped up to 70% (agriculture), 22% (industry), and 8% (domestic) of the currently available fresh water. Accordingly, an enormous volume of wastewater containing a variety of pollutants has been produced [50]. In our research we have investigated the anti-bacterial activity of ZnO nanoparticles which have been synthesized using artemisia plant extract against gram-positive bacteria, like *S. aureus* and gram-negative bacteria, like *E.coli* and *Klebsiella pneumoniae*, those bacteria have been isolated from a variety of water sources to see the impact of ZnO nanoparticles on the water remediation process. The bacterial strains were identified by means of several biochemical tests, such as catalase, coagulase, oxidase, urease, citrate utilization test, indole test and methyl red test, the procedures were very similar to those used in preceding researches [51]. The ZnO nanoparticles have been synthesized through a green synthesis approach and this approach as it mentioned before in a former studies provides a clean, cost-effective and safe way for production of nanoparticles, in addition of being an environmentally friendly process [52]. Previous researches have outlined how effective the ZnO nanoparticles as anti-bacterial and its poisonous impact against bacteria and our results which were obtained through evaluating of anti-bacterial action of ZnO nanoparticles by means of measurement of mean inhibition zone value using agar well diffusion method are concurring to what those researches have concluded [53, 54]. Many studies have confirmed about what we elucidated about the mechanism of action for ZnO nanoparticles against bacterial cell, whether that mechanism is mediated through uptake of ZnO nanoparticles or formation of reactive oxygen species [55, 56].

5. CONCLUSION

In our work, we have experimented the effectiveness of ZnO nanoparticles those formed using green synthesis method against bacteria which have been isolated from different water sources. For example, gram-positive bacteria, such as *S. aureus* and gram-negative bacteria, such as *E.coli* and *Klebsiella pneumoniae*. The effectiveness has been determined through measurement of mean inhibition zone value against each type of bacterial. The inhibition zone value against *Klebsiella pneumoniae* was 22 mm, while the value was 24 mm for *E.coli* and 20 mm against *S. aureus* and thus indicates how efficacious ZnO nanoparticles is against bacteria as an anti-bacterial

Data availability statement

The original contributions presented in the study are included in the article Material; further inquiries can be directed to the corresponding author.

Conflict of Interest Statement:

The authors declare that the research was conducted in the absence of any commercial or Financial relationships that could be construed as a potential conflict of interest.

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Author Contributions:

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