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Eco-Friendly Synthesis and Antibacterial Assessment of Zinc Nanoparticles from *Arbutus unedo* Leaf Extract

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Abstract − This study investigates the synthesis, characterization, and antibacterial effects of zinc nanoparticles (ZnNPs) synthesized via a green synthesis method using *Arbutus unedo* leaf extract. The plant extract served as both a reducing agent and a stabilizer during the ZnNP synthesis. The obtained nanoparticles were characterized using UV-Vis spectrophotometry, X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). The UV-Vis spectrum exhibited a maximum absorption band at 371 nm, consistent with the surface plasmon resonance (SPR) of ZnNPs. According to the XRD analysis, the synthesized nanoparticles possessed a hexagonal wurtzite crystal structure with an average crystallite size of 17.37 nm (ranging between 14.41 and 19.33 nm), as calculated using the Scherrer equation. SEM images revealed that the ZnNPs formed flower-like nanostructures. EDX analysis confirmed that the synthesized nanoparticles comprised 60.9% Zn, 21.2% O, 17.5% Na, and 0.4% Ca. Antibacterial tests demonstrated that ZnNPs were effective against *Escherichia coli* and *Staphylococcus aureus*. Microdilution tests conducted on bacterial cultures indicated that the antibacterial effect of ZnNPs increased up to a concentration of 500 µg/mL ($p < 0.05$). The results manifest that ZnNPs synthesized using *Arbutus unedo* leaf extract exhibit biocompatibility, eco-friendliness, and high antibacterial activity. This study highlights that biogenic ZnNP synthesis offers a sustainable and effective alternative approach.

Keywords − *Arbutus unedo, green synthesis, zinc nanoparticles (ZnNPs), antibacterial activity, biogenic nanoparticles*

1. Introduction

Nanotechnology, as an interdisciplinary field focusing on the design, production, and applications of structures at the nanometer scale, is one of the fastest-growing areas of modern science [1]. This technology has facilitated modern advancements across diverse fields, including medicine, energy, materials science, and environmental science, by providing solutions that surpass the constraints of conventional approaches [2]. Nanoparticles are among the most extensively studied components of nanotechnology. These particles are defined as materials with sizes ranging from 1 to 100 nm, exhibiting unique physical, chemical, and biological properties [3]. The high surface-to-volume ratio of nanoparticles significantly enhances their reactivity and functional properties. As a result, nanoparticles can be utilized across a wide range of applications, from drug delivery systems to catalysts [4].

Metal nanoparticles have attracted significant attention due to their strong optical, electronic, and antibacterial properties. Their synthesis can be achieved through physical, chemical, and biological methods. Physical methods involve advanced techniques such as vapor deposition, while chemical methods are typically based

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on reduction reactions [5]. However, these methods have certain disadvantages, including environmental toxicity and high costs. The green synthesis method emerges as an eco-friendly alternative for nanoparticle synthesis. With their rich phenolic and flavonoid content, plant extracts can serve as both reducing agents and stabilizers [6]. This method enhances biological compatibility by reducing the use of toxic chemicals. *Arbutus unedo*, a plant known for its antioxidant and antimicrobial properties, is a potential biomaterial for nanoparticle synthesis [7]. *Arbutus unedo* (strawberry tree) is a medicinal and aromatic plant belonging to the Ericaceae family and is native to the Mediterranean climate. Its fruit, leaves, and roots are known for their antioxidant, anti-inflammatory, and antimicrobial properties [8]. The chemical composition of *Arbutus unedo* consists of phenolic compounds, flavonoids, tannins, triterpenoids, and various volatile oils [9]. The plant's leaves are particularly rich in potent antioxidant compounds such as quercetin, kaempferol, myricetin, gallic acid, ellagic acid, and quercetin derivatives [10]. Its tannin-rich structure is associated with antibacterial effects, while flavonoids exhibit protective properties against free radicals [11]. Additionally, *Arbutus unedo* leaf extract contains high levels of ascorbic acid (vitamin C), carotenoids, and phenolic acids, making it a valuable botanical source for biomedical and pharmaceutical applications [12]. The fruit contains components such as sugars (mainly glucose and fructose), organic acids (citric acid, malic acid), volatile compounds, and anthocyanins. These components enhance the fruit's nutritional value and highlight its potential use as a functional food ingredient [13]. Studies have shown that *Arbutus unedo* extracts can be used as both bioreductant and stabilizing agents in nanoparticle synthesis. The phenolic compounds and flavonoids present in the leaf extract contribute to the reduction of metal ions and the stabilization of nanoparticle surfaces [14]. *Arbutus unedo* is a plant with strong antioxidant and antibacterial properties with its high phenolic and flavonoid content and is considered an ideal bioreductant and stabilizer for ZnNP synthesis [15]. In the literature, ZnNP synthesis has been carried out with various plant extracts, including Zingiber officinale (ginger) [16], Diospyros kaki (Persimmon) [17], and Ballota acetabulosa (thyme type) [18]. However, the unique phenolic components of *Arbutus unedo*, especially quercetin, gallic acid, ellagic acid, and flavonoids, play an essential role in the surface modification and biocompatibility of ZnNPs, providing both high yield and antibacterial activity in the synthesis process [19]. Therefore, *Arbutus unedo* is a promising plant-based source for environmentally friendly and sustainable nanoparticle synthesis.

Zinc nanoparticles (ZnNPs) have garnered significant attention due to their antibacterial activity, biocompatibility, and strong catalytic properties [20]. In recent years, growing interest in environmentally friendly and sustainable methods has led to numerous studies on the green synthesis of zinc oxide nanoparticles (ZnNPs). For instance, in one study, the green synthesis of ZnNPs was conducted using chestnut honey, and their structural and biological properties were characterized. It was reported that the natural compounds present in honey acted as reducing agents, and the synthesized ZnNPs exhibited antimicrobial properties [21]. Similarly, another study synthesized ZnNPs using an aqueous extract of Diospyros kaki L. (persimmon) peel. The nanoparticles, synthesized under varying pH and temperature conditions, were characterized using UV-Vis spectrophotometry, SEM, and EDAX analyses. The optimal conditions were determined to be pH 10 and 60 °C, and the synthesized nanoparticles were reported to have a size of 168 nm [22]. Moreover, studies have also been conducted on the green synthesis of ZnNPs using Zingiber officinale (ginger) extract, focusing on their anticancer activities [23]. Synthesis using such biological sources minimizes toxic chemicals, enabling the production of environmentally friendly and biocompatible nanoparticles. These studies demonstrate that green synthesis methods utilizing natural resources, such as plants and bee products, provide effective and eco-friendly alternatives for ZnNP production. In this context, the synthesis and investigation of the biological activities of ZnNPs using *Arbutus unedo* leaf extract will significantly contribute to the existing literature. The biological synthesis method of ZnNPs offers advantages in terms of both efficiency and environmental sustainability. This study aims to synthesize ZnNPs using *Arbutus unedo* leaf extract and investigate their antibacterial effects. It is anticipated that ZnNPs obtained through this method will represent an effective alternative from both economic and environmental perspectives. The remainder of this paper is structured as follows: Section 2 details the materials and methods used to synthesize and characterize zinc nanoparticles

(ZnNPs) from *Arbutus unedo* leaf extract. Section 3 presents the results and discussion, including the physicochemical characterization of ZnNPs and their antibacterial performance. Section 4 concludes the study by summarizing the key findings and highlighting the potential applications of ZnNPs as eco-friendly antibacterial agents.

2. Materials and Methods

The *Arbutus unedo* leaves used in this study were collected from naturally growing plants along the coastal region of Bartın Güzelcehisar. The chemicals used to synthesize zinc nanoparticles were obtained with analytical-grade purity.

2.1. Preparation of *Arbutus unedo* Extract

The collected *Arbutus unedo* leaves were thoroughly washed, shade-dried, and ground into a fine powder. The extract was prepared by boiling 10 g of dried leaves in 100 mL of distilled water at 80 °C for 30 minutes. The resulting extract was filtered using filter paper and made ready for use.

2.2. Synthesis of Zinc Nanoparticles

A 10 mM zinc acetate dihydrate solution was prepared to synthesize zinc nanoparticles. The prepared zinc acetate solution was mixed with the *Arbutus unedo* leaf extract in a 1:1 ratio. The mixture was stirred at room temperature using a magnetic stirrer, and a color change was observed, indicating the formation of zinc nanoparticles. The formed nanoparticles were precipitated using centrifugation, washed several times with distilled water and ethanol, and dried for characterization.

2.3. Characterization of Zinc Nanoparticles

The optical properties of the synthesized zinc nanoparticles were analyzed using UV-Vis spectrophotometry within the wavelength range of 200-800 nm. X-ray diffraction (XRD) was used to determine the crystal structure while scanning electron microscopy (SEM) was employed to examine the morphology and size of the nanoparticles. Additionally, the chemical composition of the nanoparticles was analyzed using energy-dispersive X-ray spectroscopy (EDX).

2.4. Antibacterial Activity

The antibacterial activities of the zinc nanoparticles were evaluated using the Broth Microdilution Method [24]. To achieve this goal, bacterial cultures of Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) were inoculated into Nutrient Broth (NB) and incubated at 37°C for 24 hours, ensuring they reached a turbidity equivalent to the 0.5 McFarland standard. A total of 200 µL was added to each well of the microtiter plates, consisting of varying concentrations of the nanoparticle suspension in glycerol (0-500 µg/mL) and 10 µL of bacterial culture inoculated into the NB. Negative controls were prepared using Nutrient Broth (NB) and glycerol without bacterial cultures, while positive controls contained glycerol but no nanoparticles.

The absorbance values of the microtiter plates were measured at the beginning of incubation (0th hour) and the end (24th hour) using a microplate reader at 600 nm. Bacterial viability was expressed as a percentage of the bacterial viability in the positive control group (which was considered 100%). Data analysis was performed using one-way ANOVA followed by the Tukey test, with statistical significance defined as $p < 0.05$.

3. Results and Discussion

The UV-Vis spectrum shown in Figure 1 demonstrates the optical properties of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract. The spectrum presents absorbance values as a function of wavelength, revealing a maximum absorption at 371 nm. This band may indicate the surface plasmon resonance (SPR) characteristic of zinc nanoparticles. Zinc nanoparticles typically exhibit UV absorption bands within the λmax range of 355 to 380 nm [25]. The UV absorption peak observed in this spectrum is a significant indicator confirming the formation of nanoparticles. Specifically, the peak at 371 nm provides insights into the size and formation mechanism of the zinc nanoparticles [26]. Furthermore, the decreasing trend in absorbance values suggests the optical stability of the nanoparticles.

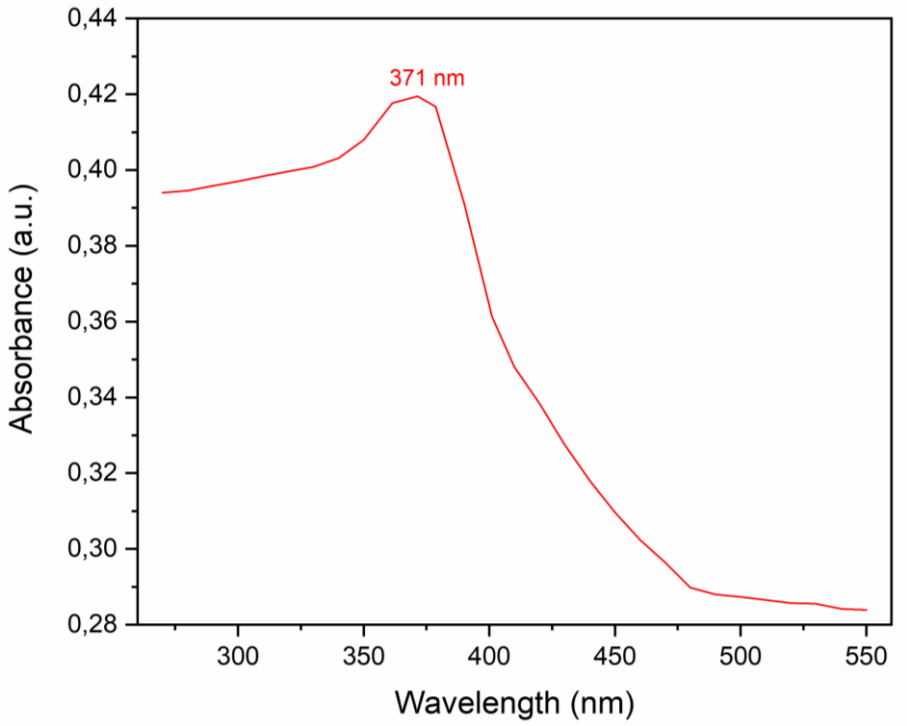

Figure 1. UV-visible absorption analysis of zinc nanoparticles produced with *Arbutus unedo* leaf extract

The FTIR analysis of zinc nanoparticles produced with *Arbutus unedo* leaf extract, shown in Figure 2, highlights the functional groups present on the nanoparticle surface. The spectrum displays transmittance values (%T) within the wavenumber range of 4000–400 cm^{-1}. Broadband is observed between 3200 and 3500 cm^{-1}, corresponding to hydroxyl (-OH) groups, which may be associated with phenolic compounds or alcohols present in the plant extract [14]. The peak at 1647 cm^{-1} corresponds to the C=O (carbonyl) group, while the peak at 1511 cm^{-1} is attributed to C=C aromatic vibrations, indicating the presence of flavonoids, tannins, and other phytochemicals [27]. The peak at 1034 cm^{-1} is associated with C-O-C stretching vibrations, suggesting the presence of polysaccharides or phenolic compounds. Characteristic vibrations in the 600–800 cm^{-1} range correspond to Zn-O bonds, providing strong evidence for nanoparticle formation [28]. This spectrum supports the role of the plant extract as both a stabilizing and reducing agent during nanoparticle synthesis. The presence of hydroxyl, carbonyl, and aromatic groups indicates the attachment of phytochemicals to the nanoparticle surface, enhancing their stability.

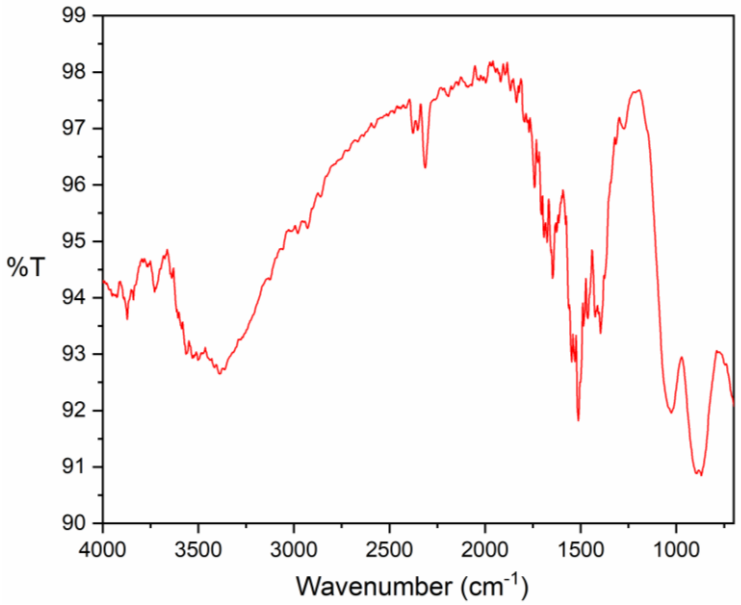

Figure 2. FTIR analysis of zinc nanoparticles produced with *Arbutus unedo* leaf extract

The XRD (X-ray diffraction) spectrum presented in Figure 3 illustrates the crystal structure and phase identification of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract. The XRD spectrum shows prominent diffraction peaks at specific 2θ values, corresponding to the hexagonal wurtzite crystal structure of zinc oxide (ZnO) nanoparticles. The diffraction pattern reveals five distinct peaks at 31.95°, 34.61°, 36.41°, 47.72°, and 56.75° 2θ angles, which correspond to the Miller indices 100, 002, 101, 102, and 110, respectively. These peaks match the JCPDS card number 36-1451 [29]. The strongest peak at approximately 36° (associated with the 101 plane) indicates the dominant crystal orientation of ZnO [30]. The additional diffraction peaks observed between 30° and 40° further support the well-crystallized wurtzite phase of ZnO [31]. Peaks outside this range may be attributed to compounds from the *Arbutus unedo* leaf extract used in the synthesis. The crystallite size can be calculated using the Scherrer equation:

$$D = K\lambda/\beta cos\theta$$

Here, D represents the crystallite size, K (~0.9, Scherrer constant), λ (X-ray wavelength), β (full width at half maximum of the peak), and θ (Bragg angle). Based on this equation, the average size of the synthesized nanoparticles was determined to be 17.37 nm, with a size range of 14.41 to 19.33 nm.

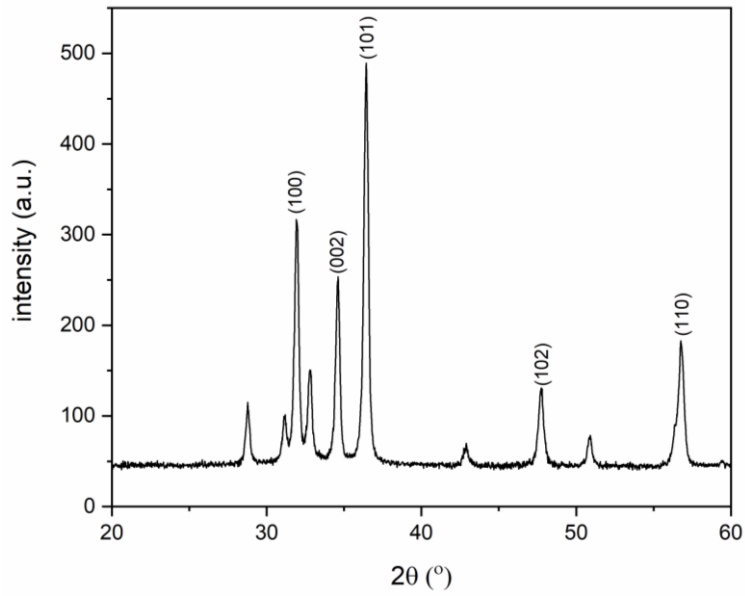

Figure 3. XRD analysis of zinc nanoparticles produced with *Arbutus unedo* leaf extract

Figure 4 presents the Scanning Electron Microscopy (SEM) images obtained to investigate the morphological characteristics of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract. The images were taken at different magnification levels (20.00kX, 50.0kX, and 100.0kX), providing crucial information on the shape, size, and distribution of the nanoparticles. SEM images reveal that the zinc nanoparticles exhibit a flower-like morphology. Such hierarchical nanostructures are commonly observed in the biogenic synthesis of ZnO and are advantageous for photocatalysis, sensors, and biomedical applications due to their large surface area and active sites [32]. At lower magnification (e.g., 20.00kX), the nanoparticles appear to be uniformly distributed with a tendency to cluster. Higher magnification images (50.0kX and 100.0kX) show that the average size of the flower-like nanostructures is on the order of several hundred nanometers. This arrangement of zinc oxide nanoparticles confirms the role of the plant extract as both a reducing and stabilizing agent during synthesis [14]. The SEM image at 100.0kX magnification highlights ZnO nanoparticles with sharp edges and well-defined crystalline structures. Visual analysis of SEM images shows that the average diameter of ZnNPs varies in the range of approximately 100–300 nm. Hierarchical flower-like ZnO nanostructures are particularly important for optoelectronic and catalytic applications, as their high surface area enhances antibacterial and sensor performance [33]. According to SEM analysis, the biogenically synthesized ZnO nanoparticles primarily consist of clustered particles with distinct morphological features. This observation suggests that the *Arbutus unedo* leaf extract influences the growth direction and final morphology of ZnO nanoparticles by coating their surface [34].

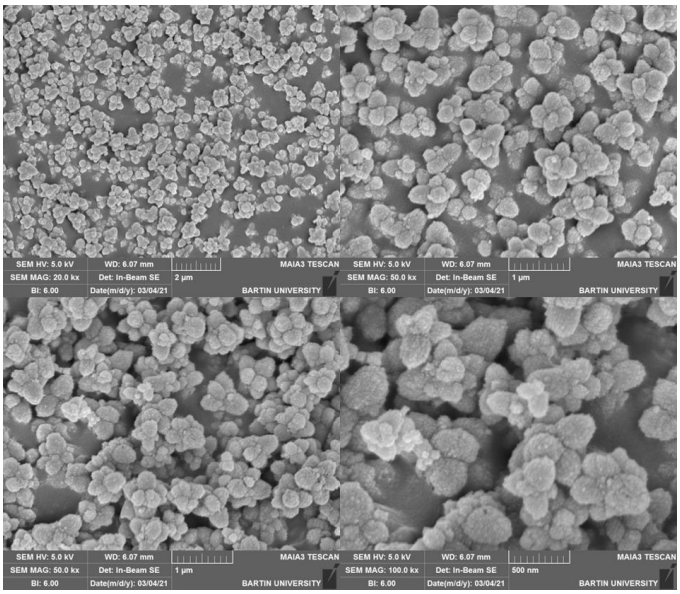

Figure 4. SEM images of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract, captured at different magnification levels (20.00kX, 50.0kX, and 100.0kX)

Energy-dispersive X-ray Spectroscopy (EDX) analysis (Figure 5) was performed to determine the elemental composition of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract. The spectrum shows that the most intense signal corresponds to the Zn element (60.9%), confirming that the synthesized nanoparticles predominantly consist of zinc oxide (ZnO). Oxygen (21.2%) further supports forming ZnO structures [35]. The high proportions of Zn and O indicate that the nanoparticles exist primarily in the ZnO form. Green synthesis using plant extracts can lead to surface modification of metal oxide nanoparticles, resulting in organic residues or phytochemicals on the surface [36]. The detected sodium (Na) and calcium (Ca) elements may originate from the plant extract or trace minerals present in the solution. *Arbutus unedo* is known to be rich in minerals, which could explain the retention of certain trace elements on the nanoparticle surface [10]. The EDX spectrum shows Zn and O as the dominant elements, suggesting that the synthesized nanoparticles are high-purity ZnO. Furthermore, the absence of other metallic impurities (such as Fe or Cu) in the spectrum indicates that the biogenic synthesis process enables clean ZnO production [27].

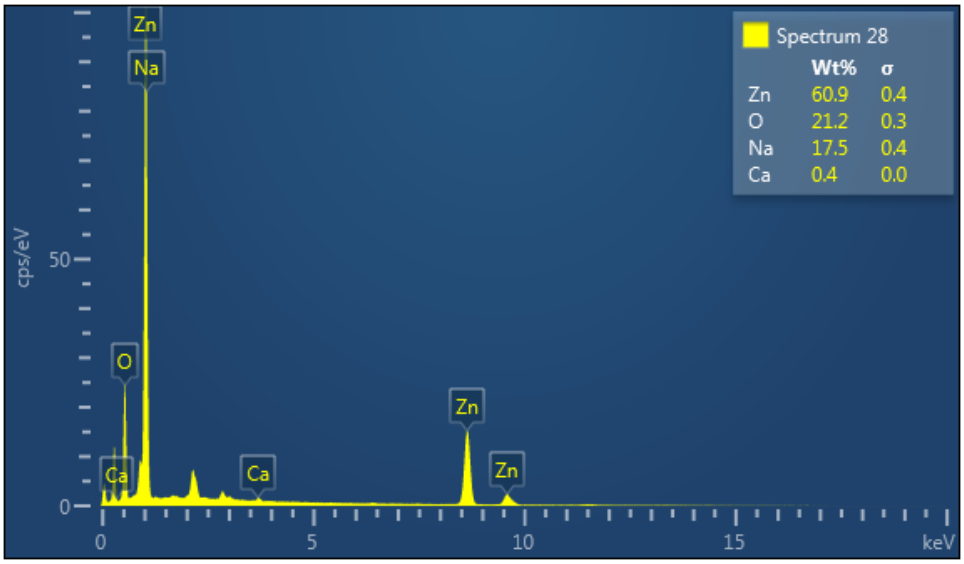

Figure 5. EDX spectrum of zinc nanoparticles synthesized using *Arbutus unedo* leaf extract

Figure 6 illustrates the antibacterial effects of zinc nanoparticles produced with *Arbutus unedo* leaf extract against *E. coli*. The tests conducted with varying nanoparticle concentrations (0–500 µg/mL) revealed a significant decrease in bacterial viability as the concentration increased ($p < 0.05$). At the highest concentration (500 µg/mL), the viability rate decreased to approximately 20%. The results of the Tukey test indicated statistically significant differences between the concentration groups. These findings demonstrate the strong antibacterial activity of ZnNPs against *E. coli*.

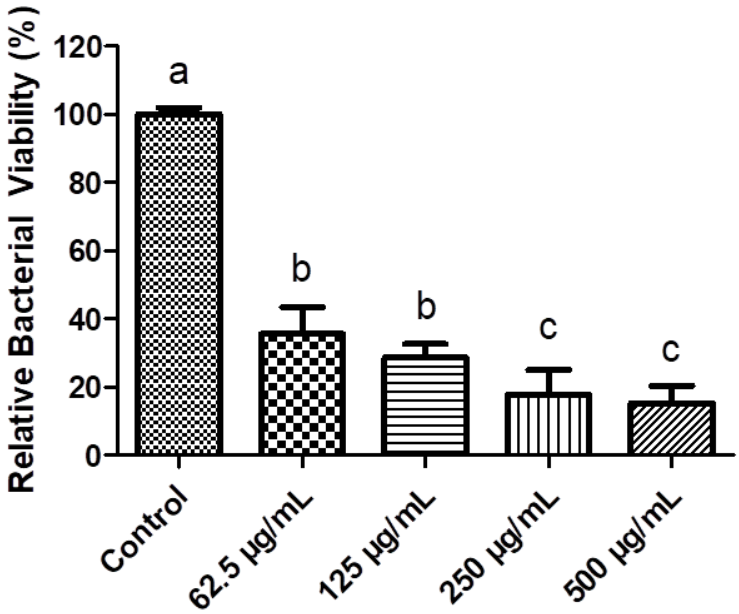

Figure 6. Antibacterial activity of zinc nanoparticles produced with *Arbutus unedo* leaf extract against *E. coli*. According to the Tukey test, different lowercase letters indicate statistically significant differences at the $p < 0.05$ level.

Figure 7 presents the antibacterial effects of the same zinc nanoparticles on *S. aureus*. It was observed that increasing concentrations of ZnNPs reduced the viability of *S. aureus*. Notably, at a concentration of 500 µg/mL, the viability decreased to approximately 20%, similar to the results observed with *E. coli*.

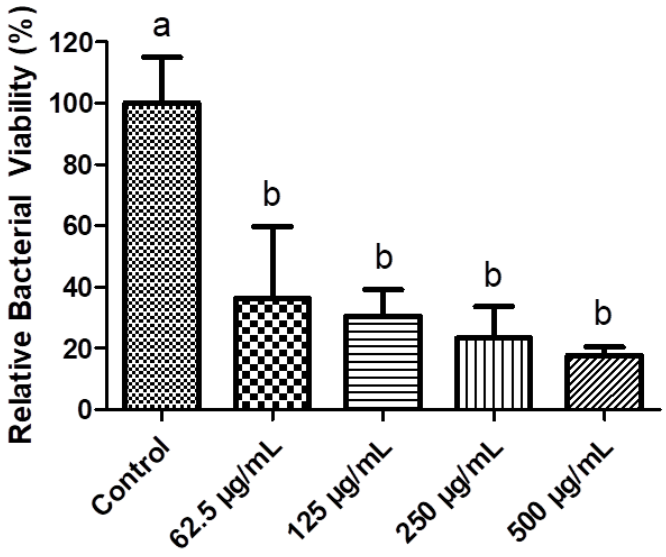

Figure 7. Antibacterial activity of zinc nanoparticles produced with *Arbutus unedo* leaf extract against *S. aureus*. According to the Tukey test, different lowercase letters indicate statistically significant differences at the $p < 0.05$ level.

It was shown that water extract of Arbutus unedo L. has antibacterial effects against both Gram-positive and Gram-negative bacteria [33, 34]. Water extract of roots from Arbutus unedo L. has a higher inhibition against *E. coli* (200 µg/mL as MIC value) than against *S. aureus* (>800 µg/mL as MIC value) [35]. On the other hand, water extract of *Arbutus unedo* L. leaves showed more inhibition on *S. aureus* growth than *E. coli* [36]. In the present study, zinc nanoparticles synthesized using water extract of Arbutus unedo leaves showed similar inhibition against *E. coli* and *S. aureus*.

The mechanisms underlying the antibacterial activity of ZnNPs include the production of reactive oxygen species (ROS) and the induction of damage to the bacterial cell membrane [37]. ROS production leads to oxidative stress on DNA, proteins, and lipids, disrupting cellular functions. Furthermore, the small size and high surface area of ZnNPs enhance their ability to bind to bacterial cell membranes, increasing their capacity to disrupt the bacterial cell wall [38]. *Arbutus unedo* contains high levels of phenolic compounds, flavonoids, and tannins [39], which are thought to act as stabilizing and reducing agents during the synthesis of ZnNPs, contributing to their antibacterial activity. The antimicrobial effects of various components of *Arbutus unedo* (fruit, leaves, and roots) have been extensively studied in the literature. For example, ethanol extracts rich in phenolic compounds derived from fruits have been reported to exhibit strong antimicrobial activity against *S. aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* [40]. Among the plant's various components, leaf extracts have been the most widely studied for their antimicrobial properties. Extracts prepared using different solvents, such as ethanol, methanol, and hot water, are effective against numerous bacterial strains, including *S. aureus*, *E. coli*, *P. aeruginosa*, *Enterococcus faecalis*, *Bacillus cereus*, and *Mycobacterium species* [41-43].

This study investigated the antibacterial activity of ZnNPs synthesized with *Arbutus unedo* plant extract on *E. coli* and *S. aureus*. When compared with similar studies conducted using different plant extracts in the literature, it is seen that the obtained ZnNPs have a strong antibacterial effect. For example, the antibacterial effect of ZnNPs synthesized with Zingiber officinale (Ginger) extract was tested on *E. coli* and *S. aureus*, and it was reported that it reduced bacterial growth by 35% at a concentration of 500 µg/mL [16]. Similarly, it was reported that ZnNPs synthesized with Diospyros kaki (Persimmon) leaf extract provided 42% inhibition against *E. coli* and 38% inhibition against *S. aureus* [17]. However, it was determined that the *Arbutus unedo*-derived ZnNPs synthesized in this study showed approximately 80% antibacterial activity against both bacterial species at 500 µg/mL.

These results show that the rich phenolic content of *Arbutus unedo* enhances the antibacterial activity by increasing the stabilization and activity of ZnNPs. It is thought that components such as quercetin, ellagic acid, and gallic acid adsorb on the ZnNP surface, increase the binding of nanoparticles to bacterial cells, accelerate the production of reactive oxygen species (ROS), and cause cellular damage [38]. Thus, it can be concluded that ZnNPs synthesized using *Arbutus unedo* exhibit higher antibacterial activity compared to other plant synthesis methods.

The results demonstrate that zinc nanoparticles synthesized using *Arbutus unedo* leaf extract exhibit a dose-dependent and strong antibacterial effect on both bacterial species. The comparable effectiveness of ZnNPs against both Gram-negative and Gram-positive bacteria highlights their potential as a broad-spectrum antibacterial agent.

4. Conclusion

The synthesized ZnNPs were thoroughly characterized using UV-Vis, XRD, SEM, FTIR, and EDX techniques. The UV-Vis spectrum confirmed the presence of ZnNPs with a surface plasmon resonance peak at 371 nm. XRD analysis revealed that the nanoparticles possessed a hexagonal wurtzite crystal structure, with an average crystallite size of 17.37 nm, ranging between 14.41 and 19.33 nm, as calculated using the Scherrer equation. SEM imaging showed that the ZnNPs exhibited a flower-like morphology, with hierarchical nanostructures known to provide large surface areas beneficial for antibacterial applications. The EDX analysis confirmed the high purity of the ZnNPs, showing 60.9% zinc and 21.2% oxygen content, along with a significant amount of sodium (17.5%) and calcium (0.4%), which may originate from the plant extract. Functional groups such as hydroxyl (-OH) and carbonyl (C=O) on the nanoparticle surface, identified by FTIR, further highlighted the role of phytochemicals in stabilizing the nanoparticles. The antibacterial activity of ZnNPs was evaluated against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) using the microdilution method. The results indicated a dose-dependent antibacterial effect, significantly decreasing bacterial viability as the ZnNP concentration increased ($p < 0.05$). At the maximum 500 µg/mL concentration, bacterial viability was reduced to approximately 20% for both bacterial species. The Tukey test further confirmed significant differences between concentration groups. The study highlights the potential of biogenic ZnNPs as sustainable antibacterial agents for applications in various biomedical and environmental fields. This study successfully demonstrated the eco-friendly synthesis of zinc nanoparticles (ZnNPs) using *Arbutus unedo* leaf extract, confirming that the plant extract acts as both a reducing and stabilizing agent, while the synthesized ZnNPs exhibited unique physicochemical properties, including a hexagonal wurtzite crystal structure, flower-like morphology, and strong antibacterial activity against both *E. coli* and *S. aureus*, which were effectively inhibited at increasing nanoparticle concentrations, highlighting the potential of biogenic ZnNPs as sustainable alternatives for biomedical and environmental applications. Future research should focus on optimizing the synthesis conditions to enhance the yield and stability of ZnNPs, investigating their cytotoxicity and biocompatibility through in vitro and in vivo studies, exploring their potential applications in various biomedical fields, such as wound healing and drug delivery, and evaluating their effectiveness in combination with other antimicrobial agents to develop synergistic strategies for combating antibiotic-resistant bacterial strains in clinical and environmental settings.

Author Contributions

All the authors equally contributed to this work. They all read and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Ethical Review and Approval

No approval from the Board of Ethics is required.

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