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Green Synthesis of Zinc Oxide Nanoparticles (ZnONPs) Using *Illicium verum* Flower Extract as a Bioreductor and Its Antibacterial Activity Against *Escherichia coli*

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Abstract: Zinc oxide nanoparticles (ZnONPs) have attracted considerable attention as antibacterial agents due to their superior physicochemical properties. This study aimed to synthesize ZnONPs via a green synthesis approach using *Illicium verum* flower extract, which is rich in phytochemical compounds, as a natural bioreductant, as well as to determine the optimal synthesis conditions and characterize the resulting nanoparticles. The synthesis was carried out using zinc acetate dihydrate, with variations in the precursor-to-extract volume ratio (1:1–1:4) and pH (7–11). Characterization was performed using UV–Vis spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), and Particle Size Analysis (PSA), while antibacterial activity against *Escherichia coli* was evaluated using the disk diffusion method. The results showed that the optimal conditions were achieved at a volume ratio of 1:2 and a pH of 10, with a maximum absorption wavelength of 385 nm. The synthesized ZnONPs exhibited an average particle size of 18.27 nm with a low polydispersity index (0.1769), and the presence of Zn–O functional groups was confirmed by FTIR analysis. Furthermore, ZnONPs demonstrated very strong antibacterial activity against *Escherichia coli*, with an inhibition zone diameter of 32.49 mm, which was higher than that of the *Illicium* verum extract. These findings indicate that *Illicium verum* extract is an effective natural bioreductant for the green synthesis of ZnONPs and highlight the potential of the synthesized nanoparticles as efficient and environmentally friendly antibacterial agents. This study also demonstrates the feasibility of utilizing *Illicium verum* flower extract for ZnONP synthesis and antibacterial applications against *Escherichia coli*.

Keywords: Antibacterial; Bioreductor; Green Synthesis; Star Anise; ZnO Nanoparticles.

Introduction

Nanotechnology is a rapidly advancing field that focuses on the manipulation of materials at the nanoscale (1–100 nm), where materials exhibit distinct properties compared to their bulk counterparts. The rapid development of nanotechnology has been driven by its wide-ranging applications in biomedical, energy, and environmental fields [1]. In this context, nanoparticles have become a primary focus due to their high surface area, which enhances reactivity and effectiveness, particularly in antibacterial applications [2]. Furthermore, controlling the size, shape, and structure of nanoparticles is a crucial factor, as these parameters directly influence the physicochemical properties and stability of the material [3].

ZnONPs have been extensively investigated due to their excellent biocompatibility, chemical stability, and notable antibacterial properties. Their antibacterial activity is primarily attributed to the generation of reactive oxygen species (ROS) and the release of Zn^{2+} ions, both of which can disrupt bacterial cell membranes and intracellular components [4], [5]. Moreover, compared to noble metal nanoparticles such as gold and silver, ZnONPs offers significant advantages in terms of cost-effectiveness, abundance, and ease of production, making it a promising candidate for large-scale applications [6]. Nevertheless, conventional synthesis approaches remain challenging, as they typically rely on hazardous chemicals and energy-intensive processes, raising concerns regarding environmental sustainability and safety [7].

As an alternative, green synthesis using plant extracts has emerged as a safer and more sustainable approach for nanoparticle production. This method utilizes bioactive compounds such as flavonoids and phenolics, which act as reducing and stabilizing agents during nanoparticle formation [8], [9]. In addition to minimizing the use of hazardous chemicals, this approach offers a simpler and more environmentally friendly synthesis pathway.

Illicium verum is one of the plants with strong potential for green nanoparticle synthesis due to its rich content of bioactive compounds, particularly flavonoids and phenolics, which play a significant role in reduction and stabilization processes [10]. These phytochemicals possess hydroxyl- and carbonyl-containing functional groups that facilitate electron transfer during nanoparticle formation and contribute to nanoparticle stabilization. Furthermore, this plant is known to exhibit various biological activities, including antibacterial properties, which may enhance the functionality of the synthesized nanoparticles.

Several previous studies have reported the synthesis of ZnONPs using various plant extracts, including pineapple peel (*Ananas comosus*) [11], Saussurea obvallata flowers [12], and mangosteen leaves (*Garcinia mangostana*) [13]. Although these studies successfully demonstrated the feasibility of plant-mediated ZnONP synthesis, variations in nanoparticle characteristics and antibacterial performance indicate that the type of plant extract plays a crucial role in

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determining the resulting nanoparticles' properties. However, the use of *Illicium verum* flower extract for ZnO nanoparticle synthesis, particularly in combination with optimization of synthesis conditions and evaluation of antibacterial activity, remains limited.

To the best of our knowledge, studies investigating the use of *Illicium verum* flower extract for ZnONP synthesis remain scarce, particularly those integrating synthesis optimization, physicochemical characterization, and antibacterial evaluation within a single experimental framework. Therefore, this study provides a systematic assessment of *Illicium verum*-mediated ZnONP synthesis and its antibacterial activity against *Escherichia coli*.

Therefore, this study aims to synthesize ZnO nanoparticles using *Illicium verum* flower extract, optimize the synthesis parameters, and characterize the resulting nanoparticles. In addition, the antibacterial activity will be evaluated against *Escherichia coli* as a model Gram-negative bacterium [14].

Research Methods

Instruments and Materials

The instruments used in this study included a centrifuge (Cence), microscope (Rehaze), analytical balances (Adventurer Ohaus and Sartorius BSA224S-CW), oven (Heraeus ST-5042), hot plate (BASCO), magnetic stirrer (Heidolph MR Hei-Standard), laminar air flow (ESCO Class II Type B2 BSC), incubator, UV–Vis spectrophotometer (Shimadzu UV-1800), Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer), Particle Size Analyzer (PSA) (Malvern), micropipettes, petri dishes, glass slides, blender, calipers, and glassware (Pyrex). The materials used included *Illicium verum* flower powder, zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$), NaOH, Mueller–Hinton Agar (MHA), *Escherichia coli* (ATCC 35218), disc paper, aquabides, aluminum foil, and plastic wrap.

Extraction of *Illicium verum* Flower

The dried powder of *Illicium verum* flower was obtained from the Materia Medica Herbal Laboratory, Batu, East Java, and extracted using the infusion method with slight modifications [15]. Briefly, 10 g of the powder was placed into a sterile beaker, followed by the addition of 100 mL of aquabides. The mixture was then covered with plastic wrap to prevent contamination. The extraction process was carried out by heating at 60°C while stirring at 1000 rpm for 90 minutes, as this method is effective for extracting secondary metabolites such as flavonoids and phenolics [16]. After extraction, the solution was filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4°C to be used as a bioreductant stock solution for the subsequent synthesis process

Green Synthesis of ZnO NPs

ZnONPs were synthesized using a green synthesis method with slight [16]. Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) was used as the precursor and reacted with *Illicium verum* flower extract at volume ratios of 1:1, 1:2, 1:3, and 1:4, with a total volume of 60 mL. The mixture was stirred at 60°C and 1000 rpm for 30 minutes. The formation of nanoparticles was confirmed using a UV–Vis spectrophotometer, and the optimal conditions were determined based on the resulting absorption spectrum. Subsequently, pH optimization was performed in the range of pH 7 to 11, as pH significantly influences nanoparticle size and stability [17]. The solution obtained under optimal conditions was centrifuged at room temperature (±25°C) at 3000 rpm for 30 minutes. The resulting precipitate was washed with aquabides to remove impurities, dried in an oven at 80°C for 3 hours, and then calcined at 400°C for 4 hours to obtain crystalline ZnONPs [16]. The synthesis steps of ZnONPs are illustrated in Figure 1.

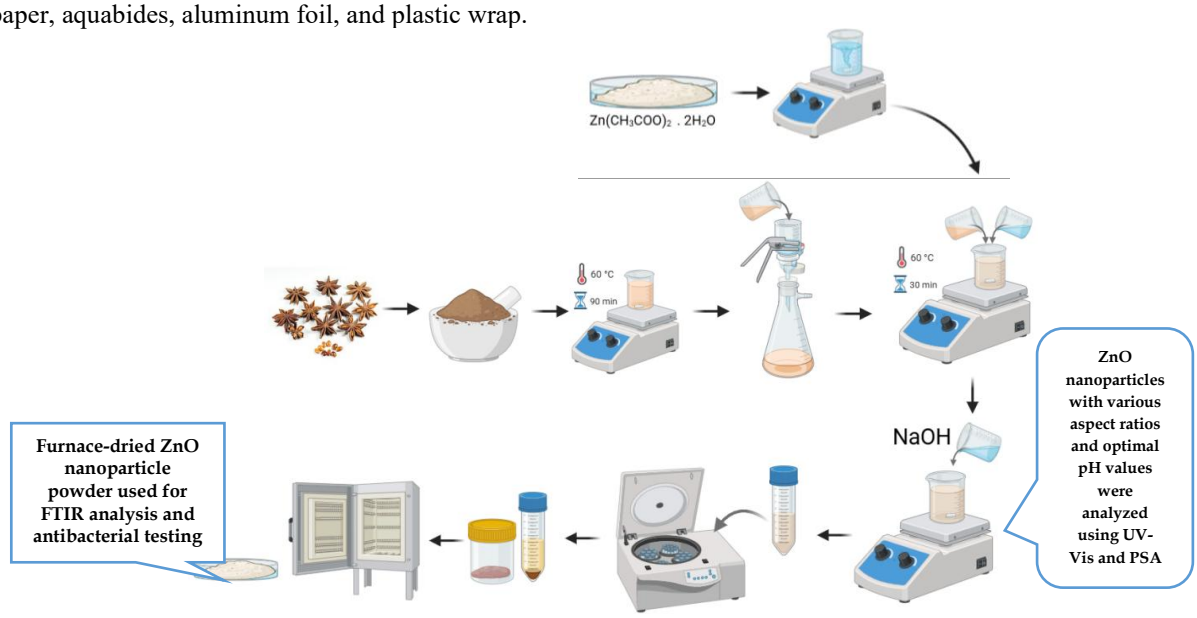

Figure 1. Preparation of ZnONPs using *Illicium verum* flower extract

Characterisation of ZnONPs

The synthesized ZnONPs were characterized using several analytical techniques. UV–Vis spectrophotometry (Shimadzu UV-1800) was employed to determine the maximum absorption wavelength as an indicator of nanoparticle formation, while Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer) was used to identify functional groups involved in the reduction and stabilization processes. In addition, particle size distribution was analyzed using a Particle Size Analyzer (PSA) (Malvern). These characterization techniques are widely applied to evaluate the optical properties, functional groups, and size distribution of ZnO nanoparticles synthesized via green methods [16].

Antibacterial Activity

The antibacterial activity of ZnONPs was evaluated using the disk diffusion method against the Gram-negative bacterium *Escherichia coli*, a standard technique for assessing antimicrobial activity. Mueller–Hinton Agar (MHA) was sterilized and poured into petri dishes, then allowed to solidify. Bacterial colonies were collected using a sterile inoculating loop and suspended in 5 mL of physiological NaCl solution until the turbidity reached the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The bacterial suspension was inoculated onto the MHA surface and evenly spread with a sterile cotton swab, then incubated for approximately 5 minutes. Paper discs soaked with the sample solutions were then placed on the agar surface. Ciprofloxacin and aquabides were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones in millimeters, and all experiments were performed in triplicate to ensure data reliability. The inhibition zone diameter data were analyzed using SPSS version 25. Normality was assessed using the Shapiro–Wilk test ($n < 50$). Normally distributed data ($p > 0.05$) were analyzed by one-way ANOVA at a 95% confidence level ($\alpha = 0.05$), followed by Duncan's Multiple Range Test for post hoc comparisons when significant differences were observed ($p < 0.05$) [18].

Results and Discussion

Optimization of ZnONPs Using *Illicium verum* Flower Extract

The optimization of zinc oxide nanoparticles (ZnONPs) synthesis using *Illicium verum* flower extract was carried out by varying the precursor-to-bioreductant volume ratio and pH, with the optimal condition determined based on the highest absorbance value obtained from the UV–Vis spectra. A higher absorbance value indicates the formation of a greater amount of nanoparticles, which is attributed to the efficient reduction of Zn^{2+} ions by secondary metabolites such as flavonoids and phenolic compounds that act as both reducing and stabilizing agents [2], [8]. However, it is important to note that a high absorbance value does not necessarily indicate a smaller particle size, as it may also be affected by nanoparticle aggregation. Therefore, further characterisation is required to confirm the size distribution and uniformity of the synthesised nanoparticles.

The results indicated that variations in the precursor-to-bioreductant volume ratio significantly influenced the formation of ZnONPs. The analysis was performed using UV–Vis spectrophotometry to determine the maximum absorption wavelength and absorbance values, which serve as indicators of nanoparticle formation.

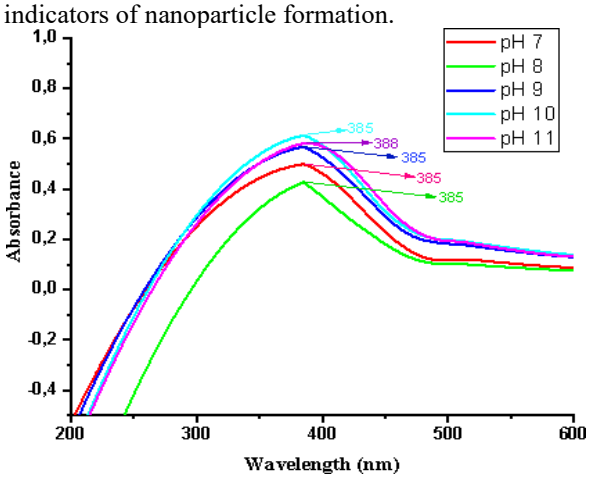

Figure 2. Optimization of precursor-to-extract volume ratio of ZnONPs using *Illicium verum* flower with UV–Vis spectrophotometer

Based on Figure 2, the UV–Vis spectra showed that the maximum absorption wavelength was observed in the range of 359–380 nm. The highest absorbance was recorded at a precursor-to-bioreductant ratio of 1:2, with a value of 0.51 at 359 nm. In contrast, other ratios exhibited lower absorbance values: 0.28 (1:1), 0.25 (1:3), and 0.39 (1:4). These results indicate that the 1:2 ratio is optimal for ZnO nanoparticle formation. The detailed data of maximum absorption wavelengths and absorbance values are presented in Table 1.

Table 1. Results of UV–Vis Spectrophotometer optimization of ZnONPs using *Illicium verum* flower Extract with Precursor-to-Extract Volume Ratio variations

Volume ratio	Wavelength (nm)	Absorbance
1 : 1	380	0.28
1 : 2	359	0.51
1 : 3	372	0.25
1 : 4	368	0.39

Based on the results, the optimal ratio was 1:2, indicating that the balance between the bioreductant and precursor plays a crucial role in nanoparticle formation. A low concentration of bioreductant leads to incomplete reduction of Zn^{2+} ions, whereas an excessive bioreductant concentration may result in excessive capping effects that inhibit nanoparticle nucleation and growth. This process follows the nucleation and growth mechanism, in which Zn^{2+} ions are reduced to Zn atoms, forming initial nuclei that subsequently grow into nanoparticles stabilized by biomolecules present in the extract, acting as capping agents [19], [15]. This mechanism is consistent with classical nanoparticle formation theories, where controlled nucleation and growth determine the final size and distribution of nanoparticles [20], [21].

After determining the optimal condition based on the precursor-to-bioreductant volume ratio, pH optimization was carried out to obtain more stable synthesis conditions. The results of the pH optimization were analyzed using UV–Vis spectrophotometry and are presented in Figure 3.

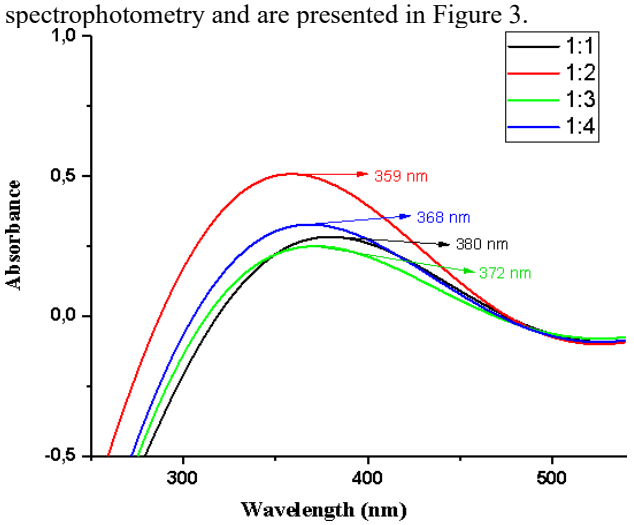

Figure 3. Optimization of pH variation of ZnONPs using *Illicium verum* flower extract with UV–Vis spectrophotometer

Based on Figure 3, the highest absorbance was observed at pH 10, with a value of 0.61 at 385 nm. In contrast, the lowest absorbance was observed at pH 8, at 0.43. Other pH variations showed absorbance values of 0.49 (pH 7), 0.57 (pH 9), and 0.58 (pH 11). These results indicate that alkaline conditions, particularly at pH 10, are optimal for the synthesis of ZnO nanoparticles using *Illicium verum* flower extract. The complete measurement data are presented in Table 2.

Table 2. Results of pH variation measurements with a UV-Vis spectrophotometer

Volume ratio	Wavelength (nm)	Absorbance
7	385	0.49
8	385	0.43
9	385	0.57
10	385	0.61
11	388	0.58

The pH optimization results showed that the optimal condition was achieved at pH 10, indicating that alkaline conditions are more favorable for ZnONPs formation. Under these conditions, Zn^{2+} ions react with OH^- to form $Zn(OH)_2$ as an intermediate species, which subsequently undergoes dehydration to produce ZnO [11]. Furthermore, an increased concentration of OH^- leads to a higher negative surface charge, enhancing electrostatic repulsion between particles and preventing aggregation. This results in smaller nanoparticles with a more homogeneous size distribution. In contrast, under acidic conditions, the activity of phenolic compounds as reducing and stabilizing agents decreases, leading to the formation of larger and less uniform particles [9].

Characterization of ZnONPs Using Star Anise Extract

The ZnO nanoparticles obtained under optimal synthesis conditions were further characterized using UV–Vis spectrophotometry, FTIR, and Particle Size Analyzer (PSA) to confirm nanoparticle formation and to evaluate their physicochemical characteristics.

Spectrophotometer UV-Vis

The ZnONPs synthesized under optimal conditions were further analyzed using UV–Vis spectrophotometry as a preliminary method to confirm nanoparticle formation. This technique was employed to identify the characteristic absorption wavelength and determine the maximum absorbance associated with ZnO nanoparticle formation. In addition, UV–Vis analysis was used to verify that the optimized synthesis conditions produced nanoparticles with appropriate optical properties.

UV–Vis spectrophotometric analysis was performed to confirm the formation of ZnONPs based on their characteristic absorption wavelength. As shown in Figure 4, the UV–Vis spectrum exhibited a maximum absorption peak at 385 nm. This absorption peak indicates the successful formation of ZnONPs under the optimal synthesis conditions.

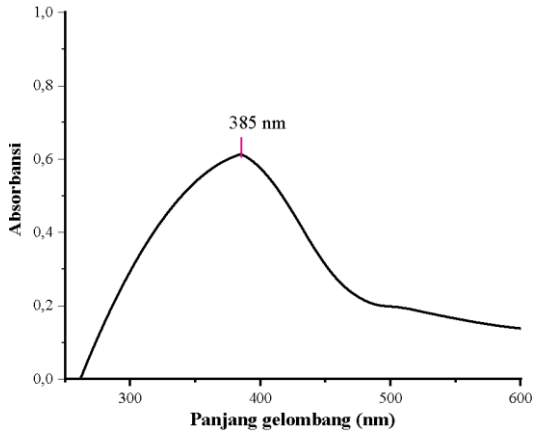

Figure 4. Optimization of pH variation of ZnONPs using *Illicium verum* flower extract with UV–Vis spectrophotometer

As shown in Figure 4, the UV–Vis spectrum indicates that ZnONPs synthesized under optimal conditions—specifically at a precursor-to-bioreductant volume ratio of 1:2 and pH 10—exhibited a maximum absorption peak at 385 nm. This absorption peak is characteristic of ZnO nanoparticles and is associated with electronic transitions from the valence band to the conduction band. These findings are consistent with previous studies reporting that green-synthesized ZnONPs typically exhibit absorption peaks in the range of 350–380 nm [22], [23].

The variation in maximum absorption wavelength compared to other studies may be attributed to differences in particle size, synthesis conditions, and the type of bioreductant used, as these parameters directly influence the optical properties of ZnONPs [24]. In semiconductor materials such as ZnO, the quantum confinement effect significantly alters the band gap as particle size changes [25]. As the nanoparticle size decreases, the band gap energy increases, resulting in a shift of absorption toward shorter wavelengths (blue shift), a characteristic of nanoscale ZnO systems [24]. This phenomenon indicates that the optical properties of ZnONPs are strongly influenced by particle size and structural characteristics developed during synthesis. Therefore, UV–Vis analysis not only confirms the successful

formation of ZnONPs but also provides preliminary insights into their size and optical behavior [17].

Spectroscopy FTIR

Fourier Transform Infrared (FTIR) analysis was performed to identify functional groups and to confirm the formation of ZnONPs. This technique enables the detection of characteristic absorption bands associated with molecular vibrations, thereby providing insight into the involvement of biomolecules in the reduction and stabilization processes during green synthesis [26]. In addition, FTIR analysis allows the identification of interactions between nanoparticles and capping agents that contribute to particle stability [26].

The FTIR analysis results are presented in Figure 5, showing the spectra of the star anise extract and the synthesized ZnONPs. Changes in the intensity of several functional groups were observed, particularly in the O–H and C–O groups after the synthesis process. These changes indicate the involvement of phytochemical compounds in the reduction and stabilization of the nanoparticles. In addition, an absorption peak appeared at 424.26 cm^{-1}, characteristic of Zn–O bonding, confirming the successful formation of ZnO nanoparticles.

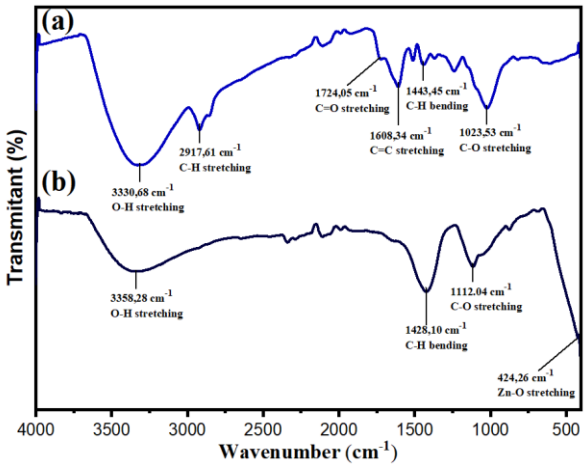

Figure 5. (a) FTIR spectrum of *Illicium verum* flower extract; (b) FTIR spectrum of synthesized ZnONPs

Based on the FTIR spectra, the synthesized ZnO nanoparticles exhibited a characteristic absorption peak at approximately 424 cm^{-1}, corresponding to Zn–O bond vibrations. This peak is a distinctive feature of ZnO nanoparticles and is typically observed in the range of 400–500 cm^{-1}, confirming the formation of the ZnO phase [27], [28]. Furthermore, the spectra revealed the presence of functional groups such as O–H, C=O, and C–O, which originate from organic compounds in the extract and play a role in the reduction and stabilization of ZnONPs [2].

Changes in peak intensity and slight shifts in FTIR bands after synthesis indicate interactions between biomolecules and the surface of ZnONPs. Bioactive compounds such as flavonoids and phenolics act as stabilizing agents by forming a protective layer on the nanoparticle surface, thereby controlling particle growth and preventing aggregation [26]. Therefore, the FTIR results not only confirm the successful formation of ZnONPs but also demonstrate the crucial role of biomolecules in the green synthesis mechanism.

Particle Size Analyzer (PSA)

Particle size characterization using PSA is presented in Figure 6. The results showed that the synthesized ZnONPs had an average diameter of 18.27 nm, with a polydispersity index (PDI) value of 0.1769. This relatively low PDI value indicates a narrow and homogeneous particle size distribution.

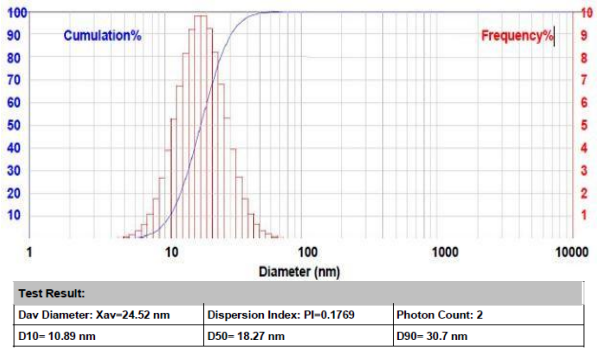

Figure 6. Results of PSA characterization of synthesized ZnONPs

Particle size characterization of ZnONPs was performed using a Particle Size Analyzer (PSA) to determine particle size distribution and the homogeneity of the nanoparticle system. PSA is a widely used technique for measuring the hydrodynamic size of particles in colloidal systems and evaluating size distribution through the polydispersity index (PDI), which reflects particle uniformity [29], [30]. Particle size is a critical parameter as it directly influences the physicochemical properties, colloidal stability, and biological activity of nanoparticles, including their antibacterial performance [31].

Based on the measurement results, the synthesized ZnONPs exhibited an average particle size of 18.27 nm with a PDI value of 0.1769, indicating that the particles are within the nanoscale range and possess a relatively homogeneous size distribution. A PDI value below 0.3 suggests a narrow size distribution and good colloidal stability [29]. The relatively small particle size can be attributed to the role of biomolecules present in the extract, such as flavonoids and phenolic compounds, which act as capping and stabilizing agents, thereby inhibiting particle growth and preventing aggregation during synthesis [32]. Furthermore, smaller nanoparticle size leads to a higher specific surface area, which enhances interaction with bacterial cells and contributes to improved antibacterial activity of ZnONPs [31].

Based on the results of this study, the synthesized ZnONPs were successfully characterized using UV–Vis spectroscopy, FTIR, and Particle Size Analysis (PSA). These techniques provided information regarding nanoparticle formation, functional groups involved in the synthesis process, and particle size distribution. However, the inclusion of X-ray Diffraction (XRD) analysis would provide valuable information on the crystallinity and phase purity of the synthesized ZnONPs, while Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM) could further elucidate the surface morphology, particle shape, and structural characteristics of the nanoparticles. Therefore, these characterization techniques

are recommended for future studies to achieve a more comprehensive understanding of the synthesized ZnONPs.

Antibacterial Activity Against *Escherichia coli*

The antibacterial activity against *Escherichia coli* is presented in Figures 7 and 8. Based on the observations, ZnONPs exhibited a larger inhibition zone compared to the star anise extract.

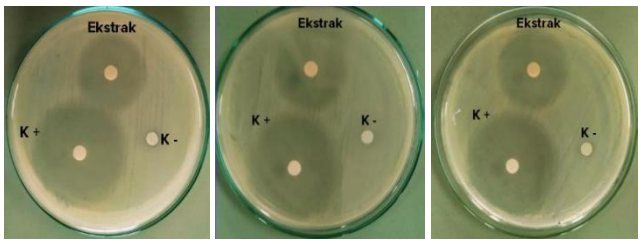

Figure 7. Antibacterial activity test of *Illicium verum* flower extract on *Escherichia coli* bacteria (a) First replication; (b) Second replication; (c) Third replication

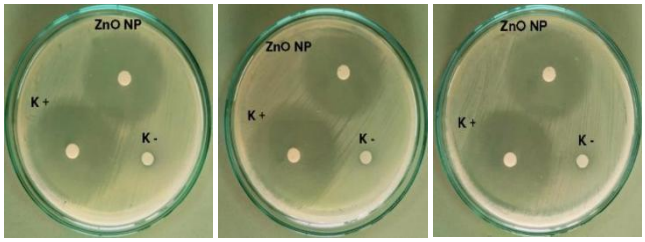

Figure 8. Antibacterial activity test of ZnONPs on *Escherichia coli* bacteria (a) First replication; (b) Second replication; (c) Third replication

The data presented in Table 3 show that the average inhibition zone diameter of ZnONPs was 32.49 mm, whereas the *Illicium verum* flower extract exhibited a smaller inhibition zone of 26.59 mm. The positive control (ciprofloxacin) showed inhibition zones ranging from 37.14 to 37.23 mm, while the negative control showed no inhibition zone. These results indicate that ZnO nanoparticles exhibit higher antibacterial activity compared to the *Illicium verum* flower extract.

Table 3. Inhibition Zone diameter of ZnONPs and *Illicium verum* flower extract against *Escherichia coli*

Sample	Inhibiiton zone diameter (mm)				Category
	1	2	3	Average	
Illicium verum flower extract	26.71	26.62	26.44	26.59 ± 0.14	Very strong
ZnO nanoparticles	32.95	32.16	32.37	32.49 ± 0.41	Very strong
Positive control (Ciprofloxacin) in *Illicium verum* flower extract samples	37.07	37.39	37.24	37.23 ± 0.16	Very strong
Positive control (Ciprofloxacin) in the ZnONPs samples	37.15	37.18	37.05	37.14 ± 0.07	Very strong
Negative control (aquades)	-	-	-	-	-

The synthesized ZnONPs exhibited higher antibacterial activity compared to the *Illicium verum* flower extract, as indicated by the larger inhibition zone against *Escherichia coli*. This enhancement suggests that transforming bioactive compounds from their extract form into nanoparticles significantly improves antibacterial effectiveness, primarily due to increased surface area and chemical reactivity. This finding is consistent with previous studies reporting that metal oxide nanoparticles exhibit stronger antimicrobial activity than their conventional counterparts [33].

Based on the Shapiro–Wilk normality test, all datasets were normally distributed ($p > 0.05$). One-way ANOVA revealed a significant difference in the inhibition zone diameters among the treatment groups ($F = 1399.344$; $p < 0.001$). Duncan's post hoc test further showed that the antibacterial activity of the synthesized ZnONPs differed significantly from that of the *Illicium verum* extract, whereas the two positive control groups were not significantly different from each other but differed significantly from both the extract and ZnONPs.

The *Illicium verum* extract exhibited the lowest mean inhibition zone diameter (26.59 mm), followed by ZnONPs (32.49 mm), while the positive control groups showed the highest inhibition zone diameters (37.13–37.23 mm). The corresponding inhibition zone diameters are presented as mean ± standard deviation (SD) in Table 3, indicating low variability among the replicate measurements. These findings indicate that the synthesis of ZnONPs significantly enhanced the antibacterial activity of *Illicium verum* extract against *Escherichia coli*, although their effectiveness remained lower than that of the ciprofloxacin positive control [34].

The enhanced antibacterial activity of the synthesized ZnONPs can be attributed to their physicochemical characteristics, particularly particle size and surface properties. The relatively small particle size, with an average diameter of 18.27 nm, plays a crucial role in enhancing antibacterial activity. Smaller nanoparticles provide a larger specific surface area, allowing more frequent interaction with bacterial cells. In Gram-negative bacteria such as *Escherichia coli*, which possess a thinner cell wall structure, nanoparticles can more easily penetrate the cell membrane [4]. Furthermore, the low polydispersity index (PDI) indicates a homogeneous particle size distribution, contributing to improved stability and consistent antibacterial performance [30], [35].

In addition to particle size and distribution, surface properties also play a key role in the antibacterial mechanism. The charged surface of ZnONPs can interact electrostatically with negatively charged bacterial cell membranes. This interaction increases membrane permeability, leading to initial structural damage and facilitating nanoparticle penetration into the cell [36], [37].

The antibacterial mechanism of ZnONPs in this study involves multiple synergistic pathways, as illustrated in Figure 9. First, the generation of reactive oxygen species (ROS), such as H_2O_2, OH^-, and O_2^-, induces oxidative stress and damages essential cellular components, including membranes, proteins, and DNA [33]. Second, the release of Zn^{2+} ions disrupts enzymatic activity and interferes with bacterial metabolic processes. Third, direct interaction

between nanoparticles and the cell membrane leads to increased permeability and leakage of intracellular components [4], [34]

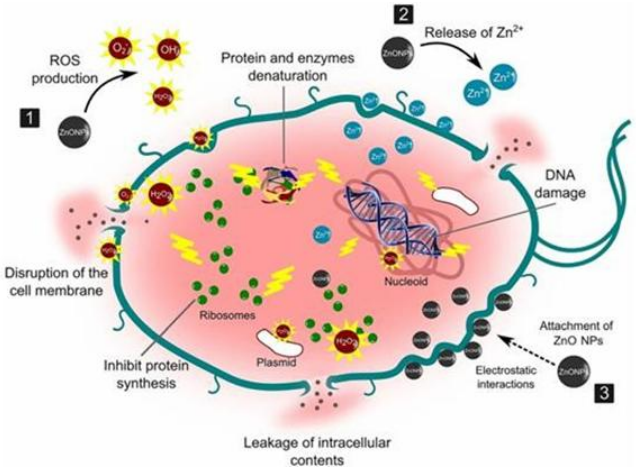

Figure 9. Mechanism of antibacterial activity of ZnO nanoparticles (adapted from Yusof et al., 2019)

Moreover, the presence of bioactive compounds from *Illicium verum* extract, which act as capping agents, further enhances antibacterial activity. Compounds such as flavonoids and phenolics not only contribute to nanoparticle formation and stabilization but also exhibit intrinsic antibacterial properties by disrupting cell membranes and inhibiting bacterial enzymes [31]. This suggests a potential synergistic effect between ZnONPs and plant-derived biomolecules.

Compared to previous studies, the antibacterial activity observed in this work is relatively high, which can be attributed to the small particle size, homogeneous distribution, and optimized synthesis conditions, particularly at the optimal pH. Therefore, ZnONPs synthesized via green synthesis using *Illicium verum* flower extract demonstrate strong potential as effective, environmentally friendly, and sustainable antibacterial agents for applications in healthcare and environmental fields.

Conclusion

Zinc oxide nanoparticles (ZnONPs) were successfully synthesized using *Illicium verum* flower extract through a green synthesis approach, with optimal conditions achieved at a precursor-to-bioreductant ratio of 1:2 and pH 10. The synthesized ZnONPs exhibited a nanoscale particle size (18.27 nm), confirmed Zn–O functional groups, and demonstrated strong antibacterial activity against *Escherichia coli*. Statistical analysis revealed significant differences in inhibition zone diameters among the treatment groups ($p < 0.05$), with ZnONPs exhibiting significantly greater antibacterial activity than *Illicium verum* extract alone but lower activity than the ciprofloxacin positive control. These findings indicate that *Illicium verum* extract is a promising natural bioreductant for the environmentally friendly synthesis of ZnONPs with potential antibacterial applications. Further studies are recommended to include XRD and SEM/TEM characterization, evaluate additional microbial strains, and investigate cytotoxicity to support future biomedical and environmental applications.

Author's Contribution

B. Ramadhani: research, data collection, wrote the draft manuscript. S. Sutoyo: helped guide and direct the author in the process of creating the article. R.Y. Arizandy & W. Setyarini: helped guide and direct the author in collecting antibacterial data.

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