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Research Paper

Green Synthesis of ZnO Nanoparticles using *Nigella sativa* Seeds Aqueous Extract and Antibacterial Activity Evaluation

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Abstract— This study aimed to determine the antimicrobial activity of ZnONPs synthesized by the green method using *Nigella sativa* extract against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. In addition, the effect of pure ZnONPs on the same bacteria was compared to the effect of ZnONPs synthesized using the green method. The synthesis of ZnONPs was carried out using the standard green method. The results indicate that ZnONPs synthesized by *N. sativa* seed extract had an effect against *S. aureus* with a zone of 19 mm obtained from the concentration of 150 mg/ml of ZnONPs synthesized using 25 ml of *N. sativa* seed extract. Also, ZnONPs showed antimicrobial activity against *S. pyogenes* in zone 19 mm at 150 mg/ml concentrations of ZnONPs synthesized using 25 ml of *N. sativa*. The synthesized ZnONPs showed activity against *K. pneumoniae* in zone 14 mm for 100 mg/ml and 150 mg/ml of ZnONPs synthesized using 25 ml of *N. sativa* extract. Moreover, ZnONPs synthesized by the green method was low effective on *P. aeruginosa*. Finally, the results exhibited low antimicrobial activity of pure ZnONPs compared to the ZnONPs synthesized by the green method, and the best effect of ZnONPs was obtained at concentrations of 150 mg/ml of ZnONPs synthesized from 25 ml of *N. sativa* extract.

Keywords— Antimicrobial activity; Zinc oxide nanoparticles; Green method; *Nigella sativa*; Bacteria

1. Introduction

The discipline of nanotechnology has had exponential growth over the last two decades and has significantly influenced the physical, chemical, earth, and biological sciences [1]. Nanotechnology is used in fields involving life, such as electronics, the environment, agriculture, and medicine [2]. Recent advances in nanotechnology have attracted the scientific community's interest because of their peculiar physical and chemical characteristics that set them apart from their bulk counterparts [3]. Metal oxides are favoured for use as sterile coatings on biomedical devices like catheters, dental implants, adhesives, biosensors, biomaterials, tissue engineering, DNA modification, drug delivery systems, and packaging due to their antibacterial, chemical, and catalytic properties [4]. The introduction of novel bacterial strains resistant to existing antibiotics poses a severe threat to the public's health, which is why there is a strong incentive to develop new bactericides [5]. Due to the significant problem of antibiotic resistance, several initiatives are being taken to discover novel antimicrobials [6]. Scientists around the world are seeking to utilize nanomaterials due to their exceptional properties, including a high surface area to volume ratio and faster chemical reactions. [7]. Zinc oxide (ZnO), which has a distinctive structure and a wide range of uses, including translucent electronics, piezoelectric sensing, catalytic processes, and chemical, and biological sensing has received much interest [8]. It is also biosafe and biocompatible [1, 9]. In addition, ZnO includes a mineral (Zn) that is necessary for people and harmless for the environment. It possesses good selectivity, heat resistance, reduced toxicity, and higher durability [1]. ZnO, a metal oxide with nanoscale dimensions, has been widely utilized for its antibacterial and anticancer properties [4]. ZnO nanoparticles (ZnONPs) have been employed as feed additives, food preservatives, and tissue healing agents. They cure meningitis, tumors, and illnesses in animals brought on by intracellular infections such as viruses and bacteria such as *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *Streptococcus* species [10].

The grassy plant *Nigella sativa*, sometimes recognized as black seed, belongs to the Ranunculaceae family [11]. Proteins, alkaloids, saponins, and essential oils comprise the remaining portion of the black seeds' composition, including dihomolionolenic acid, tocopherols, healthy fatty acids, phytosterols, and alkaloids like nigellone, and nigellimine. *Nigella sativa* has been recognized as a natural remedy for a

multitude of diseases and health conditions, including asthma, hypertension, cough, bronchitis, dizziness, diabetes, headaches, inflammation, eczema, fever, and influenza. [12]. A revolutionary method for making metal nanoparticles without hazardous compounds is biosynthesizing nanoparticles using various plant extracts [13]. A notable benefit of using plant extract to make metal oxide is the creation of valuable compounds that decrease metal ions [14]. The polyphenols in the plant extract, such as the tannins, glycosides, and flavonoids, react with the ions of zinc in solution to generate complexation followed by a hydrolysis process to form zinc hydroxide since the polyphenols include a hydroxyl group [15]. The determination of the antimicrobial activity of ZnONPs against bacteria, specifically *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *S. pyogenes*, was the purposes of this study as well as to compare the antimicrobial activity of ZnONPs produced using the green method by N. sativa extract with that of pure ZnONPs.

2. Related Work

Zinc oxide nanoparticles (ZnONPs) have been widely studied for their antimicrobial activity. The green synthesis method using Nigella sativa extract has been found to be effective in synthesizing ZnO NPs [5In recent years, numerous studies have been conducted to investigate the antimicrobial properties of ZnONPs and their efficacy in inhibiting the growth of bacteria and fungi. Although the exact mechanisms of action of ZnONPs against bacteria have not been fully determined, it is widely acknowledged that higher concentrations of ZnONPs lead to stronger inhibition. Among metal-based nanoparticles, ZnONPs have been extensively studied. Research studies, such as [16], have reported that the antibacterial effect of ZnONPs against bacteria increases with concentration and effectively impedes bacterial growth. Another study [17] highlighted the antimicrobial activity of ZnONPs against both gram-positive and gram-negative bacteria and discussed their potential application as a biocompatible coating agent for intelligent storage. In addition, [18] documented the inhibition levels of ZnONPs against S. aureus, in conjunction with improved structural integrity. Additionally, studies have noted the antibacterial properties of ZnONPs synthesized using green methods, such as against *S. aureus* and *K. pneumoniae* as reported in [18]. Furthermore, [19] has reported the antibacterial effect of ZnONPs synthesized using green methods against *S. pyogenes*, with an inhibition zone of 16mm. According to [20], ZnONPs synthesized by the green method as an antibacterial have an effect against *K. pneumoniae* with inhibition zone 12 mm. The N. sativa extract contains various bioactive compounds, such as thymoquinone and carvacrol, that have antimicrobial properties. The *N. sativa* extract also acts as a reducing and capping agent during the synthesis of ZnO NPs. Studies have shown that the N. sativa-synthesized ZnONPs exhibit higher antimicrobial activity compared to chemically synthesized ZnONPs [8]. The ZnONPs synthesized through this method have shown efficacy against a range of microorganisms, including bacteria and fungi. The antimicrobial mechanism of the N. sativa-synthesized ZnONPs is thought to be due to the production of reactive oxygen species and the disruption of the bacterial cell membrane [18]. This green synthesis method using *N. sativa* extract provides a promising alternative for the synthesis of ZnONPs with improved antimicrobial activity [16].

3. MATERIALS AND METHODS

Preparation of *Nigella sativa* Seeds extract.

Seeds of *N. sativa* were bought from the central market in Dhamar city. Freshly purchased seeds of *N. sativa* (black seeds), weighing 25 g, were ground and melted in 50 ml of distilled water with stirring of mixture for 20 minutes. Subsequently, the aqueous mixture was filtered using Whatman filter paper no. 1 to get the black seed extract [6].

Synthesis and green synthesis of ZnONPs

First, 50 ml of distilled water was used to dissolve 2.98 g of zinc nitrate (0.1M), which was then stirred for one hour. After that, desired volume of distilled water was used to dissolve sodium hydroxide (0.1M) that was stirred for ten minutes. Then, the sodium hydroxide was added to zinc nitrate with mixing by distillation with continuous mixing for three hours, the precipitate that resulted was filtered, washed with distilled water and ethanol alternately several times, dried in the oven at 150 Celsius for 24 hours, then continuously ground in a mortar before being placed in a sterilized container [21, 22]. In order to create four new substances for the green synthesis of ZnONPs with black seed extract, the same steps as those for making pure zinc were used. However, in the third step, black seed extract was added in 2 mL, 4 mL, 6 mL, and 7 mL after half an hour of mixing to synthesize four different types of ZnONPs. The nanopowders were analyzed using atomic absorption X-ray diffraction (XRD), and the size of the particles was measured by measuring the diameter of the particles. From ZnONPs synthesized by the green method using *N .sativa* seeds extract, five different concentrations of each mixture prepared, 10, 25, 50, 100, and150 mg/ml, to be used in the testing of the antimicrobial activity of ZnONPs against bacteria.

Cultivation and confirmation of bacteria

Pathogenic bacteria used to assess the antimicrobial activity of zinc oxide nanoparticles were obtained from the laboratories of Al-Jarfi, including *S. aureus, P. aeruginosa, S. pyogenes*, and *K. pneumoniae*. The implant media for the bacteria culture for confirmation were prepared according to the manufacturer's instructions. The microscopic examination of the culture and the biochemical reaction, including the catalase test, coagulase test, oxidase test, citrate utilization test, methyl red test, and indole test, carried out the confirmation of bacteria [23]. The bacteria were activated, and the MacFarland scale's bacterial suspension was prepared [24].

Antimicrobial activity of ZnONPs

The efficacy of ZnONPs against bacteria was assessed through a disc diffusion method on Muller-Hinton plates. The

medium was created according to the manufacturer's instructions, then sterilized in an autoclave at 121°C (1.5 psi/inch2) for 15 minutes. 20 ml of sterilized medium was then poured into each Petri dish. The Muller-Hinton agar plates were inoculated with the bacterial culture by streaking with a cotton swab. Four filter discs were soaked in ZnONPs, with each disc being treated with 10 µm of the substance on both sides [25]. The positive control was soaked in distilled water, while the negative control was soaked in azithromycin. The discs were placed on the plates and incubated at 37°C for 24 hours. The antibacterial activity was determined by the size of the inhibition zone, which was measured [25-27].

4. Results and Discussion

The antibacterial potency was determined against Gram-positive microorganisms (*S. aureus and S. pyogenes*) and Gram-negative microorganisms (*K. pneumoniae* and *P. aeruginosa*) through the disc diffusion method. X-ray diffraction (XRD) was utilized to examine the crystal structure, particle size and lattice parameter of ZnONPs synthesized by the co-precipitation method. Diffraction peaks were observed at 2-theta values of 31.479^0, 34.317^0, 36.688^0, 47.899^0, 56.341^0, 63.222^0, 68.418^0, 69.478^0 and 76.946^0corresponding to lattice planes (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (1 1 2),(201) and (2 0 2) respectively. As shown in Figure 1, All the diffraction peaks in the sample were identified as belonging to crystalline hexagonal ZnO with a quartzite structure as compared to powder diffraction. Notably, there were no extra peaks corresponding to any other phase of ZnO or impurities detected. Using Scherer's formula, the average crystallite size was calculated to be 15.47 nm, which was in agreement with previously reported results[28].

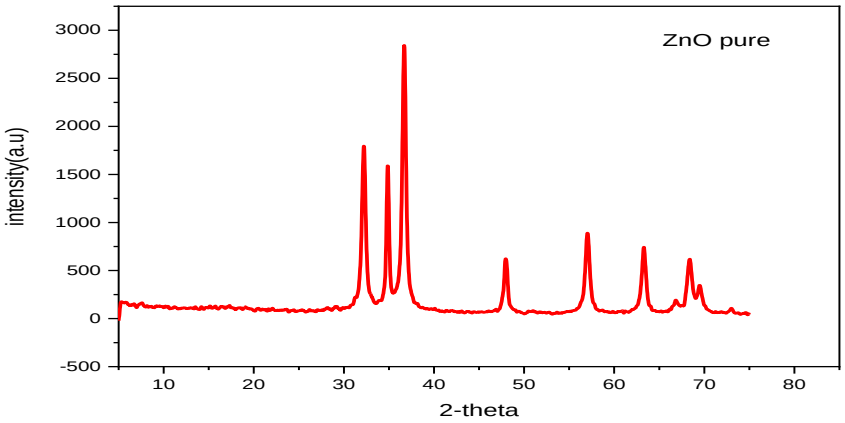

Fig 1. XRD pattern of synthesized ZnO (pure).

In this study, the results indicate that ZnONPs synthesized by the plant *N. sativa* seeds extract affected *S. aureus* bacteria, as mentioned in Table 1. The synthesized ZnONPs showed activity against *S. aureus* with zone of inhibition 19 mm at the concentration of 150 mg/ml of ZnONPs synthesized using 25 ml of *N. sativa*. In contrast, the lowest inhibition with an inhibition zone of 9 mm was recorded at the concentration of 25 mg/ml of ZnONPs synthesized using 5 ml of *N. sativa* extract. At the concentration of 10 mg/ml, the antimicrobial activity ranged from 11 to 12 mm; the highest antimicrobial activity was at the ZnONPs synthesized by 10 and 25 ml, as well as the pure, while the lowest antimicrobial activity was at the ZnONPs synthesized by 5 ml. At concentration 25, the antimicrobial activity ranged from 9 to 12 mm; the highest antimicrobial activity was at the ZnONPs synthesized by 10 and 25 ml, as well as the pure, while the lowest antimicrobial activity was at the ZnONPs synthesized by 5 ml. At the concentration of 50 mg/ml, the antimicrobial activity ranged from 11 to 15 mm; the highest antimicrobial activity was at the ZnONPs synthesized by 25 ml of *N. sativa* extract, while the lowest was at pure. At the 100 mg/ml concentration, the antimicrobial activity ranged from 11 to 15 mm; the highest antimicrobial activity was at the ZnONPs synthesized from 25 ml of *N. sativa* extract, while the lowest was at the ZnONPs synthesized from 5 ml and the pure. Moreover, at the 150 mg/ml concentration, the antimicrobial activity ranged from 12 to 19 mm; the highest antimicrobial activity was at the ZnONPs synthesized from 25 ml of *N. sativa* extract, whereas the lowest was at the pure ZnONPs. These results were consistent with those mentioned by [29], where they noted the effect of ZnONPs synthesized by the green method as an antibacterial against *S. aureus* with an inhibition zone of 18 mm obtained from the 75 concentration of ZnONPs synthesized using 5 ml of *N. sativa*. Also, the results were consistent with those mentioned by [30], where the effect of ZnONPs synthesized by the green method as an antibacterial against *S. aureus* with an inhibition zone of 16 mm obtained from the 25 concentration of ZnONPs synthesized using 5 ml of *Punica granatum*. The process of ZnONP formation is a result of a reaction between the zinc ions in the solution and the polyphenols, like flavonoids, glycosides, and tannins in the *N. sativa* extract. This reaction forms complexation, followed by hydrolysis, which leads to the creation of zinc hydroxide because of the occurrence of hydroxyl groups in the polyphenols. [2]. Also, the results were consistent with [31]. ZnONPs synthesized from plant

extracts were found to have similar significant *S. aureus* bacteria inhibition activity, with zone of inhibition 20 mm that was obtained from the concentration 50 mg/l ZnONPs synthesized using 10 ml. Also, this is similar to the results reported by [32], who found inhibition activity with an inhibition zone of 21 mm obtained from ZnONPs synthesized using 10 ml of *N. sativa* and inhibition activity with an inhibition zone of 14 mm obtained from ZnONPs synthesized using 7 ml of *N. sativa*. ZnONPs cause biochemical changes in the cell wall, cell physiology, and cell metabolism [25]. It happens when the biocompatible, synthesized ZnONPs element interacts with the microbial cell surface. According to [33], The attachment of ZnONPs to bacterial surfaces causes the release of Zn^{2+} ions that disrupt active transport inhibition and amino acid metabolism, resulting in severe harm to the enzyme system. The size and solubility of the Zn^{2+} ions utilized in the culture contribute to this characteristic [34]. The generation of Reactive oxygen species (ROS), strongly dependent on photocatalytic generation and exposure of UV, increased ZnO nanoparticles' antibacterial activity and surface [35, 36]. Nanoparticles can cross living membranes and enter cells, causing increased oxidative stress, cytokine production, and cell death. it may also be because zinc works to form hydrogen peroxide as it is a negatively charged hydroxyl radical, which can penetrate the bacterial cell membrane directly, causing injuries and preventing cell growth, thus leading to bacterial elimination. However, it causes damage to carbohydrates, nucleic acids, and lipids [37, 38].

Table 1. The inhibition zone of the pure ZnONPs and ZnONPs synthesized by green method on *S. aureus*

ZnONPs	**Zone of Inhibition (mm) of concentration ZnONPs (mg/ml)**				
	10	**25**	**50**	**100**	**150**
Zn pure	12	12	11	13	12
5 ml*	11	9	12	13	14
10 ml*	12	12	14	14	13
25 ml*	12	12	15	15	19

* = *N. sativa* seeds extract added during the preparation of ZnONPs

For *S. pyogenes*, the ZnONPs produced by plant extract from the seeds of *N. sativa* was effective against *S. pyogenes*. The synthesized ZnONPs demonstrated activity against *S. pyogenes* with zone of inhibition of 19 mm obtained from the concentrations (150 mg/ml) of ZnONPs synthesized using 25 ml of *N. sativa*. In comparison, the lowest inhibition with an inhibition zone of 10 mm was recorded at the concentration of 25 mg/ml of ZnONPs synthesized using 10 mL of *N. sativa* extract. The antibacterial activity ranged from 11 to 13 mm at a concentration of 10 mg/ml; the ZnONPs made from 25 ml of *N. sativa* extract had the maximum antimicrobial activity, while the pure and 5 ml exhibited the lowest antimicrobial activity. The antibacterial activity varied from 10 to 14 mm at a concentration of 25 mg/ml; the pure and the 10 mg/ml ZnONPs had the lowest antimicrobial activity, and the 5 mg/ml *N. sativa* extract-derived ZnONPs had the greatest. The antibacterial activity at 50 mg/ml varied from 11 to 15 mm; the ZnONPs synthesized by 25 ml of *N. sativa* extract had the maximum antimicrobial activity, whereas the ZnONPs created by 5 ml of *N. sativa* extract had the lowest. The antibacterial activity was measured at concentrations of 100 mg/ml and varied from 12 to 16 mm; the ZnONPs synthesized using 25 ml of *N. sativa* extract had the greatest antimicrobial activity, whilst those made with 10 ml of *N. sativa* extract had the lowest. The antibacterial activity varied from 12 to 19 mm at a concentration of 150 mg/ml; the ZnONPs produced by 25 ml of *N. sativa* extract had the maximum antimicrobial activity, whereas pure had the lowest. These results were in line with those indicated in [19], that investigated the antibacterial activity of ZnONPs produced using the green technique against *S. pyogenes*, with an inhibition zone of 16 mm achieved from the concentration of 100 ZnONPs produced using 20 ml of *N. sativa*. The results likewise coordinated those reported in [39], where they noted the antibacterial activity of ZnONPs produced using the green method against *S. pyogenes*, with an inhibition zone of 15 mm obtained from the 50 concentration of ZnONPs produced using 10 ml of *N. sativa*. The results also agreed with those mentioned by [30], who underlined the antibacterial activity of ZnONPs produced using the green method against *S. pyogenes*, with an inhibition zone of 14 mm from a 25 concentration of ZnONPs produced using 5 ml of *Punica granatum*. The results were consistent with those reported by [40], who demonstrated that ZnONPs produced using 20 ml of *N. sativa* have an inhibitory effect on a variety of bacteria, including *S. aureus* and *S. pyogenes* with zones of inhibition19 mm, 16 mm, 15 mm, and 13 mm, respectively. ZnONPs generally operate in three ways [1, 2, 41]; firstly, due to the integrity of the plasma membrane, which forms difficulty in material movement and gaseous exchange, nanoparticles can disrupt normal bacteria function by inducing chemical alterations in the plasma membrane. Secondly, free radicals on ZnONPs' surface finally lead to oxidative stress after penetrating the cells. Thirdly, the H_2O_2 and free radical OH-groups that ZnONPs form in aqueous circumstances are extremely poisonous and have a limited lifespan; they immediately interact with the DNA and enzymes of the cell, halting the function of all metabolic machinery and causing cell death.

Table 2. The inhibition zone of the pure ZnONPs and ZnONPs synthesized by green method on *S. pyogenes*

ZnONPs	Zone of Inhibition (mm) of concentration ZnONPs (mg/ml)				
	10	**25**	**50**	**100**	**150**
Zn	11	10	12	13	12
5ml*	11	14	11	13	14
10ml*	12	10	13	12	13
25ml*	13	12	15	16	19

K. pneumoniae is concerned as shown in Table 3, the *N. sativa* seed extracts' ZnONPs had an impact on the *K. pneumoniae* bacteria. The produced ZnONPs exhibited antimicrobial action against *K. pneumoniae*, with an inhibitory zone of 14 mm measured at concentrations of 100 mg/ml and 150 mg/ml, respectively. The ZnONPs concentration of 10 mg/ml was produced by 10 ml of *N. sativa* extract, and the pure resulted in the lowest inhibition, with an inhibition zone of 8 mm, utilizing 25 ml of *N. sativa*. The antibacterial activity was 8–10 mm at 10 mg/ml; the ZnONPs synthesized from 25 ml of *N. sativa* extract had the maximum antimicrobial activity, whereas the pure and ten ml-synthesized ZnONPs had the lowest antimicrobial activity. At a concentration of 25 mg/ml, the antimicrobial activity was between 10 and 13 mm; the ZnONPs made from 25 ml of *N. sativa* extract had the maximum antibacterial activity, whereas those made from 10 ml of *N. sativa* extract had the lowest. The antibacterial activity at 50 mg/ml varied from 10 to 13 mm; the ZnONPs synthesized using 25 ml of N. sativa extract had the greatest antimicrobial activity, while the ZnONPs made with 10 ml of *N. sativa* extract had the lowest. The antibacterial activity at a concentration of 100 mg/ml varied from 10 to 14 mm; the ZnONPs synthesized from 25 ml of *N. sativa* extract had the maximum antimicrobial activity, whereas those made from 5 ml and 10 ml of *N. sativa* extract had the lowest antimicrobial activity. The antimicrobial activity varied from 11 to 14 mm at the 150 mg/ml concentration; the ZnONPs produced by 25 ml of *N. sativa* extract had the maximum antibacterial activity, whereas the ZnONPs produced by 10 ml of *N. sativa* extract had the lowest. These results were in agreement with those mentioned in [35], where he described the antibacterial activity of ZnONPs produced using the green method against *K. pneumoniae*, which had an inhibition zone of 15 mm and was obtained from a concentration of 100 ZnONPs produced using 20 ml of *N. sativa*. On *K. pneumoniae*, antibacterial activity was evaluated; the results are listed in Table 4.4. According to [42], the inhibition zones of 17 mm from the concentration 100 of ZnONPs were obtained from 20 ml of *N. sativa*, and an inhibition zone of 13 mm from the concentration 50 of ZnONPs was obtained from 10 ml of *N. sativa*, ZnONPs synthesized by the green method demonstrated a progressive increase in the inhibition of bacterial growth. Additionally, the results were in line with those reported by [20], who noted the antibacterial activity of ZnONPs produced using the green method against *K. pneumoniae*, with an inhibition zone of 12 mm obtained from the 50-fold concentration of ZnONPs produced using 25 ml of *N. sativa*. The results were consistent with those recorded by [30], who highlighted the antibacterial activity of ZnONPs produced using the green technique against *K. pneumoniae*. They noted an inhibitory zone of 11 mm from a 25-fold concentration of ZnONPs produced using 5 ml of *Punica granatum*. Nanoparticles break down the bacterial cell wall, allowing water to enter and destroy the germs. It is also possible that zinc oxide is the cause of cell death, membrane rupturing, and cell shrinkage [8]. [19] observed the impact of zinc oxide on *K. pneumoniae* bacteria with an inhibitory zone of 12 mm, which clarified why the impact of zinc oxide on the bacterial cell membrane and genetic material, as well as the effect of zinc oxide on both.

Table 3. The inhibition zone of the pure ZnONPs and ZnONPs synthesized by green method on *K. pneumoniae*

ZnONPs	Zone of Inhibition (mm) of concentration ZnONPs (mg/ml)				
	10	25	50	100	150
Zn	8	12	12	13	13
5ml*	9	11	11	10	12
10ml*	8	10	10	10	11
25ml*	10	13	13	14	14

The results for *P. aeruginosa* showed that ZnONPs synthesized using the green approach had a low impact on *P. aeruginosa* compared to the other bacteria. The synthesized ZnONPs showed activity against *P. aeruginosa* with an inhibition zone of 12 mm obtained from the concentration of 150 mg/ml of ZnONPs synthesized using 25 ml of *N. sativa*. In contrast, the lowest inhibition with an inhibition zone of 3 mm was recorded at the concentration of 25 mg/ml of ZnONPs synthesized using 5 ml of *N. sativa* extract as in table 4. The results were disagreed with the findings of [43]. The sort of structure of bacteria and its modification due to its wide spreading may be the cause. Such behaviour is brought on by various bacteria's different cell wall structures (Kumar et al., 2017). The strength and form of bacterial cells are controlled by a porous peptidoglycan layer found in their cell walls. This layer is thicker in gram-positive bacteria than in gram-negative bacteria. Due to their supremacy over gram-negative particles, these particles have a harder time becoming harmful [44].

Table 4. The inhibition zone of the pure ZnONPs and ZnONPs synthesized by green method on *P. aeruginosa*

ZnONPs	**Zone of Inhibition (mm) of concentration ZnONPs (mg/ml)**				
	10	**25**	**50**	**100**	**150**
Zn	5	6	9	9	11
5ml*	3	5	7	7	8
10ml*	4	6	7	9	11
25ml*	8	8	10	11	12

6. Conclusion

In conclusion, the study aimed at determining the antibacterial potency of zinc oxide nanoparticles (ZnONPs) synthesized using Nigella sativa seeds extract. The disc diffusion method was used to test the activity of ZnONPs against both Gram-positive (S. aureus and S. pyogenes) and Gram-negative (K. pneumoniae and P. aeruginosa) microorganisms. X-ray diffraction was used to examine the crystal structure, particle size, and lattice parameter of ZnONPs, and the results indicated that ZnONPs synthesized by the co-precipitation method had a crystalline hexagonal structure. The average crystallite size was calculated to be 15.47 nm. The results showed that the ZnONPs had antimicrobial activity against S. aureus, with the highest activity recorded at a concentration of 150 mg/ml with an inhibition zone of 19 mm and the lowest activity at a concentration of 25 mg/ml with an inhibition zone of 9 mm. The activity was found to be consistent with previous studies, which also reported significant antimicrobial activity against S. aureus. The mechanism of action of ZnONPs is thought to involve the interaction of ZnONPs with the microbial cell surface, which leads to the release of Zn2+ ions that cause severe harm to the enzyme system, disrupt active transport inhibition and amino acid metabolism, and generate reactive oxygen species. The results of this study highlight the potential of ZnONPs synthesized using N. sativa seeds extract as a natural and effective antibacterial agent.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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Not applicable

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