Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Brassica oleracea L. (Acephala Group) based zinc oxide nanoparticles and their efficacy as antibacterial agent

Nanopartículas de óxido de zinco à base de *Brassica oleracea* L. (Acephala Group) e sua eficácia como agente antibacteriano

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Abstract

Zinc oxide nanoparticles were synthesized from the leaf extract of *Brassica oleracea L.* Acephala group (collard green) followed by their characterization using Scanning Electron Microscope (SEM), and Energy Dispersive X-ray (EDX). The antibacterial properties of zinc nanoparticles were tested against Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC ® 9027™), *Escherichia coli* (ATCC ® 8739™), *Klebsiella pneumoniae* (ATCC® BAA-1705™) and Gram-positive bacteria, *Staphylococcus aureus* (ATCC ® 6538™) and *Listeria monocytogenes* (ATCC ® 13932™), at four different concentrations (50.00 µg/ml, 100.00 µg/ml, 500.00 µg/ml and 1 mg/ml) of zinc oxide nanoparticles suspension. Results revealed that the synthesized nanoparticles exhibit strong antibacterial effects against *Pseudomonas aeruginosa*, *Listeria monocytogenes, Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli* at 500.00 µg/ml-1 mg/ml concentrations. An increase in efficacy of nanoparticles with the decrease of their size was also evident. This is a first ever report on *Brassica oleracea*, L. based nanoparticles which demonstrates that 500.00 μg^{-1} mg/ml conc. of zinc oxide nanoparticles have antibacterial activity against both Gram -ve and Gram +ve bacteria and have the potential to be considered as an antibacterial agent in future.

Keywords: *Brassica oleracea*, zinc oxide nanoparticles, Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX), antibacterial agents.

Resumo

Nanopartículas de óxido de zinco foram sintetizadas a partir do extrato foliar de *Brassica oleracea* L., grupo Acephala (couve), seguidas de sua caracterização em Microscópio Eletrônico de Varredura (MEV) e Raio X por Energia Dispersiva (EDX). As propriedades antibacterianas das nanopartículas de zinco foram testadas em bactérias Gram-negativas, *Pseudomonas aeruginosa* (ATCC® 9027™), *Escherichia coli* (ATCC® 8739™) e *Klebsiella pneumoniae* (ATCC® BAA-1705™), e bactérias Gram-positivas, *Staphylococcus aureus* (ATCC® 6538™) e *Listeria monocytogenes* (ATCC® 13932™), em quatro concentrações diferentes (50,00 µg / ml; 100,00 µg / ml; 500,00 µg / ml; e 1 mg / ml) de suspensão de nanopartículas de óxido de zinco. Os resultados revelaram que as nanopartículas sintetizadas exibem fortes efeitos antibacterianos contra *P. aeruginosa*, *L. monocytogenes*, *K. pneumonia*, *S. aureus* e *E. coli* em concentrações de 500,00 µg / ml^{-1} mg / ml. Um aumento na eficácia das nanopartículas com a diminuição de seu tamanho também foi evidente. Este é o primeiro relatório sobre nanopartículas à base de *B. oleracea* L. que demonstra que 500,00 µg-1 mg / ml de concentração de nanopartículas de óxido de zinco têm atividade antibacteriana contra bactérias Gram-negativas e Gram-positivas e que essas nanopartículas têm potencial para ser consideradas um agente antibacteriano no futuro.

Palavras-chave: *Brassica oleracea*, nanopartículas de óxido de zinco, Microscópio Eletrônico de Varredura (MEV), Raios-X por Energia Dispersiva (EDX), agentes antibacterianos.

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Received: December 18, 2021 – Accepted: May 26, 2022

1. Introduction

Due to the misuse of antibiotics, the emergence of microorganism's resistance is increasing rapidly against majority of drugs (Asai et al., 2005; Olofsson, 2006; Rossainz-Castro et al., 2016) which poses serious threat to public health. Hence the pharmaceutical companies and the researchers have focused on the introduction of alternative biocidal agents to fight and prevent microbial diseases. The use of nanostructured materials as a successful bactericidal agent is one of the available alternatives which is attracting the attention of pharmaceutical industry (Jan et al., 2013; Teodoro et al., 2018; Thill et al., 2006). As a result, in recent years, nanotechnology has emerged as an innovative field which manipulates materials on atomic scale and introduce new and unique antimicrobials to control the growth of harmful microorganisms (Pelgrift and Friedman, 2013; Aldosary et al., 2023).

A number of nanotechnology-based materials have been studied to control infectious diseases (Blecher et al., 2011; Tarusha et al., 2018; Budi et al., 2024). Among these, zinc oxide nanoparticles have obtained a significant importance due to their unique antifungal, antibacterial and UV filtering properties (Hong et al., 2008). Zinc oxide is a member of metal oxide group having photo catalytic and photo oxidizing capability against several chemical and biological species (Szabó et al., 2003). When zinc oxide nanoparticles are used as a surface coating on materials and textiles, they exhibit remarkable antibacterial and antifungal properties (Abramov et al., 2009). The antimicrobial activity of nanoparticles has been studied against pathogenic bacteria of humans, mainly *S. aureus* and *E. coli* (Akbar et al., 2017; Heinlaan et al., 2008; Jones et al., 2008). In addition, due to their antimicrobial effectiveness, the zinc oxide nanoparticles are being considered as potential agents to control the growth of pathogens in food processing atmosphere (Jin et al., 2009; Suo et al., 2017). The nanoparticles of zinc oxide possess bactericidal effect on both Gram-negative and Gram-positive bacteria and even show activity against spores that are resistant to high pressure and temperature (Rosi and Mirkin, 2005). It is evident from the literature that the effectiveness of zinc oxide nanoparticles depends on the concentration and surface area while there is a little effect of the particle shape and crystalline structure (Suntako, 2015). Furthermore, it has been revealed that smaller the size of zinc oxide nanoparticles, better the antibacterial activity (Shrivastava et al., 2007; Vayssieres et al., 2001). Hence, with increasing the concentration and surface area of the nanoparticles, the antibacterial activity is enhanced.

A variety of methods are available to synthesize the nanoparticles for their subsequent use in the antibacterial studies (Naseer et al., 2022). These methods include the synthesis of nanoparticles by employing the physical, chemical and biological treatments. However, due to some serious disadvantages associated with physical and chemical methods i.e., use of toxic chemicals and high costs but low production rate, the scientific society has turned to biological systems for synthesis and assembly of nanoparticles (Konishi et al., 2006). Plant mediated biological method of the nanoparticles connects the plant biotechnology to nanotechnology by using plant extracts (Akbar et al., 2017; Ahmad and Sharma, 2012). Such methods are cost effective as plants are nature's chemical factories and need a little or no maintenance. Therefore, the use of biological sources like plants is constantly on the rise for the synthesis of metal nanoparticles and can be preferred over the physical and chemical methods (Rajamanickam et al., 2012).

Hence, in the present study, *Brassica oleracea* was utilized for the synthesis of zinc oxide nanoparticles. The plant is traditionally used for treatment of diarrhoea, retention of urine and healing of injuries and reported to contain vitamins A, B1, C & K and other essential metabolites which may act as bioreducing agents (Kahlon et al., 2008; Miller-Cebert et al., 2009; Uddin et al., 2004; Balbach, 1993). This richness of metabolites and easy availability of plant makes it a suitable candidate to synthesize the zinc oxide nanoparticles, which was investigated in this study. Further, the effect of changing precursor concentrations (zinc salt and plant extract) and influence of morphology and size of synthesized nanoparticles on the antibacterial activity was also investigated.

2. Materials and Methods

Brassica oleracea L. Acephala group (Collard green) leaves were used in this study. All chemical reagents such as $ZnSO_4 .7H_2O$, NaOH, HCl, ethyl acetate and acetone of analytical grade were from MERCK. All glass wares (Titration flasks, Beakers, Funnels, Petri dishes and Test tubes) were of Pyrex grade and were sterilized properly.

2.1. Bacterial cultures

Five ATCC bacterial strains including *P. aeruginosa* (ATCC ® 9027™), *E. coli* (ATCC ® 8739™), *L. monocytogenes* (ATCC ® 13932™), *S. aureus* (ATCC ® 6538™) and *K. pneumoniae* (ATCC® BAA-1705™) were used to test antibacterial activity of the synthesized ZnO nanoparticles.

2.2. Preparation of the leaves extract

The fresh leaves were first washed several times with tap water in order to remove the dust particles and then with the distilled and deionized water, respectively. The leaves were then cut into very small pieces and 50.00 g of chopped leaves were taken in a clean and sterilized conical flask containing 250.00 ml of deionized water and mixed properly. The mixture was then boiled at 100 ºC for 30-45 min using a hot plate. The extract formed was cooled to room temperature and filtered using Whatman filter paper. After filtration, centrifugation was done for 30 min at 4,000 rpm in order to get a concentrated extract. The extract was stored in a refrigerator in order to use for further experiments.

2.3. Synthesis of zinc oxide nanoparticles from Brassica oleracea (collard green) leaf extract

Eco-friendly procedure was employed for the synthesis of nanoparticles using Zinc sulphate ($ZnSO_4 .7H_2O$) as a precursor salt. Three solutions of Zinc sulphate (1 mM,

2 mM and 3 mM conc.) were prepared. First of all, 10 ml leaf extract of *Brassica oleracea* was added into all three concentrations. The process was repeated by adding 20 ml of extract into another set of these concentrations. Few drops of sodium hydroxide (NaOH) solution were added into each treatment in order to increase the pH up to 10. The solution was kept under constant stirring at 50ºC using magnetic stirrer for one hour. The green color of the solutions changed to pale yellow indicating the formation of the zinc oxide nanoparticles. After completion of reaction, each treatment was kept overnight. Centrifugation of the white precipitate was done at 4,500 rpm for 15 min. The pellet was taken and washed thoroughly with double distilled water in order to remove all the ions and by-products. The obtained nanoparticles were then dried in a hot air oven at 120 ºC for 2 hours. The resulting dried material was crushed into powder and stored in airtight Eppendorf tubes for further analysis.

2.4. Characterization of zinc oxide nanoparticles

The morphology, particle size and elemental composition of the obtained Zinc oxide were determined by Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) using the facility provided by CRL, Physics Department of Peshawar University according to the standard protocol. The micrographs of the samples were obtained in SEM model JSM 5910 (JEOL Co Japan) and the elemental percentages were determined in micro analyzer model INCA 200 (UK) coupled with SEM machine.

2.5. Antibacterial activity of zinc oxide nanoparticles

The antibacterial activity of isolated zinc-based nanoparticles was tested on Nutrient agar plates using well diffusion method against different bacterial strains mentioned above. Once the optical density was adjusted (1×10^8 CFU/ml), the pathogenic bacteria were cultured on nutrient agar plates using sterile cotton swabs.

Stock suspensions of ZnO with different concentrations (50.00 µg/ml, 100.00 µg/ml, 500.00 µg/ml and 1 mg/ml) of the nanoparticles were prepared by suspending them into distilled water. Wells of 2 mm diameter were created in the media with the help of sterilized cork-borer. Different concentrations (50.00 µg/ml, 100.00 µg/ml, 500.00 µg/ml and 1 mg/ml) of the nanoparticles were loaded on the marked wells with the help of micropipette under aseptic conditions. Antibiotic Meropenem (Me) was used as a positive control for *E. coli* while Doxycycline (DO) was used as control for *P. aeruginosa*, *L. monocytogenes, S. aureus* and *K. pneumoniae*. Distilled water was loaded as negative control. Bacteria were incubated on their respective media plates at 37 ºC for 24 hours. Diameter of zone of inhibition was measured in millimeter (mm) after incubation to assess the antibacterial activity.

3. Results

3.1. Energy Dispersive X-Ray (EDX)

To confirm the synthesis and purity of zinc oxide nanoparticles, EDX was performed. Samples were processed for Zinc (Zn) and Oxygen (O) spectrum randomly with EDX. Clear peaks of Zn and O were obtained along with very small peaks of Calcium (Ca) and Phosphorous (P) as impurities which were in acceptable range (Figure 1; Table 1).

Table 1. Elemental composition of ZnO nanoparticles.

Element	Weight (%)	Atomic (%)
C K	4.50	13.27
O K	20.44	45.27
P K	0.72	0.83
Ca K	0.98	0.87
Zn K	73.35	39.76
Totals	100.00	

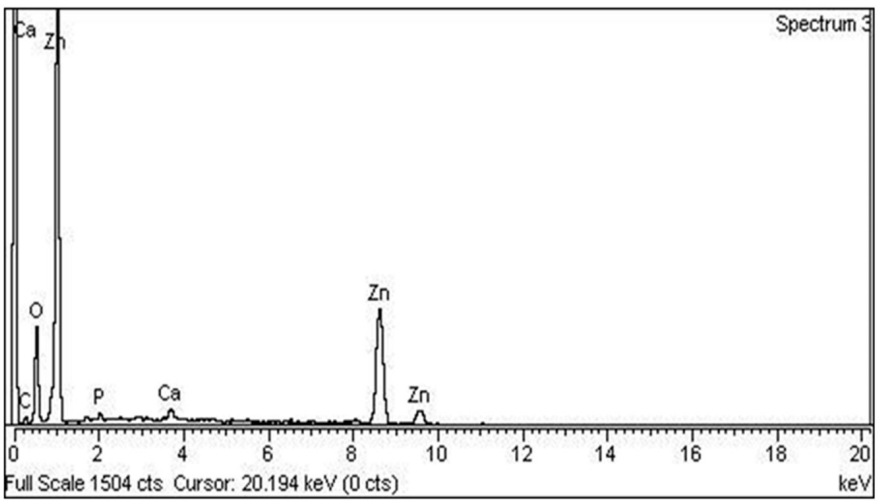

Figure 1. EDX spectra of zinc oxide nanoparticles.

3.1.1. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was performed for the analysis of effect of precursor concentrations (zinc salt and plant extract) for all six samples prepared from different concentrations. The obtained SEM results clearly revealed the influence of precursor salt ($ZnSO_4$) concentration on the size and morphology of zinc oxide nanoparticles. The size of nanoparticles changed as the concentration of $ZnSO_4$ increased. The SEM results for each sample are described below:

The first treatment for the synthesis of zinc nanoparticles was performed by mixing 90.00 ml of zinc sulphate solution (1 mM $ZnSO_4.7H_2O$) with 10 ml of *Brassica oleracea* extract. The average size of nanoparticles ranges from 10-50 nm with 0.50-01.00 µm agglomerates was recorded (Figure 2A). With increase in $ZnSO_4$ from 1 mM to 2 mM, the size of nanoparticles increased with a range 50-90 nm and 0.50-03.00 µm agglomerates (Figure 2B). Similarly, with increasing the concentration of $ZnSO_4$ solution up to 3 mM, large sized agglomerated nanoparticles were noticed having size range of 90-120 nm and 0.5-5 µm agglomerates (Figure 2C).

To assess the effect of increasing concentration of plant extract, a set of three treatments was also set up. For first treatment, 80 ml of $ZnSO_4$ solution (1 mM $ZnSO_4.7H_2O$) was mixed with 20 ml of *Brassica oleracea* leaves extract. The average size of nanoparticles of range from 50-100 nm with 0.5-2 µm agglomerates was observed (Figure 2D). Similarly, for other two treatments (80 ml of 2 mM and 3 mM zinc salt in 20 ml of plant extract separately), an average size of nanoparticles ranges from 80-120 nm with 0.2-9 µm agglomerates and an average size of nanoparticles ranges from 100.00-300.00 nm with 0.2-5 µm agglomerates were obtained for the samples, respectively (Figure 2E-2F)

3.2. Antibacterial activity of zinc oxide nanoparticles

The antibacterial activity of all six samples of ZnO nanoparticles was tested by well diffusion method against *Pseudomonas aeruginosa, Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (Figure 3A-3E). The test was performed in triplicates for each concentration. The diameter of zone of inhibition for each strain was calculated and recorded for each treatment in triplicate (Table 2). The results revealed that the presence and penetration of nanoparticles restricted the growth of tested bacteria and a clear zone, except for negative control, around the wells was evident. With an increase in the concentration of the nanoparticles, the zone of clearance also increased. Both positive and negative controls were also set up which validated the assay and the subsequently generated results.

3.3. Analysis of antibacterial activity against Pseudomonas aeruginosa

It is evident from results that ZnO nanoparticles inhibited the growth of *Pseudomonas aeruginosa* (Figure 3A) even at lowest concentration (50.00 µg/ml). The highest bactericidal activity was observed at the concentration of 1 mg/ml when it reached approximately equal to the positive control (Table 2). A slight variation between different

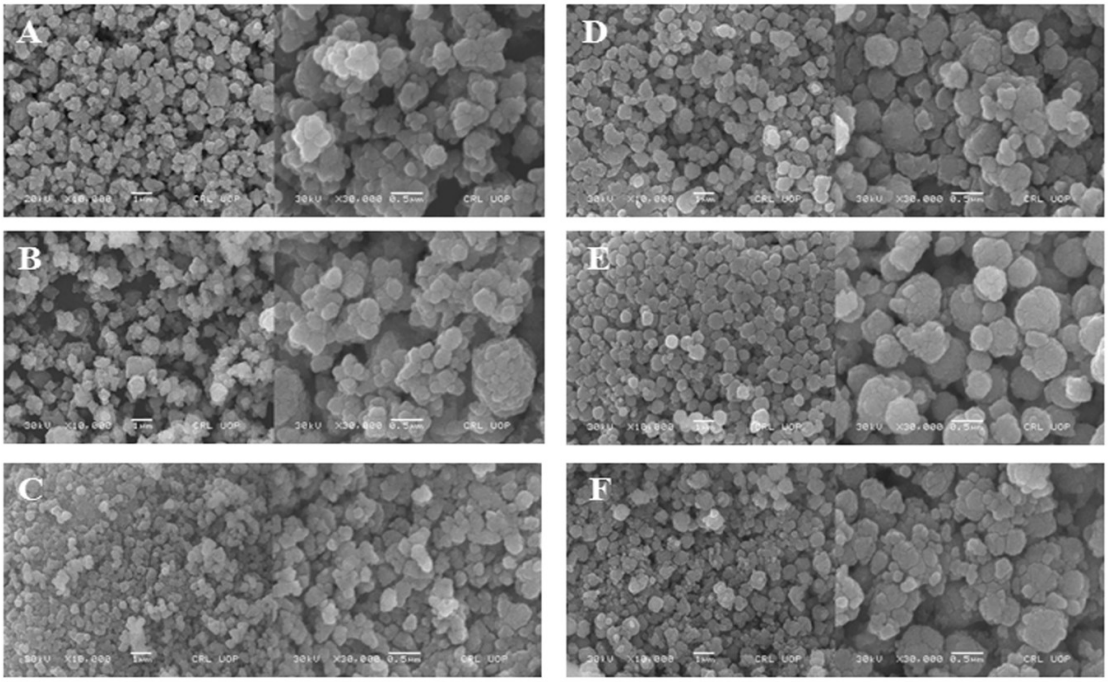

Figure 2. A. The SEM images of biosynthesized ZnO NPs (10-50 nm), B. SEM images of zinc oxide nanoparticles with little agglomeration C. Scanning electron microscopy images of zinc oxide nanoparticles for sample 3. D. SEM micrographs of zinc nanoparticles having size 50-100 nm. E. Micrographs of zinc oxide nanoparticles for sample 5. F. SEM images of ZnO nanoparticles (ZnO NPs in this case appears to be greater as compared to Figure 2E).

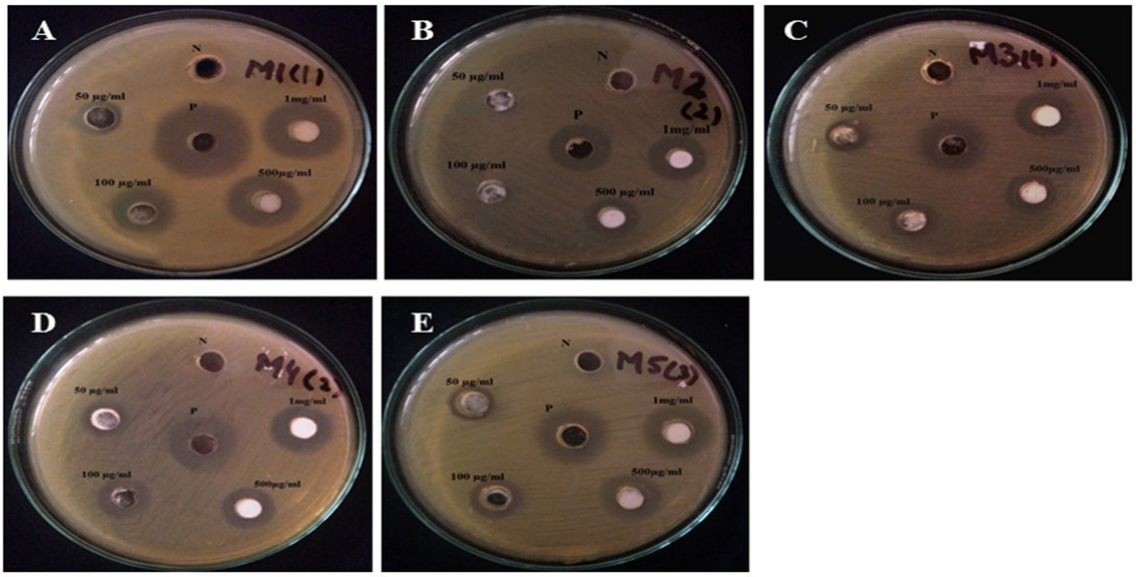

Figure 3. A. Zone of inhibition of *Pseudomonas aeruginosa* formed after treating with ZnO nanoparticles obtained from mixing 10 ml of plant extract with 1 mM $ZnSO_4$ solution. B. Zone of inhibition of *Escherichia coli* by using 2 mM solution of ZnO for 10 ml extract. C. Zone of inhibition of *Listeria monocytogenes* by using 1mM solution of ZnO for 20 ml extract. D. Zone of inhibition of *Staphylococcus aureus* by using 2 mM solution of ZnO for 10 ml plant extract. E. Zone of inhibition of *Klebsiella pneumoniae* by using 3 mM solution of ZnO for 10 ml extract 3.

molar concentrations and the amount of extract was also observed. For 10 ml extract, the zones of inhibition were either increased or remained constant with an increase in molarity of solution (1 mM-3 mM). This trend was observed for all nanoparticles concentrations (50.00 µg/ml-1 mg/ml). However, surprisingly with the increase of plant extracts (20.00 ml) for some molar solutions, the inhibitory effect was reduced with the increase in molarity. The least bactericidal effect was observed with nanoparticles obtained using 3 mM solution of Zinc sulphate. Thus, our results demonstrated that nanoparticles have strong potential to inhibit the growth of *P. aeruginosa*.

3.4. Analysis of antibacterial activity against Escherichia coli

The antibacterial efficacy of ZnO nanoparticles against *Escherichia coli* (Figure 3B) was checked using same technique and method as described in the previous section. The efficacy of nanoparticles was checked for all concentrations and zone of inhibition were recorded (Table 2). As the data indicates, ZnO nanoparticles did not show any inhibitory effect against *Escherichia coli* at lower concentrations (50.00 µg/ml and 100.00 µg/ml). The result remained same for all molar concentration when applied at higher dilutions (50.00 µg/ml and 100.00 µg/ml). An intermediate antibacterial activity was observed at 500.00 µg/ml conc. of ZnO nanoparticles which reached equivalent to positive control when a concentrated solution (1 mg/ml) of nanoparticles was applied. Surprisingly, the efficacy was reduced by increasing molar concentration of solution used for nanoparticles preparation (Table 2 top to bottom of column).

3.5. Analysis of antibacterial activity against Listeria monocytogenes

Listeria monocytogenes was also tested for the analysis of its growth inhibition by ZnO nanoparticles (Figure 3C). The antibacterial effect of ZnO nanoparticles having 1mg/ml concentration showed maximum zone of inhibition and was close to the inhibitory effect shown by positive control (Table 2). A mild to low efficacy was observed by decreasing the concentration of nanoparticles (50.00 µg/ml, 100.00 µg/ml and 500.00 µg/ml). However, in contrast to previous both results, the efficacy of nanoparticles increased with increasing the concentration of molarity of solutions.

3.6. Analysis of antibacterial activity against Staphylococcus aureus

Staphylococcus aureus is another medically important pathogen used in this study to test its ability of growth in the presence of ZnO nanoparticles (Figure 3D). The growth inhibition started at 100.00 µg/ml dilution of nanoparticles and increased with an increase in concentration (Table 2). The efficacy was just above 50% of positive control at 500.00 µg/ml concentration. However, it reached at highest level (equal to positive control) when 1 mg/ml concentration of nanoparticles was used.

3.7. Analysis of antibacterial activity against Klebsiella pneumonia

Finally, the antibacterial effect on ZnO nanoparticles was also observed against *Klebsiella pneumonia* (Figure 3E). At lower concentration of nanoparticles (50.00 µg/ml), a very low activity against tested bacteria was observed (Table 2). However, the activity disappeared by doubling

Table 2. Diameter (in mm) of zone of inhibition of selected bacteria after treatment at different concentrations of ZnO nanoparticles and molar solutions of Zinc sulphate.

S. No.	Molar Solutions	Positive control	50 μg/ml	100 μg/ml	500 μg/ml	1mg/ml
			Pseudomonas aeruginosa			
1	1mM (10ml extract)	20mm	3mm	8mm	13mm	18mm
2	2mM (10ml extract)	20mm	4mm	9mm	13mm	17mm
3	3mM (10ml extract)	20mm	4mm	9mm	15mm	18mm
4	1mM (20ml extract)	20mm	4mm	12mm	15mm	18mm
5	2mM (20ml extract)	20mm	3mm	11mm	15mm	18mm
6	3mM (20ml extract)	20mm	2mm	5mm	9mm	13mm
			Escherichia coli			
1	1mM (10ml extract)	12mm	0mm	4mm	7mm	10mm
2	2mM (10ml extract)	12mm	0mm	0mm	6mm	9mm
3	3mM (10ml extract)	12mm	0mm	0mm	2mm	5mm
4	1mM (20ml extract)	12mm	0mm	0mm	6mm	10mm
5	2mM (20ml extract)	12mm	0mm	0mm	4mm	8mm
6	3mM (20ml extract)	12mm	0mm	0mm	4mm	7mm
			Listeria monocytogenes			
1	1mM (10ml extract)	15mm	0mm	3mm	7mm	11mm
2	2mM (10ml extract)	15mm	0mm	4mm	7mm	12mm
3	3mM (10ml extract)	15mm	4mm	9mm	15mm	18mm
4	1mM (20ml extract)	15mm	2mm	4mm	8mm	13mm
5	2mM (20ml extract)	15mm	2mm	2mm	6mm	8mm
6	3mM (20ml extract)	15mm	0mm	4mm	7mm	10mm
			Staphylococcus aureus			
1	1mM (10ml extract)	11mm	0mm	3mm	7mm	9mm
2	2mM (10ml extract)	11mm	0mm	6mm	8mm	11mm
3	3mM (10ml extract)	11mm	0mm	0mm	4mm	11mm
4	1mM (20ml extract)	11mm	0mm	2mm	6mm	9mm
5	2mM (20ml extract)	11mm	0mm	0mm	6mm	10mm
6	3mM (20ml extract)	11mm	0mm	0mm	5mm	9mm
			Klebsiella pneumoniae			
1	1mM (10ml extract)	13mm	2mm	4mm	6mm	13mm
2	2mM (10ml extract)	13mm	2mm	4mm	9mm	14mm
3	3mM (10ml extract)	13mm	2mm	4mm	10mm	14mm
4	1mM (20ml extract)	13mm	0mm	3mm	8mm	14mm
5	2mM (20ml extract)	3mm	0mm	5mm	7mm	11mm
6	3mM (20ml extract)	13mm	0mm	3mm	7mm	13mm

the plant extract for same molar solutions. The activity remained constant at 100.00 μg/ml for both 10.00 ml and 20.00 ml plant extracts used. The bactericidal effects were increased by increasing the concentration of nanoparticles and reached approximately equal to or even more than the activity of positive control at 1 mg/ml concentration. However, the bactericidal activity did not seem to be changed with a change in molar concentration of solution used for particle synthesis and the amount of plant extract.

4. Discussion

The emergence of bacterial resistance against the current battery of the antibiotics has raised serious concerns among

health professionals. The pathogens have developed a variety of mechanisms to degrade, modify or inactivate the routinely prescribed antibacterial drugs. This has resulted in the sporadic reports of presence of super-bugs. In current decade, scientists have focused on exploration of alternative remedies to address this issue. Use of crude extracts/phytochemicals from plants of medical importance such as *Brassica oleracea* (Paul et al., 2012; Vale et al., 2015), *Hibiscus sabdariffa*, *Beta vulgaris* (Abdel-Shafi et al., 2019) and many others have been extensively studied in past. However, due to some limitations, the scientists have recently focused on the synthesis of nanoparticles using the extracts of medicinally important plants. Hence, synthesis of metal nanoparticles using green methods having antibacterial potential has become an alternative remedy for pathogens control. Various studies have reported that metal nanoparticles strongly inhibit the growth of pathogens due to disruption of plasma membrane which eventually leads to death of bacterial cells (Aldayel et al., 2022; Shah et al., 2021).

Among metal nanoparticles of same nature, Zinc oxide nanoparticals are one of the most effective and safe antimicrobial agents which are being used as medicine as well as preservative in packaging since long (Baum et al., 2000). Other metal nanoparticles such as gold have also been reported (Piruthiviraj et al., 2016); however due to limited antibacterial activity or effective only against a particular group of pathogens i.e., enteric bacteria, gold nanoparticles are more preferred for diagnostic and anticancer procedures (Shamaila et al., 2016). Zinc oxide nanoparticles, on other hand, are preferred for antibacterial studies due to their safety and better efficacy.

The synthesis and antibacterial activity of *Brassica oleracea L.* (Acephala Group) based Zinc oxide nanoparticles is not well-documented so far in literature; though few previous studies have determined the ZnO nanoparticle's photocatalytic activity (Osuntokun et al., 2019) as well as antimicrobial potential (Pillai et al., 2020). However, these studies did not explore the impact of different molar concentrations on the synthesis and efficacy of nanoparticles. Therefore, our study further expanded the previous understanding and focussed on biosynthesis of zinc oxide nanoparticles synthesized from different molar solutions concentrations of Zinc sulphate using leaf extract of *Brassica oleracea L.* (Acephala Group) and determining its biological activity against more bacterial types than those investigated previously (Pillai et al., 2020). The characterization study revealed that most of the obtained Zinc nanoparticles were of spherical nature along with agglomerates, which may be due to the presence of biomaterials. Also, an increase in concentration of precursors resulted in an increase of the size of nanoparticles.

The antibacterial effects of the synthesized ZnO nanoparticles were investigated later. Four nano-ZnO suspensions with different concentrations were tested, in the range of 50.00 µg/ml, 100.00 µg/ml, 500.00 µg/ml and 1 mg/ml. Diameters of inhibitory zone around the well were high when higher concentrations of zinc oxide nanoparticles were used. These results are in good agreement with the previously published studies which evaluated the efficacy of ZnO nanoparticles synthesized from different sources (Kaviyarasu et al., 2017; Wahab et al., 2010). In addition to this, the smaller sized Zinc nanoparticles (10-50 nm) showed the best antibacterial activity due to the larger surface area available for interaction with bacteria as compared to that of large agglomerates (100-300 nm). Few comprehensive studies [19, 29, 30] have also revealed the similar results (Akbar et al., 2017; Zhang et al., 2007; Rekha et al., 2010).

Almost all tested microorganisms were completely inhibited at the concentration of 1 mg/ml and 500.00 µg/ml of nano-ZnO in the present study. The nano-ZnO solution at the concentration of 500.00 µg/ml showed inhibition zones against all test organisms but no noticeable antibacterial activity was found at concentrations lower than 500.00 µg/ml. On the other hand, for each type of bacteria tested, the size of zone of inhibition was different with the same concentrations of ZnO nanoparticles. Therefore, the antibacterial effect of zinc nanoparticles was found dose dependent and was more effective against Gram negative bacteria (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) than Gram positive (*Staphylococcus aureus* and *Listeria monocytogenes*) at the both concentrations (500.00 µg/ml-1 mg/ml). A similar study has also revealed that bactericidal efficiency is affected by the type of microorganism due to the different nature of their cell structure (Singh et al., 2008).

Good effect had also been showed by *Listeria monocytogenes.* It is also evident from literature that Zinc oxide nanoparticles have a good preventive effect upon the life of *Listeria monocytogenes* (Rezaei-Zarchi et al., 2010). Nanoparticles possess large surface area and also have more contact with bacteria. The lowest concentration of ZnO nanoparticles that inhibited the bacterial growth was against *Escherichia coli* and *Staphylococcus aureus* with 50 µg/ml and 100 µl/ml. This is also in the published reports that ZnO nanoparticles also inhibited the growth of *E. coli* and *S. aureus* with minimum concentrations (Daghdari et al., 2017; Reddy et al., 2007).

5. Conclusion

Zinc oxide nanoparticles synthesized from *Brassica oleracea L.* Acephala Group (collard leaves extract) have significant antibacterial efficacy. This antibacterial activity of zinc nanoparticles against Gram negative and Gram positive bacteria is anticipated to provide strong resistance against different bacterial infections. Therefore, we expect that the work presented in this paper will provide a strong base of nanotechnology in developing new strategies against pathogens and will help to reduce health problems faced by Pakistan and other countries.

Acknowledgements

This work was supported by the department of Microbiology, Hazara University Mansehra and the Higher Education Commission of Pakistan.

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