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GREEN-SYNTHESIZED ZINC OXIDE NANOPARTICLES FROM CYMBOPOGON CITRATUS EXHIBIT ENHANCED ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY AGAINST MULTIDRUG-RESISTANT ESCHERICHIA COLI ISOLATED FROM RETAIL FISH

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ABSTRACT

The increasing prevalence of multidrug-resistant (MDR) foodborne bacteria has created an urgent need for sustainable antimicrobial alternatives capable of overcoming conventional antibiotic resistance. The present study investigated the antibacterial and antibiofilm potential of green-synthesized zinc oxide nanoparticles (ZnO NPs) prepared using *Cymbopogon citratus* leaf extract against MDR bacterial isolates recovered from retail fish. Bacterial isolates were obtained using standard microbiological methods and screened for antibiotic susceptibility to identify the most resistant isolate. Among the recovered bacteria, *Escherichia coli* exhibited the highest level of multidrug resistance and was selected for subsequent investigations. Comparative evaluation of six medicinal plant extracts identified *C. citratus* as the most active antibacterial candidate and was therefore employed for nanoparticle synthesis. The synthesized ZnO nanoparticles were characterized by UV–Visible spectroscopy and evaluated for antibacterial efficacy using agar well diffusion, minimum inhibitory concentration (MIC), growth kinetics, biofilm eradication, biofilm inhibition, MTT cell viability assay, and SDS–PAGE protein profiling. ZnO nanoparticles exhibited markedly greater antibacterial activity than the crude methanolic extract, with the DMSO formulation producing the highest inhibitory activity. Nanoparticle treatment significantly suppressed bacterial growth, reduced biofilm formation, disrupted established biofilms, and decreased bacterial cell viability. MIC analysis further demonstrated enhanced antibacterial potency of ZnO nanoparticles compared with the plant extract alone. Alterations in SDS–PAGE protein profiles suggested treatment-associated changes in bacterial protein expression under nanoparticle exposure. Collectively, these findings demonstrate that *C. citratus*-mediated ZnO nanoparticles possess superior antibacterial and antibiofilm efficacy against MDR *E. coli* isolated from retail fish and highlight their potential as an environmentally sustainable strategy for controlling foodborne bacterial contamination.

KEYWORDS: *Escherichia coli*, *Cymbopogon citratus*, zinc oxide nanoparticles, green synthesis, multidrug resistance, foodborne pathogens.

1. INTRODUCTION

Fish is widely recognised as an important part of a healthy diet because it provides high-quality protein along with essential nutrients such as omega-3 fatty acids, vitamins, and minerals. These nutrients play a key role in supporting heart health, brain function, and overall development.[1,2] In many parts of the world, especially in coastal and economically weaker regions, fish also serve as a major source of food and nutrition. Despite its benefits, fish is highly perishable and begins to deteriorate soon after it is harvested, particularly in warm and tropical climates. In local retail markets, fish is

often sold under conditions where proper refrigeration and hygiene are not maintained. As a result, it is frequently exposed to contaminated water, ice, surfaces, and repeated human handling, all of which increase the chances of microbial contamination and foodborne illness.[3]

Previous studies have shown that fish sold in such environments often contain harmful microorganisms, including Escherichia coli, Salmonella, Vibrio, Staphylococcus aureus, and Klebsiella species.[4] The detection of E. coli is especially concerning because it indicates contamination from faecal sources and poor sanitary conditions during handling and processing.[5] Likewise, Staphylococcus aureus is commonly transferred through direct contact with human skin, while Klebsiella pneumoniae is known to cause serious infections, particularly in individuals with weakened immunity. The presence of these bacteria not only reduces the quality of fish but also poses significant risks to public health.

One of the most serious challenges associated with microbial contamination in fish is the growing occurrence of multidrug-resistant (MDR) bacteria. The excessive and often uncontrolled use of antibiotics in aquaculture has contributed to the development of resistant bacterial strains in aquatic environments.[6] These bacteria can persist in fish and eventually enter the human food chain, where they may spread resistance genes to other microorganisms. In addition to resistance, many of these bacteria are capable of forming biofilms on fish surfaces and processing equipment. Biofilms act as protective layers that help bacteria survive harsh conditions, making them more difficult to remove and less sensitive to antimicrobial treatments.[7] This makes the control of such pathogens increasingly difficult in food processing and handling systems.

Although antibiotics have traditionally been used to control bacterial infections, their effectiveness is declining due to the rapid emergence of resistance. Moreover, their use is associated with several drawbacks, such as the presence of harmful residues, disruption of beneficial microbial populations, and limited effectiveness against biofilm-forming bacteria. These limitations highlight the need for alternative approaches that are not only effective but also safe and environmentally sustainable.

In this context, plant-based antimicrobial agents have gained increasing attention as promising alternatives. Plant extracts contain a wide range of bioactive compounds, including phenolics, flavonoids, alkaloids, and terpenoids, which are known for their antimicrobial, antioxidant, and antibiofilm properties.[8] These compounds can act in different ways, such as damaging bacterial cell membranes, interfering with enzyme systems, and disrupting metabolic activities. Because of their multiple modes of action, it is more difficult for bacteria to develop resistance against them. Additionally, plant-derived antimicrobials are generally considered to be safer, biodegradable, and environmentally friendly, making them suitable for use in food safety applications.

An emerging advancement in this field is the use of plant extracts for the green synthesis of nanoparticles. Unlike conventional methods, which often require toxic chemicals and high energy input, green synthesis offers a simpler, safer, and more sustainable approach.[9] In this process, plant extracts function as natural reducing and stabilising agents, enabling the formation of nanoparticles while also enhancing their biological activity. The phytochemicals present in the extracts not only assist in nanoparticle formation but also improve their stability and effectiveness.

Among different types of nanoparticles, zinc oxide nanoparticles (ZnO NPs) have received considerable attention due to their strong antimicrobial properties, stability, and biocompatibility. ZnO nanoparticles can inhibit bacterial growth through several mechanisms, including the generation of reactive oxygen species (ROS), disruption of cell membranes, and the release of zinc ions that interfere with essential cellular functions.[10] Because they act on multiple targets within the microbial cell, they are effective against a wide range of pathogens, including multidrug-resistant strains, and are less likely to promote resistance. In addition to their antibacterial activity, ZnO nanoparticles are also effective in preventing biofilm formation by interfering with bacterial communication systems and disrupting the structure of biofilms.

Despite these promising developments, there is still a lack of studies focusing on the application of plant-mediated ZnO nanoparticles against bacteria isolated directly from real food sources such as retail fish. Most existing research is based on laboratory strains, which may not accurately represent the behaviour of bacteria found in natural environments. Furthermore, limited attention has been given to evaluating both antibacterial and antibiofilm activities together in such systems.[11]

Therefore, the present study aims to assess the level of microbial contamination in retail fish, identify multidrug-resistant bacterial isolates, and evaluate the antibacterial and antibiofilm effectiveness of zinc oxide nanoparticles synthesised using lemongrass (*Cymbopogon citratus*) extract. By combining the benefits of plant-based antimicrobials with nanotechnology, this study aims to provide a practical, sustainable solution for controlling foodborne pathogens and improving food safety.

2. MATERIAL AND METHODS

2.1 Collection of Fish Samples

A total of ten fresh fish samples were collected from local retail markets under aseptic conditions to assess surface-associated microbial contamination. The samples were selected randomly to ensure diversity and minimise

sampling bias. Immediately after collection, surface sampling was performed to prevent external contamination. Sterile cotton swabs were used to collect microbial flora from the scales of each fish by gently and uniformly rubbing the surface. Each swab was then transferred into sterile zip-lock bags, properly labelled with sample number, date, and location. The samples were transported to the laboratory promptly for further microbiological analysis.[12,13]

2.2 Isolation of Bacteria

Bacterial isolation was performed using the streak plate method on UTI agar plates under aseptic conditions in a laminar airflow cabinet. The swab samples were streaked carefully to obtain well-isolated colonies. The inoculated plates were incubated at 37°C for 24 hours in an inverted position under aerobic conditions.[14,15]

2.3 Preparation of Bacterial Cultures

Nutrient broth was prepared, dispensed into culture tubes, and sterilised by autoclaving at 121°C for 15 minutes at 15 psi. After cooling, well-isolated colonies of *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were inoculated into separate tubes and incubated at 37°C for 24 hours to obtain actively growing cultures.[14,16]

2.4 Antibiotic Susceptibility Testing

The antibiotic susceptibility of bacterial isolates was determined using the Kirby–Bauer disc diffusion method. Fresh bacterial cultures were spread uniformly on nutrient agar plates to form a lawn. Antibiotic discs (Ampicillin, Tetracycline, Streptomycin, and Chloramphenicol) were placed aseptically on the surface. Plates were incubated at 37°C for 18–24 hours, and zones of inhibition were measured in millimetres.

2.5 Morphological Characterisation (Gram Staining)

Gram staining was performed to differentiate bacterial isolates based on cell wall structure. Smears were prepared on clean slides, heat-fixed, and stained sequentially with crystal violet, iodine, ethanol, and safranin. Slides were observed under a microscope using oil immersion to determine Gram reaction and morphology.

2.6 Physiological Characterisation

2.6.1 Effect of NaCl Concentration

Nutrient broth supplemented with varying NaCl concentrations (0.1%, 1%, 2%, and 4%) was prepared. Each tube was inoculated with *E. coli* and incubated at 37°C for 24 hours. Growth was assessed based on turbidity.

2.6.2 Effect of Temperature

Bacterial cultures were incubated at 4°C, room temperature, 37°C, and 70°C. Growth was analysed after 24 hours using optical density measurements.

2.6.3 Effect of pH

Nutrient broth was adjusted to pH levels of 1, 3, 5, 7, and 9. After inoculation with *E. coli*, cultures were incubated at 37°C and analysed spectrophotometrically.

2.6.4 Catalase Test

Catalase activity was determined by adding hydrogen peroxide to bacterial colonies. Immediate bubble formation indicated a positive result.

2.7 Preparation of Plant Material

Leaves of selected medicinal plants (*Azadirachta indica*, *Cymbopogon citratus*, *Murraya koenigii*, *Moringa oleifera*, *Justicia adhatoda*, and *Curcuma longa*) were collected, washed, and shade-dried. The dried materials were ground into fine powder and stored in sterile containers.

2.8 Preparation of Plant Extracts

Methanolic extracts were prepared by mixing 5 g of powdered plant material with 50 mL of methanol. The mixture was kept at 4°C with continuous stirring for efficient extraction. The extract was concentrated and stored at 4°C.

2.9 Antibacterial Activity Assay

The antibacterial activity of plant extracts was evaluated using the agar well diffusion method. Nutrient agar plates were inoculated with *E. coli* to form a bacterial lawn. Wells were created and filled with plant extracts. Plates were incubated at 37°C for 24 hours, and inhibition zones were observed.

2.10 Thin Layer Chromatography (TLC)

TLC was performed using silica gel plates to analyse phytochemicals in lemongrass extract. Samples were spotted and developed using solvent systems of n-hexane and ethyl acetate. Spots were visualised under UV light, and Rf values were calculated.[17]

2.11 Column Chromatography

Lemongrass extract was separated using silica gel column chromatography. Fractions were collected using a solvent system and further analysed by TLC.

2.12 Phytochemical Screening

Standard qualitative tests were performed to detect alkaloids, flavonoids, proteins, carbohydrates, phenolics, tannins, glycosides, saponins, and terpenoids based on colour changes or precipitate formation.

2.13 Green Synthesis of ZnO Nanoparticles

Zinc oxide nanoparticles were synthesised using zinc nitrate and lemongrass extract. The reaction mixture was stirred at 60–70°C and adjusted to alkaline pH using NaOH. After incubation, the precipitate was collected, dried, and powdered.[18,19]

2.14 Solvent Selection for Nanoparticles
ZnO nanoparticles were dispersed in DMSO, ethanol, and water. Antibacterial activity was evaluated using the well diffusion method to determine the most effective solvent.

2.15 UV–Visible Spectroscopy
Nanoparticles dispersed in DMSO were analysed using a UV–Vis spectrophotometer in the range of 200–800 nm to confirm nanoparticle formation.

2.16 Growth Kinetics Study
The growth of *E. coli* was analysed in the presence of lemongrass extract and ZnO nanoparticles. Optical density was measured at regular intervals to monitor bacterial growth patterns.

2.17 Biofilm Eradication Assay
Biofilm formation and inhibition were assessed using a microtiter plate method. Treated and untreated samples were stained with crystal violet, and absorbance was measured.

2.18 Antibiofilm Assay
The ability of treatments to inhibit biofilm formation was evaluated by incubating bacterial cultures with plant extract and nanoparticles, followed by staining and absorbance measurement.

2.19 Cell Viability (MTT Assay)
Cell viability was determined using the MTT assay. After treatment, MTT reagent was added, and absorbance was measured to evaluate metabolic activity.

2.20 Minimum Inhibitory Concentration (MIC)
MIC was determined using serial dilution in microtiter plates. Optical density readings were used to identify the lowest concentration inhibiting bacterial growth.

2.21 SDS-PAGE Protein Profiling
Protein profiling of *Escherichia coli* was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Whole-cell and extracellular protein fractions were prepared from untreated and treated bacterial cultures. Briefly, bacterial cells were harvested by centrifugation, washed with acetone to remove residual medium components, and processed for protein extraction. Extracellular proteins were recovered separately from the corresponding culture supernatants. Protein samples were mixed with SDS-PAGE loading buffer and heated at 95°C for 5 min to ensure complete protein denaturation before electrophoresis. The denatured proteins were resolved on polyacrylamide gels under standard electrophoretic conditions and subsequently stained for visualisation. Comparative analysis of protein banding patterns was performed to evaluate changes in protein expression following treatment.

3. RESULTS

3.1 Collection of Fish Samples
Surface swabbing revealed a high microbial load on fish samples, indicating significant contamination. This suggests that retail fish carry surface-associated microorganisms due to exposure to open market conditions.

3.2 Isolation of Bacteria from the Surface of the Fish

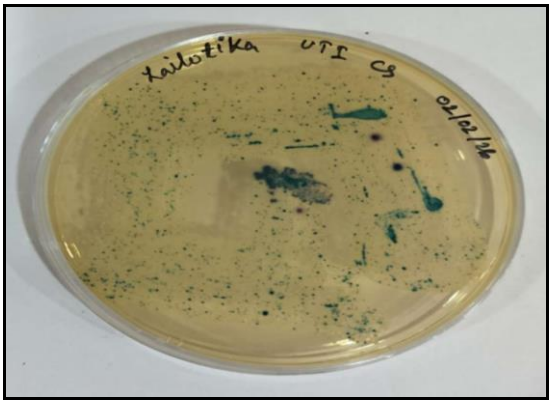

Figure 1: Isolation of bacteria on UTI agar plate.

Distinct colonies on UTI agar confirmed a diverse microbial population, with colour differentiation. This plate of Lailantika (Tilapia) showed the most prominent and well-developed colonies.

3.3 Preparation of Nutrient Broth and Inoculation of Bacterial Culture

Figure 2: Inoculation of bacterial cultures on nutrient agar.

All bacterial isolates showed successful growth in nutrient broth, indicated by turbidity. *Escherichia coli* exhibited the highest growth, followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*.

3.4 Kirby-Bauer Disc Diffusion Assay for Determination of Antibiotic Susceptibility Testing

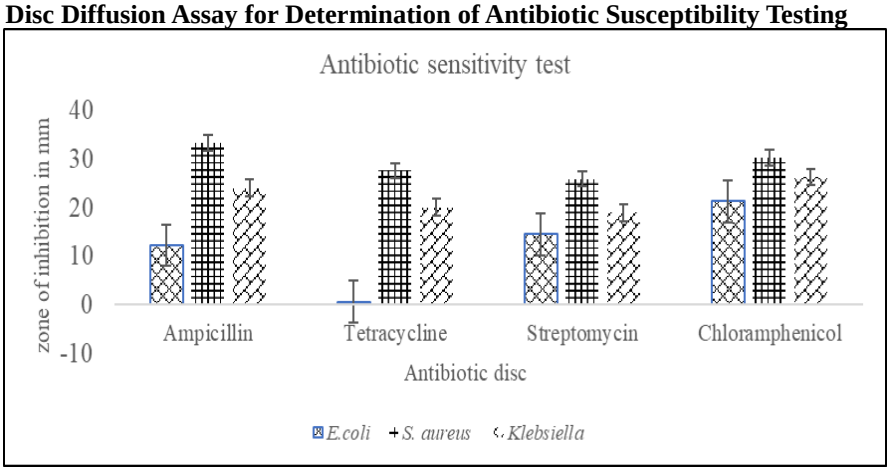

Figure 3: Antibiotic sensitivity test.

Escherichia coli showed the lowest zones of inhibition, indicating the highest resistance, while *Staphylococcus aureus* was highly susceptible, and *Klebsiella pneumoniae* showed moderate sensitivity to the tested antibiotics.

3.5 Morphological Characterisation of Bacterial Isolates by Gram Staining

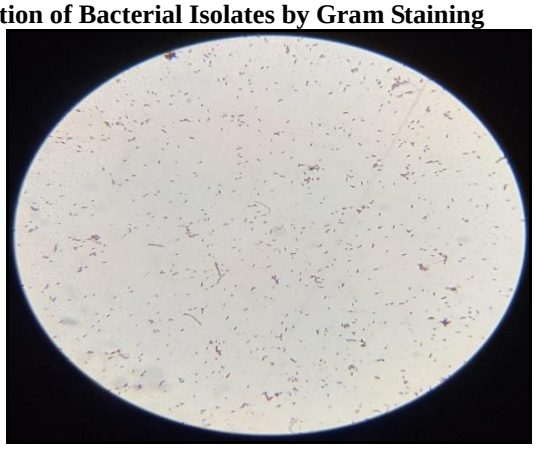

Figure 4: Gram strain of E. coli at 40x magnification.

The isolate appeared pink after Gram staining, confirming it as Gram-negative, with rod-shaped (bacilli) morphology consistent with *Escherichia coli*.

3.6 Physiological Tests

3.6.1 Growth at Different NaCl Concentrations

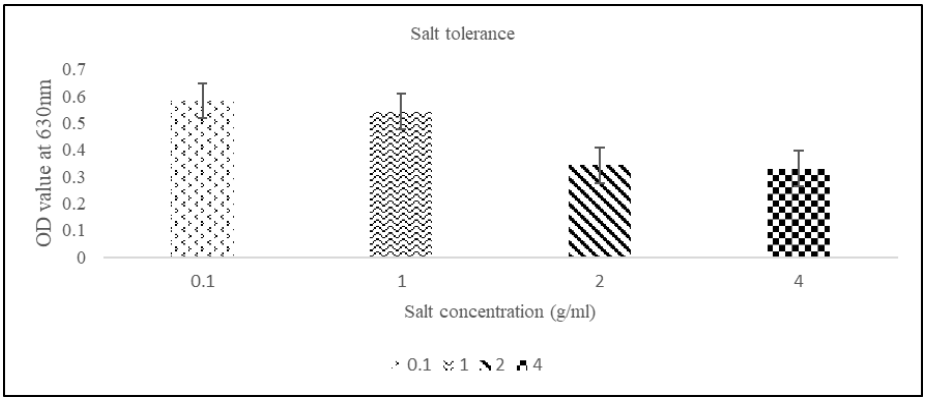

Figure 5: Effect of NaCl concentration on bacterial growth.

Escherichia coli showed maximum growth at 0.1 g/mL NaCl, while growth gradually decreased with increasing salt concentration, with minimum growth observed at 4 g/mL NaCl.

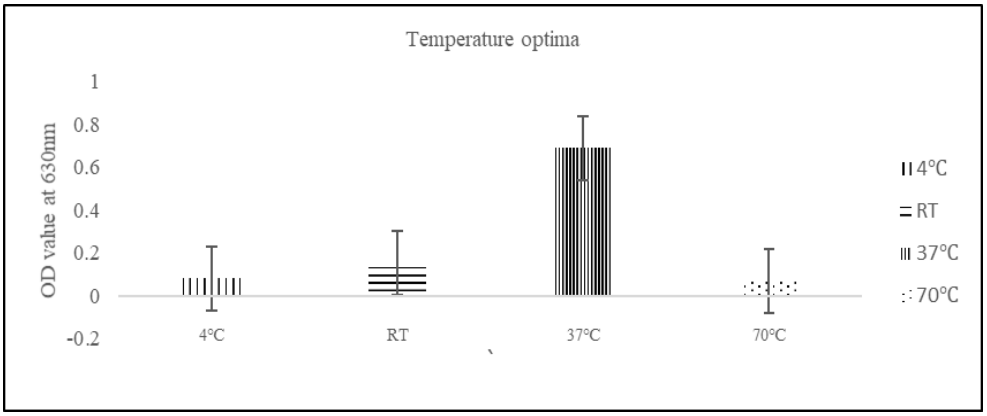

Figure 6: Effect of temperature on bacterial growth.

3.6.2 Growth at Different Temperatures

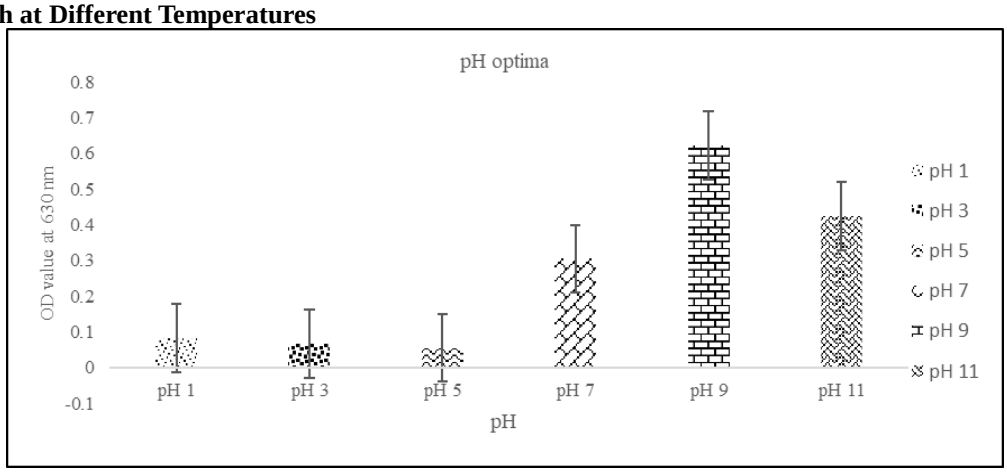

Figure 7: Effect of pH on bacterial growth.

Escherichia coli showed maximum growth at 37°C, moderate growth at room temperature, very low growth at 4°C, and no significant growth at 70°C.

3.6.3 Growth at Different pH

Escherichia coli showed maximum growth at pH 9, moderate growth at pH 7, reduced growth at pH 11, and minimal growth under acidic conditions (pH 1, 3, and 5).

3.6.4 Determination of Catalase Activity

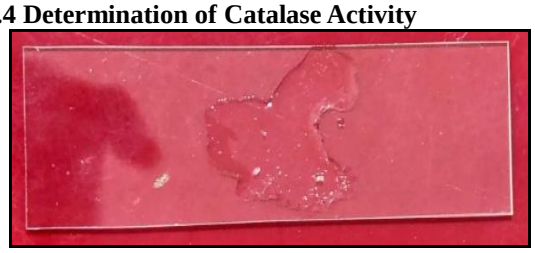

Figure 8: Determination of catalase activity in bacterial isolates.

The catalase test showed immediate bubble formation, confirming that the bacterial isolate is catalase positive and consistent with the characteristics of *Escherichia coli*.

3.7 Preparation of Plant Material from the Selected Medicinal Plant

Figure 9: Preparation of plant materials from selected medicinal plants.

The plant materials were successfully cleaned, shade-dried, powdered, and stored under sterile conditions for further phytochemical extraction and antimicrobial analysis.

3.8 Selection of Solvent and Preparation of Plant Extracts for Antibacterial Evaluation

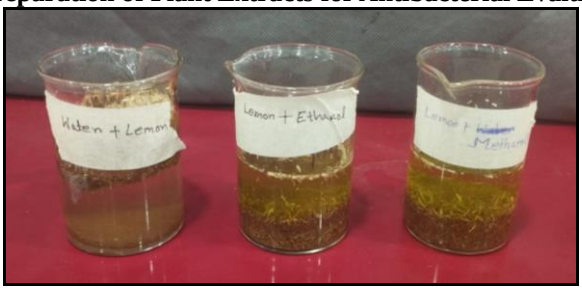

Figure 10: Selection of solvent and preparation of plant extracts.

Methanolic extraction successfully yielded concentrated plant extracts containing bioactive phytochemicals suitable for antimicrobial evaluation.

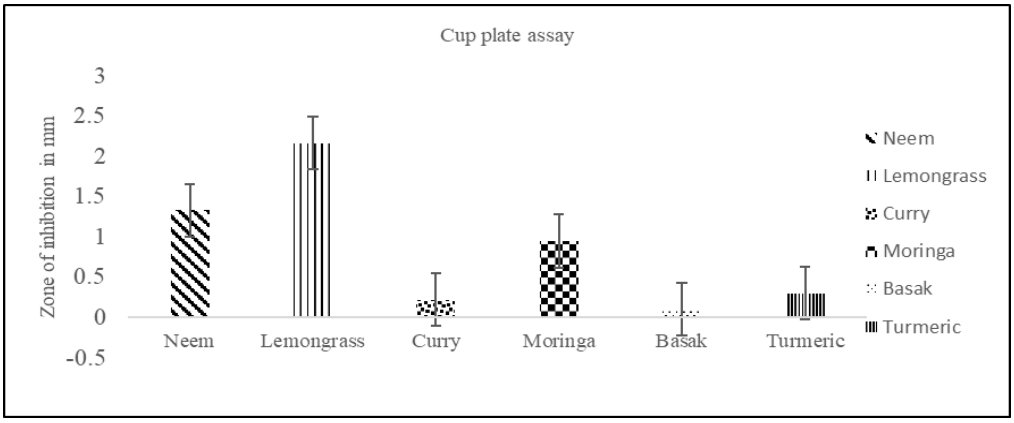

Figure 11: Comparative analysis of the antimicrobial activity of various plant extracts.

3.9 Antibacterial Activity By Cup Plate (Agar Well Diffusion) Assay

Among all tested plant extracts, *Cymbopogon citratus* showed the highest zone of inhibition against *Escherichia coli*, indicating the strongest antibacterial activity.

3.10 Thin Layer Chromatography (TLC) Analysis of Lemongrass Methanolic Extract

Table 1: Summary of TLC results.

Fraction No	Solvent Front (cm)	Spot Distance (cm)	RF Value
1	5.4	1.8	0.22
1	5.4	2.5	0.46
1	5.4	4.9	0.91
2	5.5	3.5	0.61
2	5.5	4.1	0.75
2	5.5	4.7	0.86

3.11 Column Chromatography of Methanolic Lemongrass Extract Followed by TLC Analysis

Table 2: TLC analysis of fractions.

Fraction No	Solvent Front (cm)	Spot Distance (cm)	RF Value
1	5.0	4.8	0.96
1	5.0	4.0	0.80
2	5.2	3.6	0.69
2	5.2	3.3	0.63
3	5.3	4.9	0.92
3	5.3	4.7	0.88
4	4.9	4.5	0.91
4	4.9	1.5	0.30
5	6.0	5.3	0.88
5	6.0	5.1	0.85

3.12 Phytochemical Test of Lemongrass Methanolic Extract

Table 3: Qualitative phytochemical screening of lemongrass extract.

Sl. No.	Phytochemical Group	Test Performed	Observation	Results
1	Alkaloids	Dragendorff's Test	Reddish-brown precipitate	positive
2	Reducing sugars	Benedict's Test	Brick-red precipitate	negative
3	Carbohydrates	Molisch's Test	Violet ring at the interface	positive
4	Proteins	Biuret Test	Violet / Purple color	negative
5	Phenolics	Ferric Chloride Test	Bluish/ Black color	positive
6	Tannins	5% Ferric Chloride Test	Dark Green / Blue-Black color	positive
7	Cardiac Glycoside	Keller-Kiliani Test	Brown ring at the interface	positive
8	Saponins	Froth Test	Stable Foam Formation	Positive (mild foam)
9	Terpenoids	Salkowski Test	Reddish brown interface	positive

3.13 Green-Synthesis of Zinc Oxide Nanoparticles

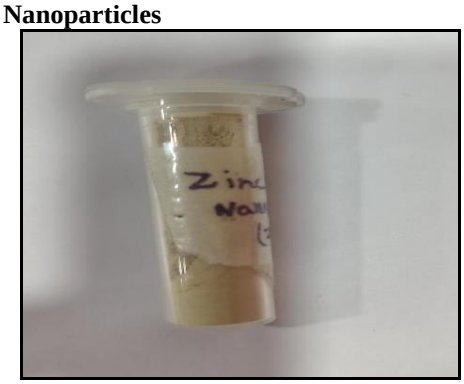

Figure 12: Successfully synthesized and dried ZnO nanoparticle product.

Green synthesis of ZnO nanoparticles using *Cymbopogon citratus* aqueous extract was successfully achieved, as confirmed by the formation of a white precipitate and the final ZnO nanoparticle powder after alkaline treatment and drying.

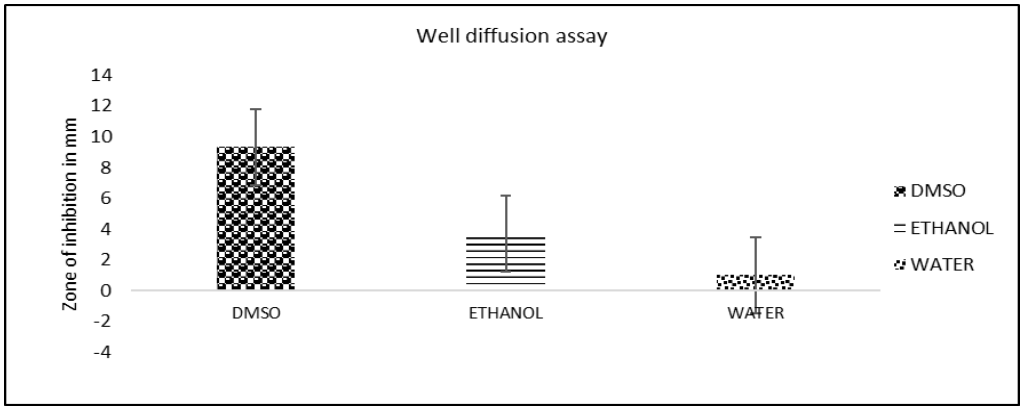

Figure 13: Selection of solvent for ZnO nanoparticles.

3.14 Well Diffusion Assay to Select the Solvent for Nanoparticles

ZnO nanoparticles prepared in DMSO showed the highest antibacterial activity against *E. coli*, producing the largest zone of inhibition, while ethanol showed moderate activity and distilled water showed the least.

3.15 UV-VIS Spectroscopic Analysis

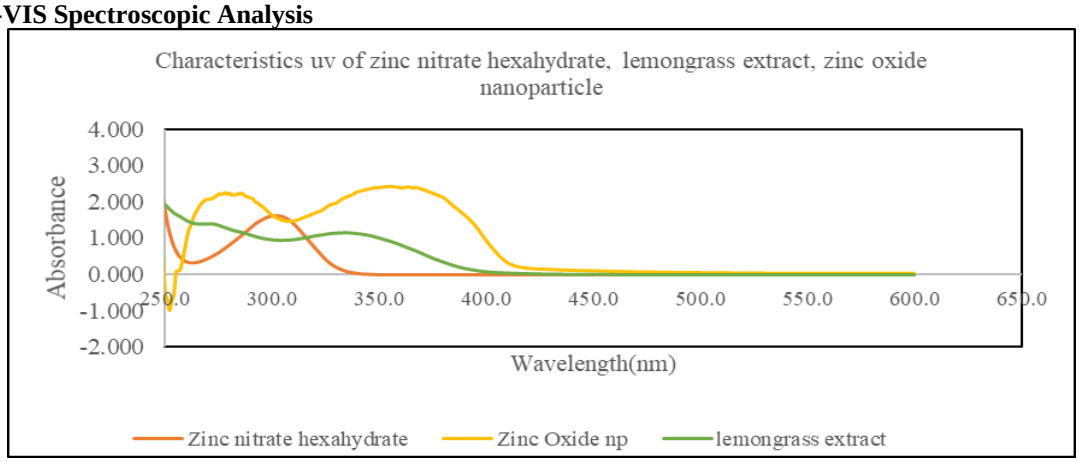

Figure 14: UV-Vis spectrophotometric analysis of precursors and green-synthesised zinc oxide nanoparticles.

UV–Visible spectroscopy confirmed the successful formation of ZnO nanoparticles, showing a characteristic UV absorption peak with an estimated band gap of ~3.25 eV and a particle size of approximately 18 nm.

3.16 Growth Kinetics Study of *Escherichia Coli* in the Presence of Lemon Methanolic Extract and Zinc Oxide Nanoparticle

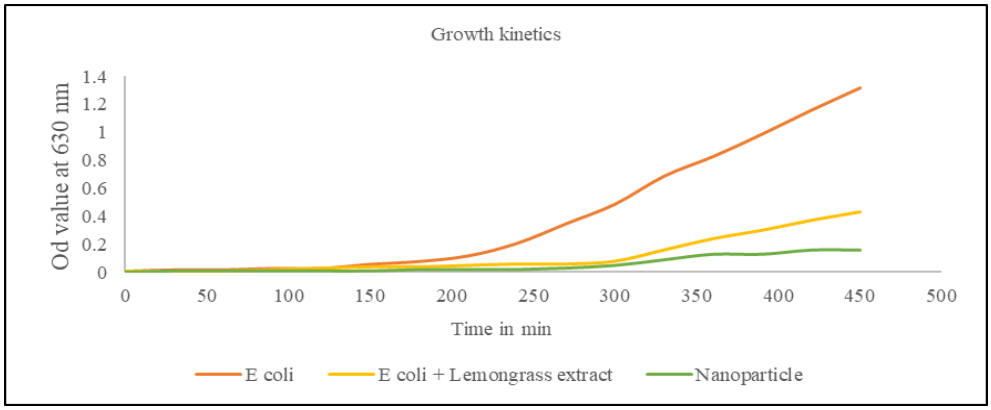

Figure 15: Growth kinetics of Escherichia coli monitored via turbimetric analysis in the presence of lemon methanolic extract and zinc oxide nanoparticles.

Turbidimetric analysis demonstrated that ZnO nanoparticles showed the highest antibacterial activity by significantly inhibiting the growth of *E. coli* compared to the control and lemongrass extract-treated samples.

3.17 Biofilm Eradication Assay

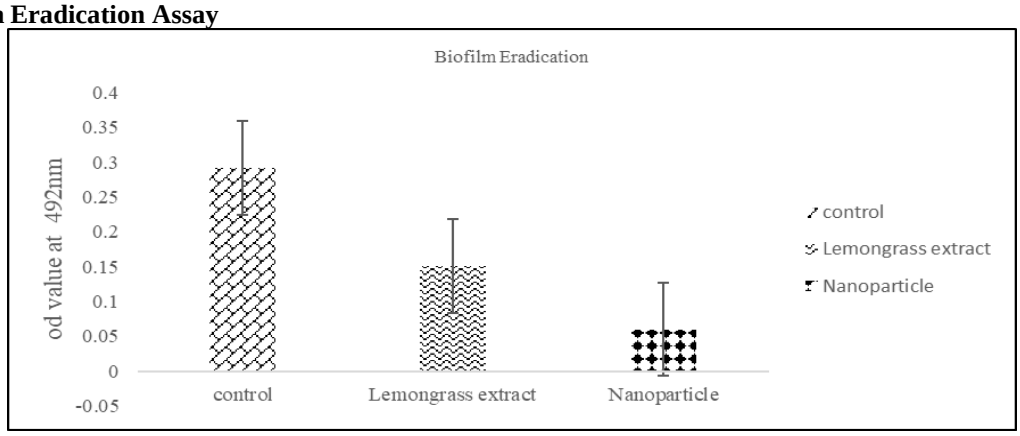

Figure 16: Biofilm eradication assay for control, lemongrass extract and nanoparticle treatments against E. coli.

The biofilm eradication assay demonstrated that green-synthesised ZnO nanoparticles exhibited significantly greater antibiofilm activity against *E. coli* than lemongrass extract, effectively disrupting established biofilms and markedly reducing biofilm biomass.

3.18 Antibiofilm Assay

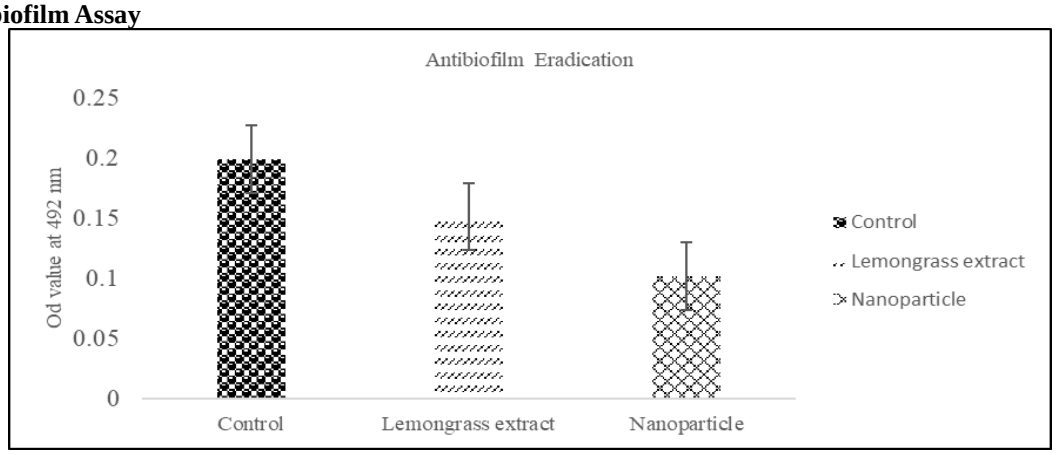

Figure 17: Antibiofilm eradication assay for Comparison of the effectiveness of lemongrass extract and nanoparticles in reducing Escherichia coli biofilm density.

Comparative analysis showed that green-synthesised ZnO nanoparticles exhibited significantly higher antibiofilm activity than lemongrass extract, producing the lowest optical density values and demonstrating superior inhibition of *E. coli* biofilm formation.

3.19 Cell Viability Assay (MTT Assay)

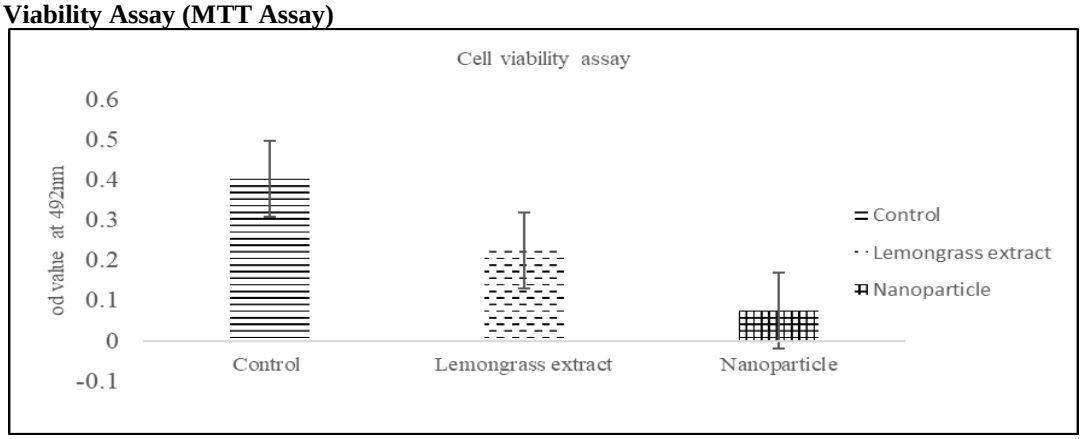

Figure 18: Cell viability of Escherichia coli treated with lemongrass extract and green-synthesised zinc oxide nanoparticles.

The MTT cell viability assay showed that green-synthesised ZnO nanoparticles significantly reduced the viability of *E. coli* compared to lemongrass extract, demonstrating superior cytotoxic and antibacterial activity.

3.20 Determination of Minimum Inhibitory Concentration (MIC)

Table 4: Minimum inhibitory concentration (MIC) of lemongrass extract and nanoparticle.

Dilution	Lemongrass extract	Nanoparticle
1/2dilution	(-)	(-)
1/4dilution	(-)	(-)
1/8dilution	(-)	(-)
1/16dilution	(+)	(-)
1/32dilution	(+)	(-)
1/64dilution	(+)	(+)

['+' indicates lesser effect of factor on cell; '-' indicates more effect of factor on cell]

a3.21 SDS-PAGE Protein Profiling

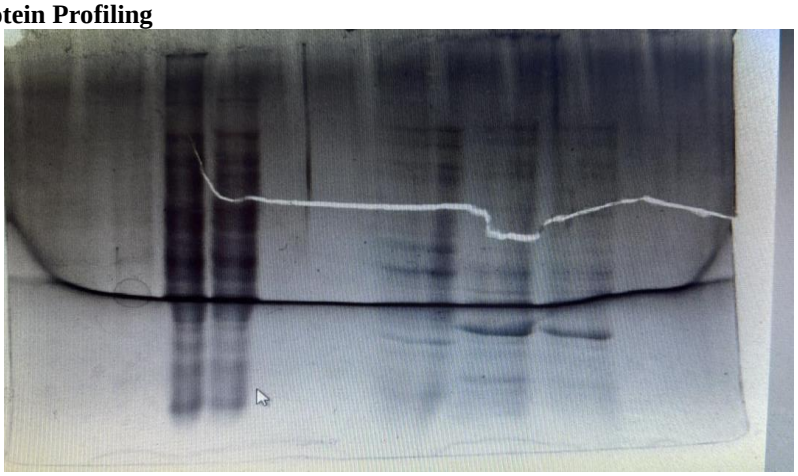

Figure 19: SDS PAGE analysis.

4. DISCUSSIONS

The occurrence of multidrug-resistant (MDR) bacteria in retail fish represents an emerging food safety concern because contaminated aquatic food products can serve as reservoirs for clinically relevant pathogens. The isolation of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* from retail fish samples in the present study indicates microbial contamination that may arise from handling, processing, storage, or environmental exposure. The use of chromogenic UTI agar facilitated rapid presumptive identification of these organisms, consistent with previous reports demonstrating its effectiveness for differentiating clinically important bacterial species.[20] The predominance of MDR E. coli among the recovered isolates is particularly significant, as this organism is frequently associated with both foodborne transmission and urinary tract infections, thereby representing an important public health concern.[21,22]

The antibiotic susceptibility profile demonstrated marked variation among the isolated bacteria, with E. coli exhibiting the highest level of multidrug resistance. Such resistance patterns are consistent with the increasing prevalence of intrinsic and acquired resistance mechanisms, including β-lactamase production, altered membrane permeability, efflux systems, and target modification, which collectively reduce the efficacy of conventional antibiotics.[23] The selection of MDR E. coli for subsequent investigations therefore provided an appropriate model for evaluating alternative antimicrobial approaches.

Among the medicinal plants investigated, *Cymbopogon citratus* exhibited the strongest antibacterial activity against the selected isolate. Methanolic extraction efficiently recovered phytochemicals with known antimicrobial potential, while TLC and qualitative phytochemical analyses confirmed the presence of phenolics, flavonoids, tannins, terpenoids, alkaloids, and glycosides. These classes of secondary metabolites have previously been associated with antibacterial activity through disruption of membrane integrity, interference with metabolic pathways, and inhibition of bacterial proliferation.[24] The phytochemical diversity observed in the present study therefore provides a plausible basis for the antibacterial activity exhibited by the crude extract.

Green synthesis of ZnO nanoparticles using C. citratus extract was successfully achieved under alkaline conditions, where plant-derived phytochemicals acted as reducing and stabilizing agents during nanoparticle formation. UV–Visible spectroscopic analysis confirmed the formation of ZnO nanoparticles with optical characteristics consistent with nanoscale materials. The estimated particle size of approximately 18 nm indicates successful synthesis of nanoparticles possessing a high surface-area-to-volume ratio, a property widely recognized for enhancing antimicrobial performance.

The antibacterial assays consistently demonstrated that ZnO nanoparticles exhibited superior activity compared with the crude methanolic extract. Larger inhibition zones, lower MIC values, and greater suppression of bacterial growth collectively indicate that nanoparticle formulation significantly improved antibacterial efficacy. The enhanced performance of the DMSO-dispersed nanoparticles further suggests that improved dispersion increased the interaction between nanoparticles and bacterial cells. Similar observations have been reported for ZnO nanoparticles, where increased surface reactivity contributes to enhanced antibacterial activity.[19]

An important outcome of the present investigation was the pronounced inhibition and eradication of bacterial biofilms by the synthesized ZnO nanoparticles. Biofilm-associated bacteria are inherently more tolerant to antimicrobial agents because the extracellular polymeric matrix restricts antimicrobial penetration and protects embedded cells. Compared with the crude extract, ZnO nanoparticles produced substantially greater inhibition of biofilm formation as well as more effective disruption of preformed biofilms. These findings are consistent with previous studies demonstrating the ability of ZnO nanoparticles to interfere with bacterial adhesion, biofilm development, and extracellular matrix integrity.[19] The

corresponding reduction in bacterial viability observed in the MTT assay further supports the enhanced antibacterial effectiveness of the nanoparticle formulation.

Protein profiling by SDS–PAGE revealed detectable alterations in the banding patterns of treated bacterial cells compared with the untreated control, indicating that exposure to ZnO nanoparticles influenced the bacterial protein profile. Although the present study did not identify individual proteins, these observations suggest that nanoparticle treatment induces measurable physiological responses at the cellular level, complementing the antibacterial, antibiofilm, and growth inhibition results obtained in this investigation.

Overall, the findings demonstrate that green-synthesized ZnO nanoparticles derived from *Cymbopogon citratus* possess significantly greater antibacterial efficacy than the crude plant extract against MDR *E. coli* isolated from retail fish. The integration of medicinal plant phytochemistry with green nanotechnology represents a promising and environmentally sustainable strategy for controlling multidrug-resistant foodborne pathogens. These findings provide a scientific basis for further investigations into the application of plant-mediated ZnO nanoparticles in food safety, antimicrobial therapy, and related biomedical fields.

5. CONCLUSION

This study highlights the presence of microbial contamination in retail fish and emphasises the growing concern of multidrug-resistant (MDR) bacteria in the food supply. Pathogenic organisms such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were identified, indicating poor hygiene and the potential risk of foodborne infections. The observed antibiotic resistance, particularly in *E. coli*, further demonstrates the declining effectiveness of conventional antibiotics and the urgent need for alternative antimicrobial strategies.

Lemongrass (*Cymbopogon citratus*) extract showed notable antibacterial and antibiofilm activity, mainly due to its rich phytochemical composition. Compounds such as flavonoids, terpenoids, and phenolics contribute to disrupting bacterial cells and inhibiting their growth and communication systems. Its natural origin and lower toxicity make it a promising and sustainable antimicrobial option.

The study also demonstrated that green-synthesised zinc oxide nanoparticles significantly enhance antimicrobial effectiveness. Their small size and large surface area allow better interaction with bacterial cells, leading to membrane damage and inhibition of vital cellular functions. The improved activity observed in DMSO formulations suggests better dispersion and bioavailability.

Overall, plant-mediated ZnO nanoparticles offer an eco-friendly and effective alternative to traditional antibiotics, with strong potential for applications in food safety and healthcare.

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