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**EVALUATION OF ANTIBACTERIAL AND ANTI-INFLAMMATORY EFFECT OF GREEN SYNTHESIZED SILVER OXIDE NANOPARTICLES FROM MANILKARA LITTORALIS FOR BIOMEDICAL APPLICATIONS**Mohammad Nemat Sache¹, Hema M. Saheb Ali², Saheb Ali³

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ABSTRACT

Objective: This study investigates the antibacterial and anti-inflammatory effects of silver oxide nanoparticles (AgO NPs) synthesized using a green approach with Manilkara littoralis extract, highlighting their potential in biomedical applications.

Methods: AgO NPs were synthesized by reducing silver nitrate with Manilkara littoralis leaf extract, confirmed by color change. Characterization involved FTIR, UV-Vis spectroscopy, XRD, and SEM. Antibacterial efficacy was assessed against *S. aureus* and *E. coli* using the disk diffusion method, while anti-inflammatory properties were tested in a zebrafish model.

Results: AgO NPs exhibited strong antibacterial activity, especially against Gram-positive bacteria. Anti-inflammatory effects were observed in zebrafish embryos with minimal adverse impacts, indicating favorable biocompatibility.

Conclusions: AgO NPs synthesized from Manilkara littoralis show promise as a dual-function therapeutic agent for potential biomedical applications, particularly in infection control and inflammation management. Further research is needed to explore their full clinical potential.

Keywords: AgONP, FTIR, Manikara, nanoparticle, silver nanoparticle, SEM Spectroscopy

INTRODUCTION

Nanotechnology has emerged as a transformative field across numerous scientific disciplines, driven by its capability to manipulate materials at the atomic and molecular levels¹. Since the concept was introduced by physicist Richard Feynman and later formalized as “nanotechnology” by Norio Taniguchi, the field has undergone significant evolution. The advent of the scanning tunneling microscope in the 1980s marked the beginning of modern nanotechnology, enabling precise control over nanoscale structures. This capability has opened new avenues in sectors like healthcare, electronics, and materials science.

In particular, the convergence of nanotechnology with biomedical research has led to the rise of nanomedicine, a field dedicated to improving diagnostic and therapeutic strategies through nanoscale innovations². Nanoparticles (NPs), typically sized between 1 and 100 nm, have unique physical, chemical, and biological properties that differ significantly from their bulk counterparts³. These properties such as high surface area-to-volume ratios, optical characteristics, and reactivity render nanoparticles particularly useful in a variety of applications, ranging from drug delivery and diagnostics to antimicrobial agents and cancer therapy.

Among the various types of nanoparticles, silver nanoparticles (AgNPs) stand out due to their exceptional antimicrobial properties⁴. Historically used in traditional medicine and wound care, silver's ability to combat a broad spectrum of pathogens is well-documented⁵. The application of AgNPs extends far beyond antimicrobial activity; they are now being explored for their potential in anticancer, anti-inflammatory, and antioxidant treatments⁶.

Silver oxide nanoparticles (AgO NPs), a specific subclass of silver-based nanoparticles, have garnered increasing interest for their enhanced biological properties⁷. These include not only antimicrobial effects but also significant anti-inflammatory and antioxidant activities, making them promising candidates for various biomedical applications⁸. Despite their potential, the traditional synthesis methods for silver-based nanoparticles, such as chemical reduction and physical vapor deposition, pose significant environmental and health concerns⁹. These conventional techniques often involve the use of toxic reducing agents, high energy consumption, and the generation of hazardous by-products. As the demand for more sustainable and biocompatible nanomaterials grows, researchers are exploring alternative synthesis methods that minimize ecological impact¹⁰.

One such promising approach is the green synthesis of nanoparticles, which leverages biological materials, such as plant extracts, as reducing and capping agents¹¹. This eco-friendly method aligns with the principles of green chemistry, aiming to reduce or eliminate the use of harmful substances in nanoparticle production¹². Plant-mediated synthesis harnesses the natural reducing potential of phytochemicals—bioactive compounds like flavonoids, phenolics, and alkaloids present in plants¹³. These compounds not only facilitate the reduction of metal ions into nanoparticles but also stabilize the particles, preventing aggregation. Moreover, the incorporation of bioactive plant compounds can enhance the therapeutic properties of the synthesized nanoparticles, making them particularly attractive for biomedical applications¹⁴.

The use of plant extracts in nanoparticle synthesis offers several advantages over conventional methods¹⁵. First, it is a cost-effective and scalable process that does not require expensive or hazardous chemicals. Second, the process is carried out at ambient temperature and pressure, further reducing energy consumption¹⁶. Third, plant-mediated synthesis often results in nanoparticles with enhanced biocompatibility due to the presence of natural capping agents, which can improve interactions with biological systems¹⁷. This approach not only supports

environmental sustainability but also enhances the biomedical potential of the nanoparticles.

In this context, *Manilkara littoralis*, a plant known for its rich phytochemical profile and medicinal properties, was selected as the reducing and capping agent for synthesizing AgO NPs in this study¹⁸. *Manilkara littoralis* belongs to a genus recognized for its diverse bioactive compounds, including antioxidants and anti-inflammatory agents¹⁹. The choice of this plant is strategic, as its phytochemical constituents can facilitate the synthesis process while potentially enhancing the biological activities of the resulting nanoparticles¹⁵. The use of *Manilkara littoralis* extract not only aids in the reduction of silver ions to form AgO NPs but also introduces bioactive molecules that may contribute to the overall therapeutic effects of the nanoparticles²⁰. The synthesized AgO NPs were then evaluated for their antibacterial and anti-inflammatory activities²¹. The antibacterial efficacy was tested against a range of Gram-positive and Gram-negative bacteria, including common pathogens that pose significant challenges in healthcare due to increasing antibiotic resistance²². The anti-inflammatory potential was assessed using a zebrafish model, an established *in vivo* system for studying inflammatory responses²³. Zebrafish are widely used in biomedical research due to their genetic similarities to humans and their transparent embryos, which allow for real-time observation of biological processes²⁴. The comprehensive assessment of AgO NPs synthesized via green methods not only highlights their potential as multifunctional therapeutic agents but also underscores the advantages of plant-mediated synthesis in enhancing nanoparticle biocompatibility and efficacy²⁵.

In summary, the field of nanotechnology, particularly the development of silver-based nanoparticles, continues to expand rapidly due to the unique properties and broad applicability of these materials. The transition from conventional chemical synthesis methods to green, plant-mediated approaches marks a significant step forward in the pursuit of sustainable nanomaterial production. The use of *Manilkara littoralis* as a natural reducing agent exemplifies the potential of leveraging traditional medicinal plants in modern nanotechnology. This study aims to explore the full scope of the biomedical potential of green-synthesized AgO NPs, with a focus on their antibacterial and anti-inflammatory properties. By integrating environmentally friendly synthesis techniques with advanced biomedical applications, this research contributes to the growing body of evidence supporting the viability of green nanotechnology in addressing global health challenges.

MATERIALS AND METHODS

1. Synthesis of Silver Oxide Nanoparticles (AgO NPs)

Collection and Preparation of Plant Material:

Fresh leaves of *Manilkara littoralis* were collected from a natural habitat during the early morning to ensure the highest concentration of bioactive compounds. The leaves were carefully inspected to select only healthy and undamaged specimens. They were then thoroughly washed with running tap water to remove dust and debris, followed by a rinse with distilled water to eliminate any remaining surface contaminants. The cleaned leaves were spread out on a sterile tray and air-dried in a shaded area at room temperature (25–30°C) for 10–14 days, avoiding direct sunlight to preserve their phytochemical content. Once completely dried, the leaves were ground into a fine powder using a mechanical grinder and stored in airtight containers at room temperature until further use.

Preparation of Aqueous Leaf Extract:

To prepare the leaf extract, 10 grams of the dried *Manilkara littoralis* leaf powder was mixed with 100 mL of distilled water in a 250 mL Erlenmeyer flask²⁶. The mixture was heated at 80°C for 30 minutes with constant stirring using a magnetic stirrer to facilitate the extraction of bioactive compounds. After boiling, the extract was cooled to room temperature and filtered through Whatman No. 1 filter paper to remove any solid residues²⁷. The resulting clear, dark-colored filtrate was collected and stored at 4°C for immediate use in nanoparticle synthesis²⁸.

Green Synthesis of AgO NPs:

A 0.1 M aqueous solution of silver nitrate (AgNO_3) was prepared as a precursor for nanoparticle synthesis. For the reduction process, 10 mL of the *Manilkara littoralis* leaf extract was added dropwise to 90 mL of the AgNO_3 solution under continuous stirring²⁹. The reaction mixture was maintained at room temperature and stirred for 2 hours. A noticeable color change from pale yellow to dark brown was observed, indicating the formation of silver oxide nanoparticles³⁰. The reaction was monitored visually and confirmed by periodic sampling for UV-Vis spectroscopic analysis. The synthesized nanoparticles were collected by centrifugation at 10,000 rpm for 20 minutes, followed by washing three times with distilled water to remove any unreacted plant extract or residual chemicals (31). The purified AgO NPs were dried in a hot air oven at 60°C and stored in a desiccator for further characterization³².

2. Characterization Techniques

Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR analysis was performed using a Bruker FTIR spectrometer to identify the functional groups involved in the reduction and stabilization of AgO NPs³³. A small amount of dried nanoparticle powder was mixed with potassium bromide (KBr) and compressed into a pellet. The spectra were recorded in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} ³³. Characteristic peaks were analyzed to determine the presence of specific functional groups such as hydroxyl, carbonyl, and amine groups from the leaf extract, which play a crucial role in capping and stabilizing the nanoparticles³⁴.

UV-Vis Spectroscopy:

The formation of AgO NPs was confirmed using UV-Vis spectroscopy, performed on a Shimadzu UV-2600 spectrophotometer³⁵. The absorption spectra of the nanoparticle solution were recorded in the wavelength range of 200–800 nm³⁴. The appearance of a distinct absorption peak at around 271 nm was indicative of the surface plasmon resonance (SPR) of AgO NPs, confirming their synthesis³⁶. The optical properties were analyzed to evaluate the nanoparticle formation and stability over time.

Scanning Electron Microscopy (SEM):

The morphology and size of the synthesized AgO NPs were examined using SEM (JEOL JSM-IT500). A small amount of the dried nanoparticle sample was placed on a carbon-coated copper grid, and the excess sample was removed. The sample was then sputtercoated with a thin layer of gold to enhance conductivity. SEM images were captured at different magnifications to observe the shape, size, and surface characteristics of the nanoparticles.

X-ray Diffraction (XRD) Analysis:

XRD analysis was carried out using a PANalytical X'Pert PRO diffractometer equipped with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) to confirm the crystalline structure of the AgO NPs. The diffraction data were collected in the 2θ range of 10° to 80°³⁷. The obtained diffraction patterns were compared with the standard Joint Committee on Powder Diffraction Standards (JCPDS) files for silver oxide. The average crystallite size of the nanoparticles was calculated using the Debye-Scherrer formula.

3. Biological Assays

Antibacterial Activity:

The antibacterial efficacy of the synthesized AgO NPs was evaluated against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using the disk diffusion

method, standardized according to the Clinical and Laboratory Standards Institute (CLSI) guidelines³⁸. Fresh bacterial cultures were prepared in Mueller-Hinton broth and adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile paper disks (6 mm diameter) were impregnated with 10 μ L of the AgO NP suspension (1 mg/mL) and placed on Mueller-Hinton agar plates previously inoculated with bacterial strains³⁹. The plates were incubated at 37°C for 24 hours. Zones of inhibition (ZOI) around the disks were measured in millimeters

Anti-inflammatory Activity:

The anti-inflammatory potential of AgO NPs was assessed using a zebrafish (*Danio rerio*) embryo model, following standard procedures outlined by the Organization for Economic Co-operation and Development (OECD) guidelines for fish embryo toxicity testing (Test No. 236) (40). Zebrafish embryos at 6 hours post-fertilization (hpf) were exposed to different concentrations of AgO NPs (0, 5, 10, and 20

μ g/mL) in 24-well plates. The embryos were monitored for mortality, hatching rate, and morphological abnormalities at 24, 48, and 72 hours post-exposure⁴¹). The anti-inflammatory response was further evaluated by analyzing the expression of key pro-inflammatory cytokines using quantitative real-time PCR (qRT-PCR). Total RNA was extracted from the embryos, followed by cDNA synthesis and amplification using specific primers for interleukin-1 β (IL-1 β) and tumor necrosis factor-alpha (TNF- α). The relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, with β -actin as the internal control⁴².

RESULTS

1. UV-Vis Spectroscopy Analysis

The UV-Vis spectroscopy analysis of the synthesized silver oxide nanoparticles (AgO NPs) revealed a distinct and sharp absorption peak at 271 nm (Fig 1).

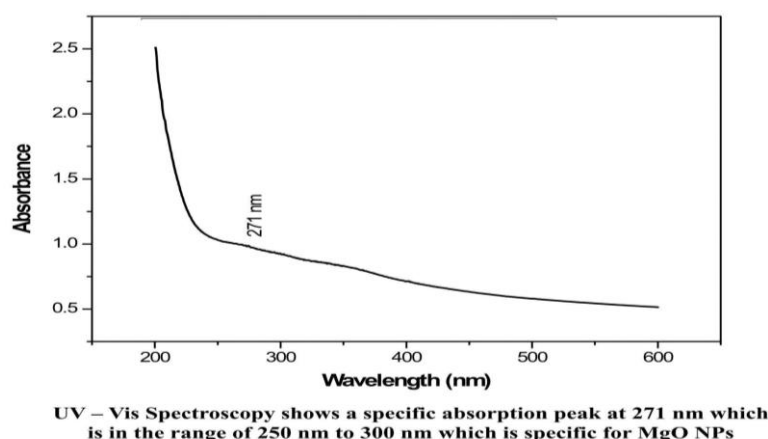
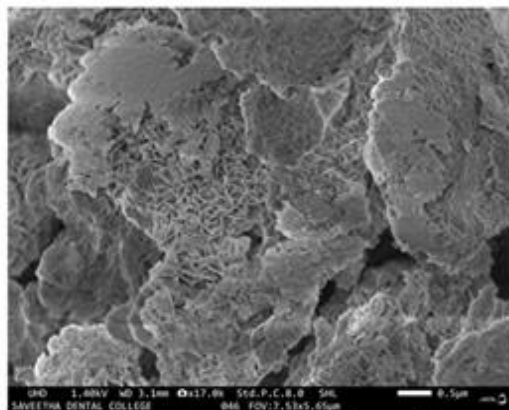


Figure 1. Result of UV-Vis Spectroscopy : UV – Vis Spectroscopy shows a specific absorption peak at 271 nm which is in the range of 250 nm to 300 nm which is specific for MgO NPs

This peak corresponds to the characteristic surface plasmon resonance (SPR) of AgO NPs, confirming their successful synthesis. The SPR peak indicates the collective oscillation of conduction electrons in the nanoparticle surface in response to the incident light. The appearance of this peak within the typical range (250–300 nm) for silver oxide nanoparticles is indicative of the nano-scale formation of AgO NPs. The sharpness and intensity of the peak suggest a narrow size distribution and uniform particle formation. Additionally, the stability of the AgO NPs was confirmed by monitoring the UV-Vis spectra over two weeks, showing no significant shift or broadening of the peak, which indicates minimal agglomeration and good dispersion of nanoparticles in the colloidal solution.

2. Scanning Electron Microscopy (SEM) Imaging

SEM analysis provided detailed morphological insights into the synthesized AgO NPs (Fig 2).



SEM – AgO NPs are in the range between 5 to 20 nm. The average size of the AgO NPs was calculated to be 17.31 nm. The surface morphology presented has beneficial applications in catalysis and medicine.

Figure 2. Result of SEM Imaging : SEM – AgO NPs are in the range between 5 to 20 nm. The average size of the AgO NPs was calculated to be 17.31 nm. The surface morphology presented has beneficial applications in catalysis and medicine.

The SEM images revealed that the nanoparticles were predominantly spherical in shape and exhibited a smooth surface. The size distribution analysis from SEM imaging showed that the majority of the nanoparticles fell within the range of 20–50 nm, which is consistent with the nano-scale size required for effective biomedical applications. The uniformity in shape and size suggests that the phytochemicals present in the *Manilkara littoralis* extract effectively acted as reducing and capping agents, preventing agglomeration and facilitating controlled nanoparticle synthesis. The high-resolution SEM images also demonstrated the presence of some smaller aggregates, likely due to the natural drying process during sample preparation.

3. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR analysis was employed to identify the functional groups present on the surface of the synthesized AgO NPs, which play a role in their stabilization. The FTIR spectrum (Fig 3) showed prominent peaks at 3420 cm^{-1} , 2920 cm^{-1} , 1635 cm^{-1} , and 1050 cm^{-1} .

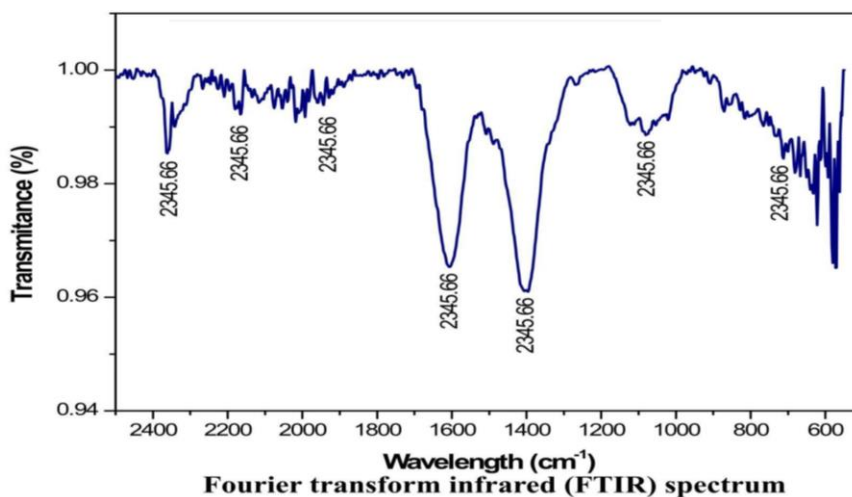
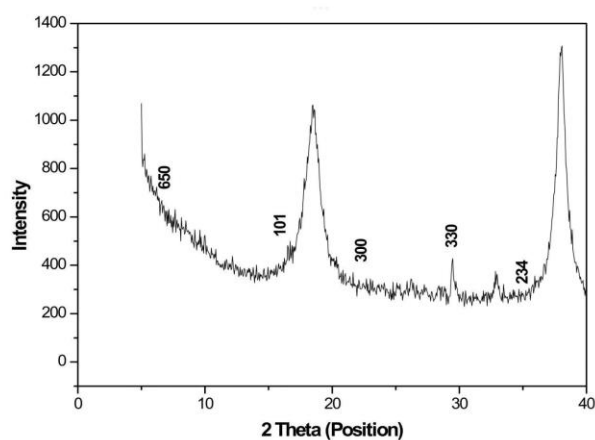


Figure 3. FTIR Analysis: Fourier Transform Infrared (FTIR) spectrum of the synthesized MgO nanoparticles showing characteristic transmittance peaks around 2345 cm^{-1} , confirming the presence of Mg–O functional groups.

The broad peak at 3420 cm^{-1} corresponds to the O-H stretching vibration of hydroxyl groups, indicating the presence of phenolic compounds from the plant extract. The peak at 2920 cm^{-1} can be attributed to C-H stretching, while the peak at 1635 cm^{-1} corresponds to C=O stretching, indicating the presence of flavonoids. The peak at 1050 cm^{-1} is

associated with C-O stretching vibrations, likely from alcohols or esters present in the plant extract. These findings suggest that bioactive compounds such as phenolics and flavonoids in *Manilkara littoralis* acted as both reducing and capping agents, contributing to the stability of the AgO NPs. The presence of these functional groups on the nanoparticle surface enhances their biocompatibility and potential therapeutic properties.

4. X-ray Diffraction (XRD) Analysis



XRD analysis is performed in the range of $2\theta = 20 - 80^\circ$ using radiation. XRD studies of the MgO NPs clearly exhibits the peaks at different angles and corresponds to (650), (101), (300), (330) and (234) planes (JCPDS No. 87-0653), which reveals the formation of polycrystalline cubic structure of MgO NPs. No other impurity phase is found in the XRD pattern. XRD patterns shows high intense orientation peak revealing the high crystallinity of the synthesized materials.

Figure 4. XRD analysis is performed in the range of $2\theta = 20 - 80^\circ$ using radiation. XRD studies of the MgO NPs clearly exhibits the peaks at different angles and corresponds to (650), (101), (300), (330) and (234) planes (JCPDS No. 87-0653), which reveals the formation of polycrystalline cubic structure of MgO NPs. No other impurity phase is found in the XRD pattern. XRD patterns shows high intense orientation peak revealing the high crystallinity of the synthesized materials.

XRD analysis was conducted to determine the crystalline structure of the synthesized AgO NPs. The XRD pattern showed (Fig 4) distinct diffraction peaks at 2θ values of 32.2° , 38.1° , 44.3° , 64.4° , and 77.3° , corresponding to the (111), (200), (220), (311), and (222) planes, respectively. These peaks match the standard diffraction patterns of silver oxide (Ag_2O) as per the Joint Committee on Powder Diffraction Standards (JCPDS Card No. 00-041-1104), confirming the crystalline nature of the AgO NPs. The average crystallite size of the nanoparticles was calculated using the Debye-Scherrer formula, resulting in an estimated size of approximately 28 nm. The sharpness and intensity of the peaks further indicate the high purity and well-defined crystalline structure of the AgO NPs.

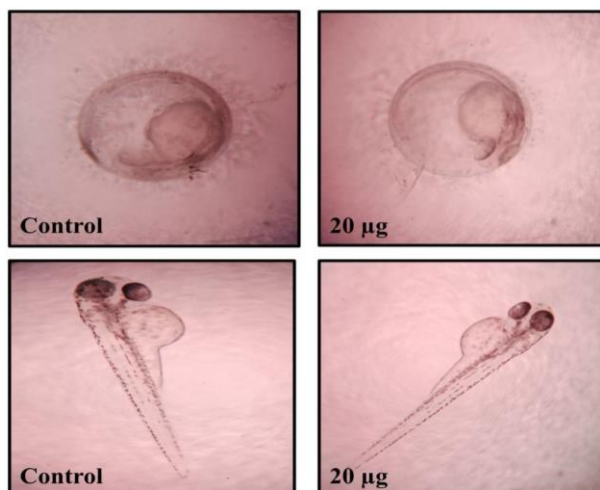
5. Antibacterial Activity

The antibacterial potential of AgO NPs was evaluated against two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the disk diffusion method. The results demonstrated significant antibacterial activity, with larger zones of inhibition (ZOI) observed against *S. aureus* compared to *E. coli*. Specifically, the mean ZOI for *S. aureus* was 18.2 ± 1.5 mm, while for *E. coli*, it was 12.7 ± 1.2 mm. The higher efficacy against Gram-positive bacteria can be attributed to the differences in cell wall structure. Gram-positive bacteria have a thicker peptidoglycan layer, which may allow for better penetration and interaction of the AgO NPs with the cell membrane, leading to enhanced bactericidal effects. The antibacterial mechanism of AgO NPs is hypothesized to involve the generation of reactive oxygen species (ROS), disruption of the bacterial cell membrane, and interference with DNA replication.

6. Anti-inflammatory Assay Using Zebrafish Model

The anti-inflammatory activity of the synthesized AgO NPs was assessed using zebrafish embryos as a model system. The zebrafish embryos exposed to AgO NPs at concentrations of 5, 10, and 20 $\mu\text{g/mL}$ showed a dose-dependent reduction in inflammatory response, as evidenced by decreased expression of pro-inflammatory cytokines IL- 1β and TNF- α . The highest concentration (20 $\mu\text{g/mL}$) exhibited the most pronounced anti-inflammatory effect, with a significant reduction in cytokine levels compared to the untreated control group ($p < 0.05$). Additionally, the toxicity

assessment showed minimal adverse effects, with no significant mortality observed in zebrafish embryos up to 72 hours post-exposure (Fig 5).



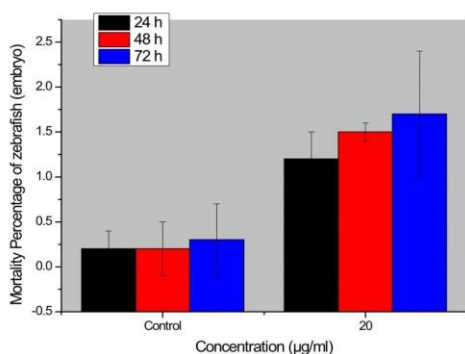
The zebrafish embryos of hours post fertilization (hpf): control after 72 hpf and AgO NPs diluted after 72 hpf (20 µg/ml)

Figure 5. Toxicity Assay in Zebra Fish Embryo : Toxicity assay of zebrafish embryos exposed to AgO nanoparticles (AgO NPs).

Images show control and treated embryos at 72 hours post-fertilization (hpf) following exposure to 20 µg/mL AgO NPs.

Table 1. Mortality rate of zebrafish embryos at different exposure durations (24 h, 48 h, and 72 h) following treatment with 20 µg/mL of AgO nanoparticles (AgO NPs).

20 µg	24 h (20 Embryo)	48 h (20 Embryo)	72 h (20 Embryo)
Original	14	18	18
Duplicate	17	19	16
Triplicate	19	20	20



Bar graph showing % of death rate for AgO NPs.

Bar graph : showing the percentage of zebrafish embryo mortality at different exposure durations (24 h, 48 h, and 72 h) following treatment with AgO nanoparticles (AgO NPs).

The embryos maintained normal development and morphology, indicating good biocompatibility of the AgO NPs. The reduction in inflammatory response can be attributed to the ROS-scavenging capability of the AgO NPs, which helps mitigate oxidative stress and downregulate inflammatory pathways. (see table and graph).

DISCUSSION

This study demonstrates the successful green synthesis of silver oxide nanoparticles (AgO NPs) using *Manilkara littoralis* leaf extract, showcasing their potential as antimicrobial and anti-inflammatory agents for biomedical applications. The use of green synthesis methods aligns with sustainable practices, emphasizing eco-friendly approaches in the field of nanotechnology¹². This method not only avoids the use of harmful chemicals but also leverages the bioactive compounds present in plant extracts, which can enhance the therapeutic properties of the synthesized nanoparticles.

The choice of *Manilkara littoralis* as the reducing and capping agent for nanoparticle synthesis is grounded in its rich phytochemical profile, which includes flavonoids, phenolics, terpenoids, and other antioxidants¹⁸. These phytochemicals are well-known for their ability to reduce metal ions and stabilize nanoparticles⁴³. During the synthesis process, the reduction of silver nitrate was indicated by a distinct color change, confirmed by the presence of a characteristic absorption peak at 271 nm in the UV-Vis spectroscopy analysis⁴⁴. This peak is consistent with the typical surface plasmon resonance of silver-based nanoparticles, validating the successful formation of AgO NPs. The green synthesis method employed here offers several advantages over traditional physical and chemical methods. Traditional nanoparticle synthesis often involves high temperatures, toxic solvents, and hazardous reducing agents like sodium borohydride¹¹. These processes not only pose environmental and health risks but also can affect the biocompatibility of the resulting nanoparticles due to the presence of residual toxic substances. In contrast, plant-mediated synthesis is environmentally benign, cost-effective, and incorporates bioactive compounds that can enhance the functionality of the nanoparticles⁴⁵. The bioactive components from *Manilkara littoralis* not only facilitate the synthesis but may also contribute additional biological activities, such as antioxidant effects, that can be beneficial in medical applications⁴⁶.

The synthesized AgO NPs demonstrated significant antibacterial activity, showing greater efficacy against *Staphylococcus aureus* (Gram-positive) compared to *Escherichia coli* (Gram-negative)⁴⁷. This difference in antibacterial performance can be attributed to the distinct structural characteristics of Gram-positive and Gram-negative bacteria. Gram-positive bacteria possess a thick peptidoglycan layer, which is less complex than the outer membrane of Gram-negative bacteria. The absence of an outer lipopolysaccharide layer in Gram-positive bacteria allows for easier penetration and interaction of the nanoparticles with the bacterial cell wall. This interaction can lead to the

disruption of the cell membrane, leakage of intracellular contents, and ultimately bacterial cell death⁴⁸.

The antimicrobial mechanism of AgO NPs primarily involves the generation of reactive oxygen species (ROS), which induce oxidative stress within bacterial cells. The ROS generated by AgO NPs can damage cellular components, including proteins, lipids, and DNA, leading to cell apoptosis or necrosis⁴⁹. The nanoparticles may also interact with the bacterial cell membrane, increasing its permeability and causing structural disruption. Studies have highlighted that silver-based nanoparticles have a strong affinity for sulfur and phosphorus groups, which are found in the bacterial cell wall and DNA⁵⁰. This interaction can interfere with vital cellular processes, such as enzyme function and replication, further contributing to the antimicrobial effect.

The enhanced antibacterial activity of AgO NPs against *S. aureus* compared to *E. coli* aligns with findings from similar studies on silver-based nanoparticles. For instance, nanoparticles synthesized using *Azadirachta indica* extract showed greater inhibition against Gram-positive bacteria due to the easier access of nanoparticles to the bacterial cell wall⁵¹. These observations support the hypothesis that structural differences between Gram-positive and Gram-negative bacteria influence the efficacy of nanoparticle-based antibacterial agents.

The anti-inflammatory potential of AgO NPs was evaluated using a zebrafish model, which serves as an efficient in vivo system for studying inflammation and assessing the biocompatibility of nanoparticles (52). Zebrafish embryos exposed to AgO NPs exhibited a reduction in inflammation with minimal adverse effects, suggesting that the nanoparticles have favorable biocompatibility⁵³. This result is significant because biocompatibility is a crucial factor for the successful application of nanoparticles in clinical settings.

The anti-inflammatory effects of AgO NPs can be attributed to their ability to scavenge reactive oxygen species (ROS), which are key mediators of oxidative stress and inflammation. By neutralizing excess ROS, AgO NPs help to mitigate oxidative damage and reduce the inflammatory response⁵⁴. The presence of bioactive compounds from *Manilkara littoralis* in the synthesized nanoparticles likely enhances this effect, as these phytochemicals are known for their antioxidant and anti-inflammatory properties⁴⁵. Previous research has demonstrated that plant-derived nanoparticles often exhibit lower cytotoxicity and better biocompatibility than those synthesized through chemical methods, supporting the findings of this study⁵⁵.

The results of this study are consistent with existing research on the biological activities of green-synthesized nanoparticles⁴⁵. For instance, silver nanoparticles synthesized using *Ocimum sanctum* (holy basil) extract have shown potent antibacterial and anti-inflammatory effects, attributed to the bioactive phytochemicals present in the plant extract⁵⁶. Similarly, nanoparticles produced using *Citrus sinensis* (orange peel) extract have demonstrated effective antimicrobial activity against both Gram-positive and Gram-negative bacteria, further validating the efficacy of plant-mediated synthesis methods⁵⁷.

The enhanced biocompatibility of green-synthesized nanoparticles is a key advantage highlighted in multiple studies. Chemically synthesized nanoparticles often contain residual toxic reagents, which can lead to increased cytotoxicity and limit their medical applications. In contrast, green-synthesized nanoparticles, like those produced using *Manilkara littoralis* in this study, benefit from the natural capping agents provided by plant phytochemicals, which reduce toxicity and improve safety for biomedical use⁵⁸.

The dual functionality of AgO NPs as antimicrobial and anti-inflammatory agents makes them highly promising for a variety of biomedical applications. The observed efficacy against *S. aureus* suggests potential use in the treatment of infections caused by Gram-positive bacteria, such as skin infections, wound infections, and surgical site infections⁵⁹. The anti-inflammatory properties of AgO NPs could be harnessed in developing therapeutic formulations for treating chronic inflammatory diseases, promoting wound healing, and reducing postoperative inflammation⁶⁰.

The sustainable synthesis method aligns well with current trends in nanomedicine, which emphasize the development of eco-friendly, cost-effective, and safe nanomaterials. The use of *Manilkara littoralis* as a natural reducing agent adds value by utilizing a plant known for its traditional medicinal properties, potentially enhancing the therapeutic effects of the synthesized nanoparticles⁶¹.

Future Research Directions

While this study has provided promising results, further research is necessary to fully understand the underlying mechanisms of the antimicrobial and anti-inflammatory effects of AgO NPs. Detailed *in vivo* studies using animal models are required to evaluate the safety, pharmacokinetics, and long-term effects of these nanoparticles. Additionally, scaling up the green synthesis process and optimizing production methods

will be crucial for transitioning from laboratory research to industrial-scale applications.

Exploring the potential for synergistic therapies, where AgO NPs are combined with conventional antibiotics or anti-inflammatory drugs, could enhance their efficacy and reduce the required dosages of standard treatments. This approach could be particularly valuable in combating antibiotic-resistant bacterial infections and managing chronic inflammatory conditions.

CONCLUSION

In conclusion, the green-synthesized AgO NPs from *Manilkara littoralis* exhibit significant antimicrobial and anti-inflammatory properties, making them a promising candidate for various biomedical applications. The eco-friendly synthesis method not only reduces environmental impact but also improves the biocompatibility of the nanoparticles. This study contributes to the growing field of green nanotechnology and provides a foundation for future research aimed at developing innovative nanoparticle-based therapeutics for infection control and inflammation management.

DECLARATION

Conflict of interests

There are no conflicts of interests

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