In vitro biological evaluation of silver nanoparticles synthesized using *zingiber officinale* and *ocimum gratissimum* herbal formulation

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ABSTRACT

Introduction: The biomedical potential of silver nanoparticles (Ag NPs) synthesized with *Zingiber officinale* and *Ocimum gratissimum* herbal formulation was investigated in this study. The study aims to reveal their applications in various biomedical fields. The study evaluates the antioxidant, thrombolytic, and antimicrobial potential of *Zingiber officinale* and *Ocimum gratissimum* herbal formulation-mediated Ag NPs.

Materials and Methods: Biogenically synthesized silver nanoparticles (Ag NPs) from an herbal formulation containing *Zingiber officinale* and *Ocimum gratissimum* were tested at various concentrations using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The absorbance was measured at 517 nm to quantify DPPH free radicals. With Ag NP concentrations, the H_2O_2 test exhibited increased activity. This work evaluated the antibacterial activity of Ag NPs mediated by *Zingiber officinale* and *Ocimum gratissimum* against *Staphylococcus aureus, Streptococcus mutans, Candida albicans*, and *Enterococcus faecalis*.

Results: The utilization of herbal formulations from *Z. officinale* and *O. gratissimum* to synthesize Ag NPs revealed considerable therapeutic effectiveness. At a concentration of 50 µl, the maximal inhibition was 76%, which is comparable in effectiveness to that of standard ascorbic acid. Significant blood clot dissolution was observed during thrombolytic testing at a concentration of 100µg/ml, indicating promising prospects for the treatment of thrombotic disorders. Nanoparticles dose-dependently inhibited *E. faecalis, C. albicans, S. aureus*, and *S. mutans* in antibacterial testing. These results show the potential of the nanoparticles as supplementary or alternative treatments to conventional antibiotics, particularly in light of the increasing prevalence of antibiotic resistance.

Conclusion: The further investigation of nanoparticles into their mechanisms and efficacy in therapeutic applications, positioning *Zingiber officinale* and *Ocimum gratissimum* formulation-mediated Ag NPs as viable candidates in developing antioxidant, thrombolytic, and antimicrobial treatments.

KEYWORDS:
Green synthesis, silver nanoparticles, oral pathogens, Zingiber officinale, Ocimum gratissimum, biomedical applications

INTRODUCTION

Nanotechnology has transformed many areas of science and medicine by providing new and positive ways to diagnose and treat illnesses. Among nanomaterials, silver nanoparticles (Ag NPs) have attracted much interest in the medical field because of their anti-inflammatory, antibacterial, and antioxidant properties.[1] These characteristics make them especially well-suited for use in biological applications. Traditional methods of synthesizing nanoparticles often involve chemical or physical processes, which may pose hazards and incur high costs. However, green synthesis methods utilizing plant formulation offer an eco-friendly and cost-effective alternative.[2] Plant-mediated synthesis of nanoparticles connects the reducing properties of phytochemicals present in the formulation, resulting in stable and biocompatible nanoparticles. There are several medicinal and dental uses for silver due to its antibacterial characteristics, which have been known for a long time.[3] Ag NPs are an attractive choice for treating bacterial infections because they have more antibacterial action than bulk silver.[4] Although their special characteristics might lead to physiological reactions in living systems by interacting with these materials. The size-dependent physicochemical features of nanoparticles boost their applicability in many applications.[5] Different approaches to produce Ag NPs have been successfully combined into a range of biomaterials, enabling their use in many biological applications. Their effects on dental use and wound healing have been extensively investigated. The unique properties of Ag NP-based biomaterials enable the healing of acute and chronic wounds and greatly limit the development of germs at wound sites.[6]

The mechanisms underlying their antibacterial action involve interactions with bacterial cell walls, oxidative stress induction, and inhibition of microbial growth.[7] In addition to their antimicrobial properties, Ag NPs also possess free radical scavenging activity. Free radicals generated during normal cellular metabolism or due to environmental factors can cause cellular damage, leading to various health issues.

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Antioxidants, including Ag NPs, help neutralize these free radicals, thereby protecting cells from oxidative stress-induced damage.[8] Furthermore, the thrombolytic and anticoagulant properties of Ag NPs present a quiet area of research, especially in the context of thrombocytopenia,[9] it is a condition characterized by a low platelet count, which can lead to bleeding disorders and increased thrombosis risk. Studies have shown that Ag NPs can influence platelet function and thrombin generation, highlighting their potential in modulating blood coagulation processes.[10] This dual role of Ag NPs, as both pro-coagulant and anti-platelet agents, the complexity of their interaction with the hematological system, and the need for a thorough investigation to connect their therapeutic potential safely. Ginger (Zingiber officinale) has been employed as a culinary seasoning for over 2000 years.[11] The roots and extracts derived from this plant contain polyphenol chemicals, including 6-gingerol and its derivatives, which have significant antioxidant properties. Ocimum gratissimum is commonly referred to as a scent leaf.[12] The incorporation of Ocimum oil, derived from the leaf essential oil of Ocimum gratissimum, has been seen in several formulations as topical antiseptics and for the management of small wounds, boils, and pimples.[13] This research focuses on the effects of Zingiber officinale and Ocimum gratissimum formulation-mediated Ag NPs on thrombolytic activity, free radical scavenging activity, and antimicrobial activity.

MATERIALS AND METHODS

Preparation of Plant Extract

0.5 grams of each *Zingiber officinale* and *Ocimum gratissimum* were added to 100 ml of pure water. The heating mantle was set at 50 °C and boiled for 10 minutes. A muslin cloth was used to filter out the mixture. The filtered extracts of *Z. officinale* and *O. gratissimum* were mixed with a solution of silver nitrate ($AgNO_3$) to start the synthesis process for the Ag NPs.

Synthesis of Ag NPs

The volume of 10 mL of the plant formulation was combined with 90 mL of 1 mM $AgNO_3$ under continuous stirring. The reaction mixture was subsequently incubated at room temperature in the absence of light to inhibit the photoactivation of silver nitrate. The formation of Ag NPs was confirmed by the transformation of the clear solution to a brown color after 48 h.

Characterization of Ag NPs

UV-visible spectroscopy, an analytical method for analyzing the optical characteristics of NPs, was used to characterize the synthesized NP solution. The NPs solution (3 mL) was placed in a cuvette and scanned using a double-beam UV-Vis spectrophotometer. Spectrophotometric scans from 250 nm to 650 nm allowed a complete evaluation of the NP solution's visible spectrum absorbance in a time-dependent manner, such as 3, 12, 24, 30, 36, and 48 h. Graphical analysis was conducted for the recorded results. The morphologies of the synthesized Ag NPs were analyzed using a scanning electron microscope.

Antioxidant Activity

DPPH Method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) test was used to measure the antioxidant activity of AgNPs produced by biogenic synthesis methods. Different concentrations (10, 20, 30, 40, and 50 µL) of silver nanoparticles derived from *Z. officinale* and *O. gratissimum* herbal formulations were combined with 1 ml of 0.1 mM DPPH in methanol and 450 µL of 50 mM Tris HCl buffer (pH 7.4). The mixture was then incubated for 30 min. DPPH free radicals were determined by measuring the absorbance at 517 nm. Ascorbic acid was used as a standard.[14]

The percentage of inhibition was calculated using the formula:

Percentage of inhibition = (Absorbance of control – Absorbance of the test sample) / Absorbance of control × 100

H_2O_2 Assay

The H_2O_2 scavenging activity of the biosynthesized AgNPs was assessed. 40 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). A solution of the test sample (Ag NPs) and a standard sample of ascorbic acid at varying concentrations (10, 20, 30, 40, and 50 µg/mL) were individually added to 0.6 mL the H_2O_2 solution. After 10 min of incubation in the dark, the absorbance of the reaction solution was spectrophotometrically measured at 230 nm. Ascorbic acid was used as a standard.[15,16] The percentage of H_2O_2 scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Thrombolytic Activity

The thrombolytic activities of *Z. officinale* and *O. gratissimum* formulation-mediated AgNPs were evaluated using previously established protocols. Preformed blood clots were treated with 100µg/ml *Z. officinale* and *O. gratissimum* formulation-mediated AgNPs placed on a slide for analysis. All the experiments were performed at a controlled temperature of 30 ± 2°C. The process of thrombolysis, which refers to the dissolution of blood clots, was carefully monitored throughout the experiment.[17]

Antibacterial Activity

To assess the antibacterial efficacy of *Z. officinale* and *O. gratissimum* – Ag NPs against strains such as *E. faecalis, C. albicans, S. aureus,* and *S. mutans,* Mueller-Hinton agar was chosen for its reliability in determining zones of inhibition. The procedure began with the preparation and sterilization of Mueller-Hinton agar, which was autoclaved for 15 min at 121°C under a pressure of 15 lbs. Sterile 9 mm polystyrene tip was used to create the wells in the solidified agar. Subsequently, the test organisms were evenly swabbed across the agar surface. Different concentrations of AgNPs were added to each well. The plates were kept warm for 24 h at 37 °C. Once the incubation time was completed, the zones of inhibition were carefully measured. These images show the areas around the wells where Ag NPs stopped bacterial growth.[18]

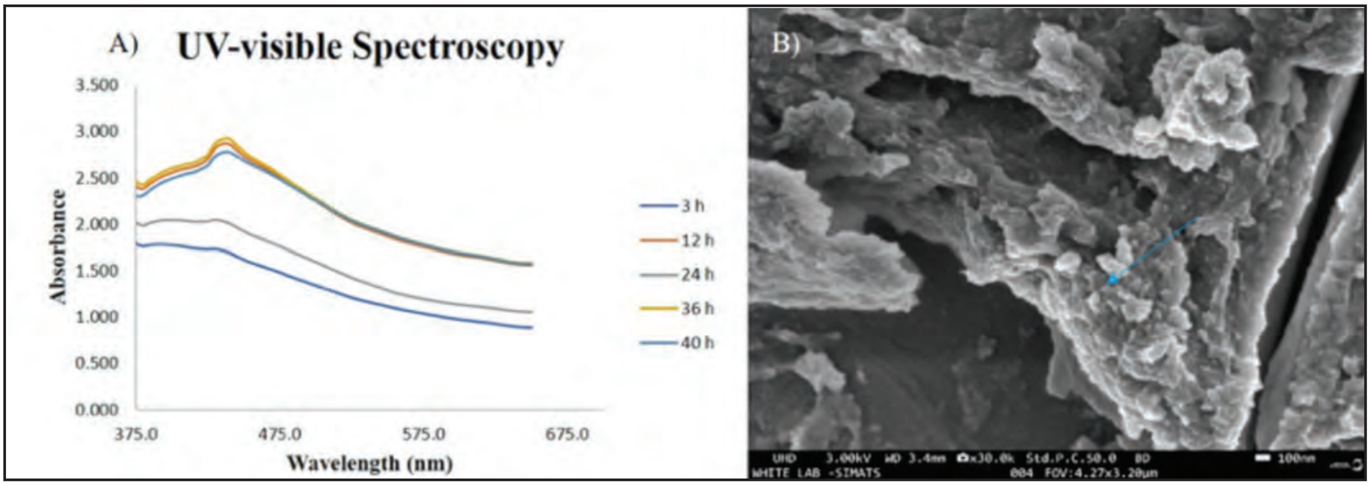

Fig. 1: A) UV-visible spectroscopy of Ag NPs. B) SEM image of AgNPs synthesized from Zingiber officinale and Ocimum gratissimum herbal formulation.

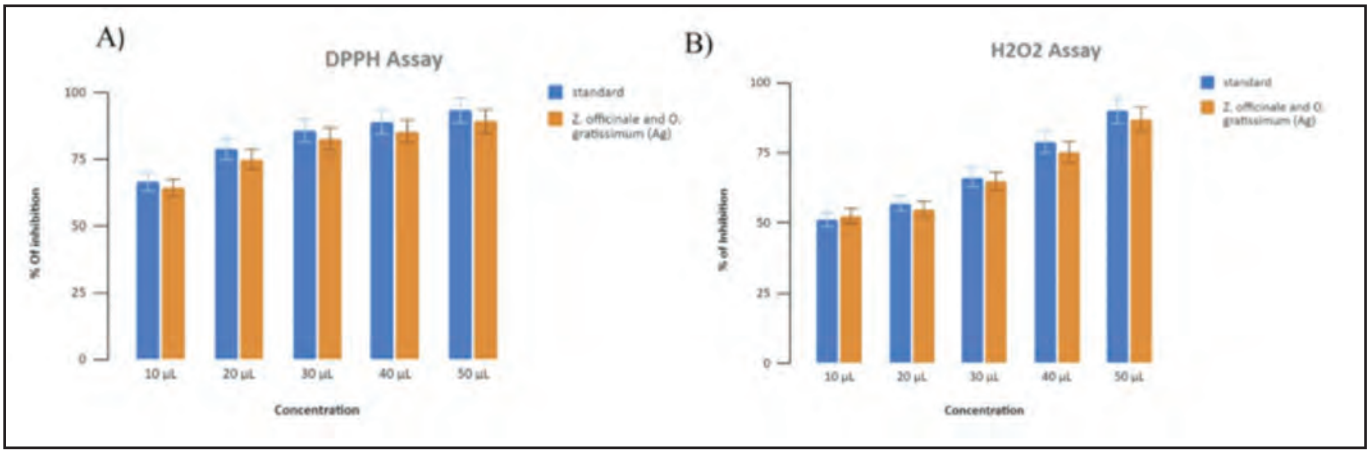

Fig. 2: A) DPPH radical scavenging activity of Zingiber officinale and Ocimum gratissimum mediated Ag nanoparticles. B) H_2O_2 activity of Zingiber officinale and Ocimum gratissimum mediated AgNPs

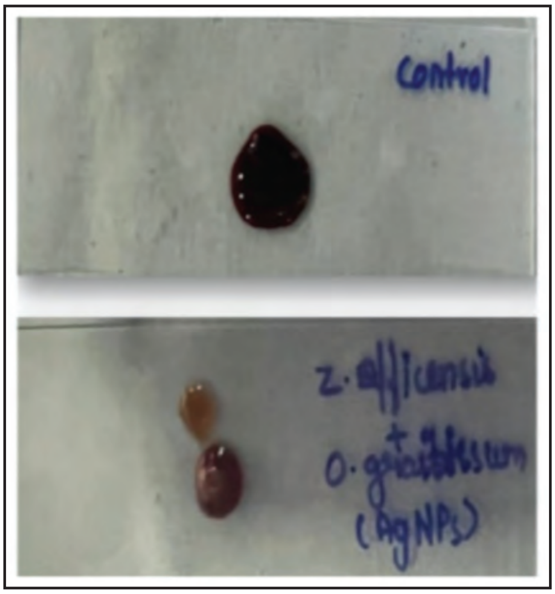

Fig. 3: Thrombolytic activity of Zingiber officinale and Ocimum gratissimum mediated silver nanoparticles

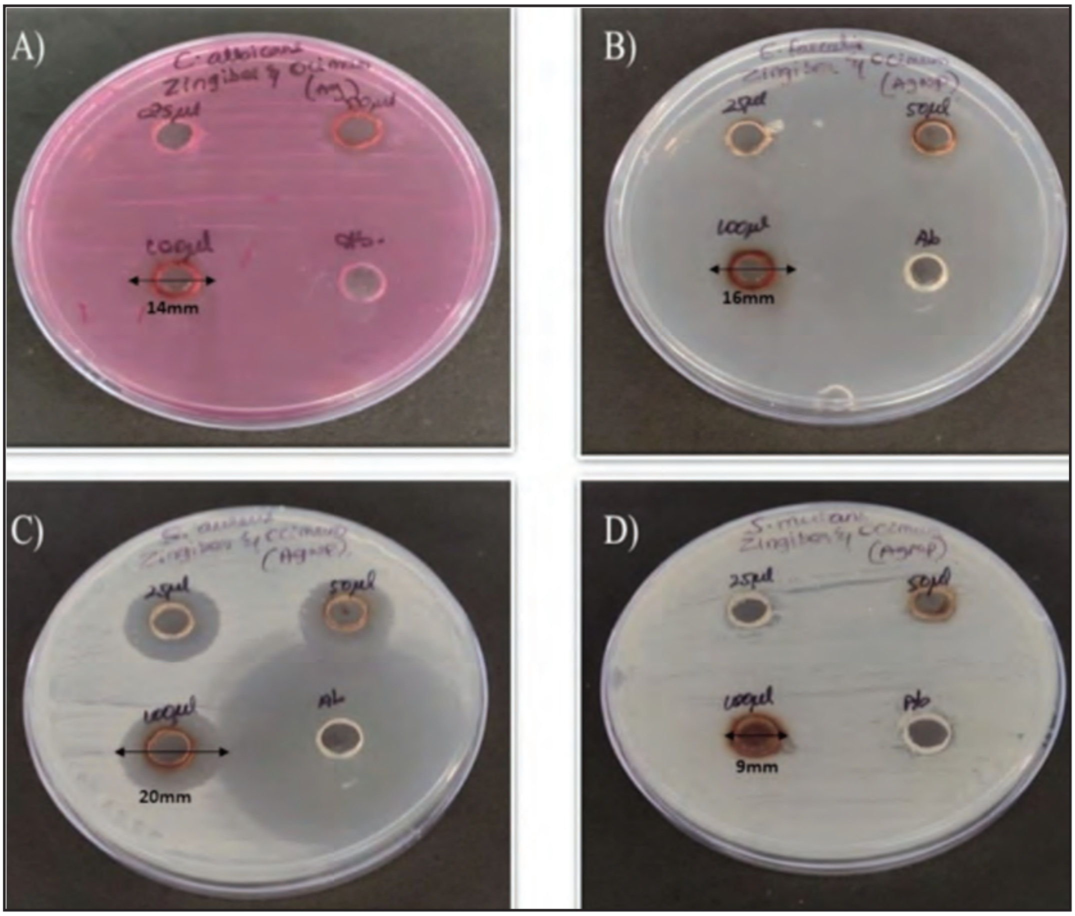

Fig. 4: Antimicrobial activity of Zingiber officinale and Ocimum gratissimum mediated Ag NPs against oral pathogens A) C. albicans B) E. faecalis C) S. aureus D) S. mutans

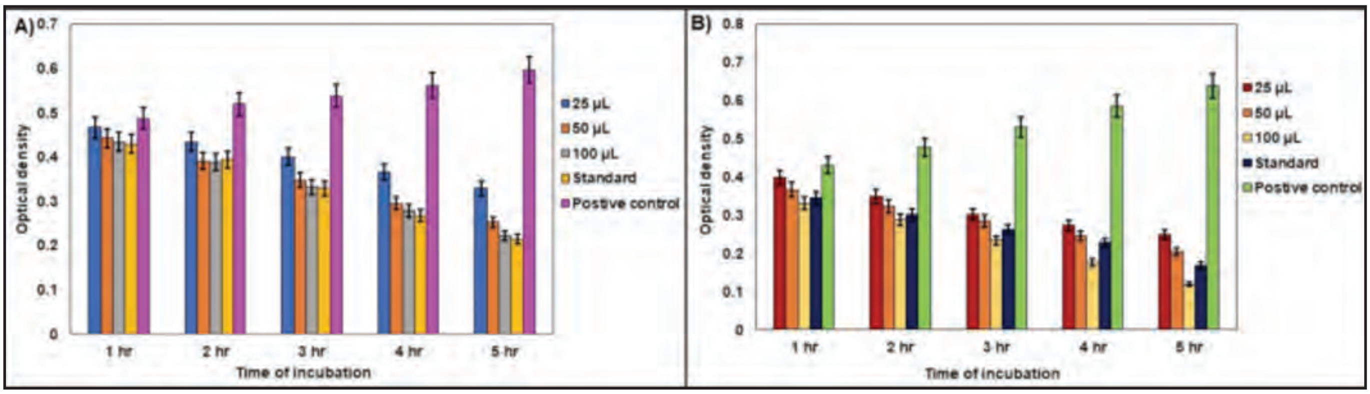

Fig. 5: Antimicrobial activity of Zingiber officinale and Ocimum gratissimum mediated Ag NPs against Oral pathogens

RESULTS

Green Synthesis of Ag NPs

Effective synthesis of Ag nanoparticles using Zingiber officinale and Ocimum gratissimum was carried out. The presence of Ag nanoparticles was indicated by the brown color. Visual observation of Ag nanoparticles has confirmed that they are a green and environmentally benign herbal formulation.

Analysis of UV–Visible Spectroscopy

In UV-visible spectroscopy, the synthesized Ag NPs exhibited considerable absorbance variation over time over a wide wavelength range (250–650 nm). After 3 h, the absorbance levels were low, suggesting reduced aggregation or particle size. However, the absorbance increased after 12 h and peaked at 440 nm (Figure 1A). The spectroscopic results indicated that the growth or aggregation processes produced valuable optical changes in the Ag NPs throughout the experiment. As Ag NPs interact strongly with UV light, their maximal absorption indicates their size and concentration variation. This shows that the Ag NPs attained a fixed size or aggregation state, strongly absorbing and scattering UV light. The synthesized AgNPs had a consistent size distribution and were spherical, as revealed by SEM examination (Figure 1B). Nanoparticles have little possibility of clumping or clustering because they are evenly distributed throughout the substrate. The surface morphology of the AgNPs was evident in the high-resolution SEM images, which showed smooth, spherical particles free of extraneous flaws. The spherical shape of the nanoparticles indicates that the synthesis technique successfully produced monodisperse AgNPs. The SEM images reveal a steady size distribution, indicating a continuous synthesis process.

Antioxidant activity

The antioxidant efficacy of Zingiber officinale and Ocimum gratissimum formulation-mediated AgNPs was assessed using DPPH and H_2O_2 assay, revealing a dose-dependent enhancement in antioxidant activity. In the DPPH assay, the antioxidant activity of the formulation-mediated Ag NPs demonstrated a significant increase, with the highest inhibition observed at 76% for a concentration of 50 µL, a considerable increase from the 51% inhibition at 10 µL. This activity was compared against the standard antioxidant ascorbic acid, which showed an 80% inhibition (Figure 2A). Similarly, the H2O2 assay indicated a progressive increase in activity with higher concentrations of herbal formulation -Ag NPs a significant scavenging rate of 76.84% at 50 µL compared to 51 % at 10 µL, with ascorbic acid as the control showing an 81% scavenging rate at 50 µL (Figure 2B). These findings indicate the antioxidant potential of formulation-mediated AgNPs.

Thrombolytic activity

The thrombolytic activity of Z. officinale and O. gratissimum herbal formulation-mediated AgNPs showed that blood clots were treated with 100 µg/ml concentration, and a significant degree of thrombolysis was observed (Figure 3). The potential of Ag NPs as promising agents for the treatment of thrombotic conditions.

Antimicrobial activity

A study examining the antimicrobial activity of Z. officinale- and O. gratissimum-mediated Ag NPs showed a clear zone of inhibition against various pathogens. Three different concentrations of Ag NPs were tested: 25, 50, and 100 µL. Against E. faecalis, the inhibition zones increased with the concentration of Ag NPs, with measurements of 11 mm, 14 mm, and 16 mm for the increasing concentrations, respectively, compared with a significantly larger zone of 35 mm observed for the antibiotic control. A similar dose-dependent activity was noted against C. albicans, with zones of inhibition measuring 9, 12, and 14 mm, notably against S. mutans, with a zone of inhibition of 9 mm across all concentrations. The maximum zones of inhibition observed for S. aureus against AgNPs were 18, 19, and 20 mm at all concentrations. (Figures 4 and 5).

DISCUSSION

A detailed investigation of the various uses of AgNPs mediated by herbal formulations from *Z. officinale* and *O. gratissimum* explains their potential in the thrombolytic, antibacterial, and antioxidant domains. These biogenic Ag NPs are effective radical scavengers, as shown by their antioxidant effectiveness in DPPH and H_2O_2 experiments.[14,15] Their activity was compared with that of ascorbic acid, a well-established standard for antioxidant measurements. This demonstrates the potential of these nanoparticles to reduce oxidative stress, and suggests a possible direction for the development of antioxidant treatments. These results highlight the natural antioxidant qualities of plant formulations and the increased effectiveness of nanoparticle mediation, recommending further research into their potential medicinal uses.[16]

Regarding thrombolytic activity, the significant disintegration of blood clots treated with these AgNPs suggests that they may be useful as new medicines to treat thrombotic disorders. This clot dissolution highlights the therapeutic potential of using plant herbal formulation-mediated nanoparticles to improve clot breakdown, particularly when compared with the AgNO3 control solution. These nanoparticles significantly affect the treatment of illnesses marked by undesired blood clot formation and require further investigation into the underlying processes and therapeutic optimization.[17]

Evaluation of antimicrobial activity against pathogens including *C. albicans, S. aureus*, and *E. faecalis* shows distinct effectiveness and significant antibacterial potential of Ag NPs produced from Zingiber officinale and Ocimum gratissimum. This capacity, which presents a viable substitute or addition to conventional antibiotic therapies, is vital in light of growing antibiotic resistance.[18] The effectiveness of the nanoparticles against *S. aureus* and *E. faecalis* was significant; however, it was not as strong against S. mutans. This suggests that there may be a complex connection between nanoparticles and the structures or processes of microbial cells.[19] This emphasizes the complex relationships between microorganisms and nanoparticles, and how focused studies are required to maximize the antibacterial activity of nanoparticles while maintaining their safety for therapeutic usage.[20,21,22]

CONCLUSION

Our results highlight the broad-spectrum effectiveness of Ag NPs mediated by Ocimum gratissimum and Zingiber officinale herbal formulations for thrombolytic, antioxidant, and antibacterial applications. Their action is dose-dependent, which emphasizes the significance of concentration in attaining the intended therapeutic effects, and provides a crucial criterion for further study and application development. These nanoparticles provide a viable option for various therapeutic interventions because of their proven capacities, providing new approaches to enduring problems in the treatment of thrombosis, infection prevention, and oxidative stress management.

CONFLICT OF INTEREST

The authors declare that no conflicts of interest would prejudice the impartiality of this scientific work.

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