Ocimum gratissimum mediated synthesis of AgNPs – An *in vitro* analysis of anti-inflammatory and antimicrobial effects

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ABSTRACT

Introduction: Silver nanoparticles (AgNPs) are effective against almost all kinds of pathogenic organisms. The green synthesis of AgNPs utilizing extracts from medicinal plants is being researched to examine the therapeutic advantages of AgNPs because the chemical production of AgNPs is more toxic. In this study, the stem extract of *Ocimum Gratissimum* (OG) also known as *Karunthulasi* or wild basil for green synthesis of AgNPs and evaluating their anti-inflammatory and antimicrobial effects.

Materials and Methods: The produced nanoparticles were characterized using UV-visible spectroscopy. The Bovine Serum Assay (BSA) and Egg Albumin (EA) assays were used to assess the anti-inflammatory effects. The protein denaturation of AgNPs was calculated and compared to a standard to determine the anti-inflammatory activity of green synthesized AgNPs. Using varying concentrations of OG-mediated AgNPs in Mueller Hinton Agar (MHA), the antimicrobial effects of OG have been investigated against *E. coli*, *S. aureus*, and *Pseudomonas sp.* Additionally, by measuring optical density, the time-kill curve analysis for *E. Coli* and *S. Aureus* has been examined from one hour for up to five hours.

Results: The green synthesized AgNPs were developed successfully using a plant Ocimum gratissimum. The synthesized AgNPs exhibited a maximum absorption peak at 440 nm and SEM analysis revealed that the synthesized AgNPs were spherical and oval. The result findings of the anti-inflammatory activity reveal that AgNPs have great potential when compared to the standard. At the concentration of 50 µg/mL, AgNPs exhibit 76% in BSA assay and 74% in EA assay, where the standard shows 80% inhibition. The antimicrobial activity showed a zone of inhibition around 19mm for E. coli and a 20mm zone of inhibition for *S. aureus* and *Pseudomonas sp.*, which shows the efficacy of AgNPs. The time-kill assay shows that the optical density of *E. coli* and *S. aureus* was reduced to 0.1 after 5 hours of incubation, which shows the potential of green synthesized AgNPs.

Conclusion: OG-mediated AgNPs have both anti-inflammatory and antimicrobial effects. Anti-inflammatory effects are better when compared to standard drugs. Antimicrobial effects are better for Gram-negative bacteria.

KEYWORDS:
Silver nanoparticles, biomedical applications, time-kill curve analysis, Nanobiomedicine

INTRODUCTION

The evidence for the use of Silver and other metals as a treatment modality has been mentioned in the history of medicine as early as the 2nd century BC in Indian Charaka Samhita and by Hippocrates for treatment of wounds and as preservatives.[1,2] Silver nitrate was also widely used in the 1800s for wound healing in burns, in wound dressings, and in suture materials. After the Second World War, the use of silver declined during the era of antibiotics.[3] The advent of silver nanoparticles in therapeutics gained momentum in recent years. Nanoparticles as the name denotes, are less than 100 nm in size and are used extensively in the field of biomedical research. Its longevity in diagnosis and treatment is explained by its unique properties, which include its high surface area to volume ratio, robust response to living cells, stability at high temperatures, programmable surface plasmon resonance,[4] and translocation into the cells' cellular uptake and effect of nanoparticle depends on its physical and chemical characteristics like composition, shape, size, charge, surface coating, pH, and width-to-height ratio,[5] But at the same time, the small size of nanoparticles results in a large surface area making them more toxic.[6] Nonchemical or biological Nanoparticle synthesis using the bottom-up method is also called green synthesis. During green synthesis, secondary metabolites in plant extracts such as vitamins, flavonoids, enzymes, polyphenols, and polysaccharides act as reducing agents. They have a donated electron outer layer, thereby reducing the metal ions to a zero-oxidation state (ex-Ag+ to Ag0).[7,8]

Green synthesis using medicinal plant extracts is preferred because of their eco-friendly nature, simplicity, and low cost. There are several advantages to green synthesis over chemical synthesis. The biologically active components in plant extracts strongly influence the size and distribution of metallic nanoparticles.[9,10] They also form a cover on the surface of the nanoparticles (capping) and prevent them from agglomerating or clustering together because of their small size, thereby giving them more stability. In addition, plant extracts with medicinal value can act synergistically with nanoparticles and enhance the therapeutic effects of

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metal nanoparticles. It reduces the harmful side effects of chemically synthesized metal nanoparticles.[11] It can also be combined with most of the existing antibiotics, and can increase its effectiveness and reduce dosage, thereby reducing side effects. Most importantly, green synthesis helps overcome the increasing problem of antimicrobial resistance.[12,13] AgNPs exert their antimicrobial effect in several ways by interacting with living cells and causing cell death. It increases cell membrane permeability, causes sulfide bond breakage, protein misfolding, aggregate formation, iron imbalance, and increased production of reactive oxygen species such as hydroxyl ions.[14] Each of which has specific biological compounds. Several plant extracts are being studied and used to form AgNPs. In this study, we have used the extract of Ocimum Gratissimum (OG), OG is widely known as clove basil, African basil, wild basil, and in the Indian subcontinent as Karunthulasi, Ramthulasi and belongs to Lamiaceae or mint family.[15] OG plant extracts have been shown to have anti-inflammatory, antimicrobial effects antidiabetic, antidiarrheal, antiprotozoal, cytotoxic, hepatoprotective, and many other medicinal effects.[16] In this present study, the synthesis of AgNPs using Ocimum Gratissimum evaluated its anti-inflammatory and antimicrobial/antibacterial effects against wound pathogens. The time-kill curve assay can be considered an indicator of effectiveness against antibiotic resistance.

MATERIALS AND METHODS

Preparation of AgNPs with Ocimum gratissimum Stem Extract

The Ocimum gratissimum plant was shade-dried and ground into powder. A beaker containing 100 mL distilled water and 1 g of Ocimum gratissimum was added and heated in a heating mantle at 60–70 °C for 15–20 minutes. Precursor solution: To prepare the precursor solution, 1 mM of silver nitrate (0.0169 g) was combined with 80 mL of distilled water,[17] and 20 ml of filtered plant extract was added, mixed well, and placed in an orbital shaker.

In vitro Antimicrobial Assay

The antimicrobial action of Ocimum Gratissimum green-synthesized AgNPs was assessed using the agar-well diffusion method, a commonly used method. Bacteria were cultured on Mueller-Hinton agar (MHA) plates, which served as the growth medium. The cultured bacteria were incubated for 24 h and the bacterial load was standardized using McFarland standards. Cultures were spread uniformly on MHA plates and wells were created. Green synthesized AgNPs (25, 50, and 100 micrograms) were loaded into bacterial culture wells. After incubation for 24 h at 37 °C, the inhibition zones were measured and compared with the inhibitory zones of known standard antibiotics, which served as positive controls.[18]

Bovine Serum Albumin Denaturation Assay (BSA Assay)

Using this approach, the anti-inflammatory properties of AgNPs, mediated by Ocimum gratissimum stem extract, were evaluated. AgNPs of different fixation (10µL, 20µL, 30µL, 40µL, and 50µL) mediated by Ocimum gratissimum extract were added to 0.45 mL of bovine serum albumin (1% aqueous solution), and the mixture's pH was adjusted to 6.3 using a small amount of 1N hydrochloric acid. Double-beam Mult spectrophotometry was used to measure the absorbance at 660 nm after the samples were cooled after a 20-minute incubation time at room temperature and a 30-minute heating session in a water bath at 55 °C. Diclofenac sodium was used as a reference standard. Dimethyl sulphoxide (DMSO) was used as the control.[19]

Egg Albumin Denaturation Assay [EA Assay]

2.8 mL of freshly prepared phosphate-buffered saline with a pH of 6.3 and 0.2 mL of egg albumin from a hen's egg were combined to create a 5 mL solution. For the Ocimum gratissimum stem extract-mediated AgNPs, specific quantities were generated independently using the same procedure as the Bovine Serum Albumin assay.[20]

Time Kill Curve Assay

Minimum inhibitory concentrations (MICs) are the lowermost antibacterial concentrations in mg/L or µg/mL that completely prevent visible growth of the tested microbes. For the MIC test, 100 µL of MHB was added to all columns in the microtiter plate along with the bacterial inoculum at a concentration of 5×10^5 CFU/ml. A stock solution of (500 µg/mL) OG-mediated AgNPs was prepared, 25, 50, and 100 µL were added to the last column in the microtiter plate, and serial double dilutions were made backward.[21,22] Different concentrations of nanoparticles were used to test the time-kill curve analysis using *S. aureus* and *E. coli*.

For the time-kill assay, Mueller Hinton broth was prepared, sterilized, and added to five test tubes. Three different concentrations of *Ocimum gratissimum* green-synthesized AgNPs were added to the first three tubes at 25, 50, and 100 µL (1,2- and 4-times the MIC), the standard drug was added to the fourth tube (Amoxyrite), and the fifth tube was considered as the control. Bacterial suspensions (*Staphylococcus aureus* and *E. coli*) were added to all five test tubes at a concentration of 5×10^5 CFU/mL. The tubes were incubated for various time intervals (1h, 2h, 3h, 4h, 5h) and the percentage of dead cells was calculated at a wavelength of 600 nm at regular time intervals using optical density values.[23]

RESULTS

Synthesis of AgNPs and their characterization analysis

The precursor solution was mixed with 20 mL of stem extract and centrifuged at 110 revolutions per minute for a full day. After 24 hours of incubation, the color of the solution changed from yellow to brown as a result of surface plasmon resonance, which excited the free electrons of the reaction mixture. This confirms the reduction of silver nitrate to AgNPs. The synthesized nanoparticles were characterized by UV-visible spectroscopy. Higher absorption is directly proportional to the higher yield of AgNPs in the colloidal solution. The color change was dependent on the incubation time (6–24 h) as well as the size and shape of the nanoparticle as shown in Figure 1A. The UV-visible absorption spectra of the synthesized AgNPs are shown in Figure 1B. The UV-visible region of wavelengths from 350 to 650 nm has a distinctive absorption peak at 440 nm in the spectrum. Numerous parameters, such as the size, shape, and degree of aggregation of nanoparticles, influence the position of the SPR peak. Larger nanoparticles display peaks at longer

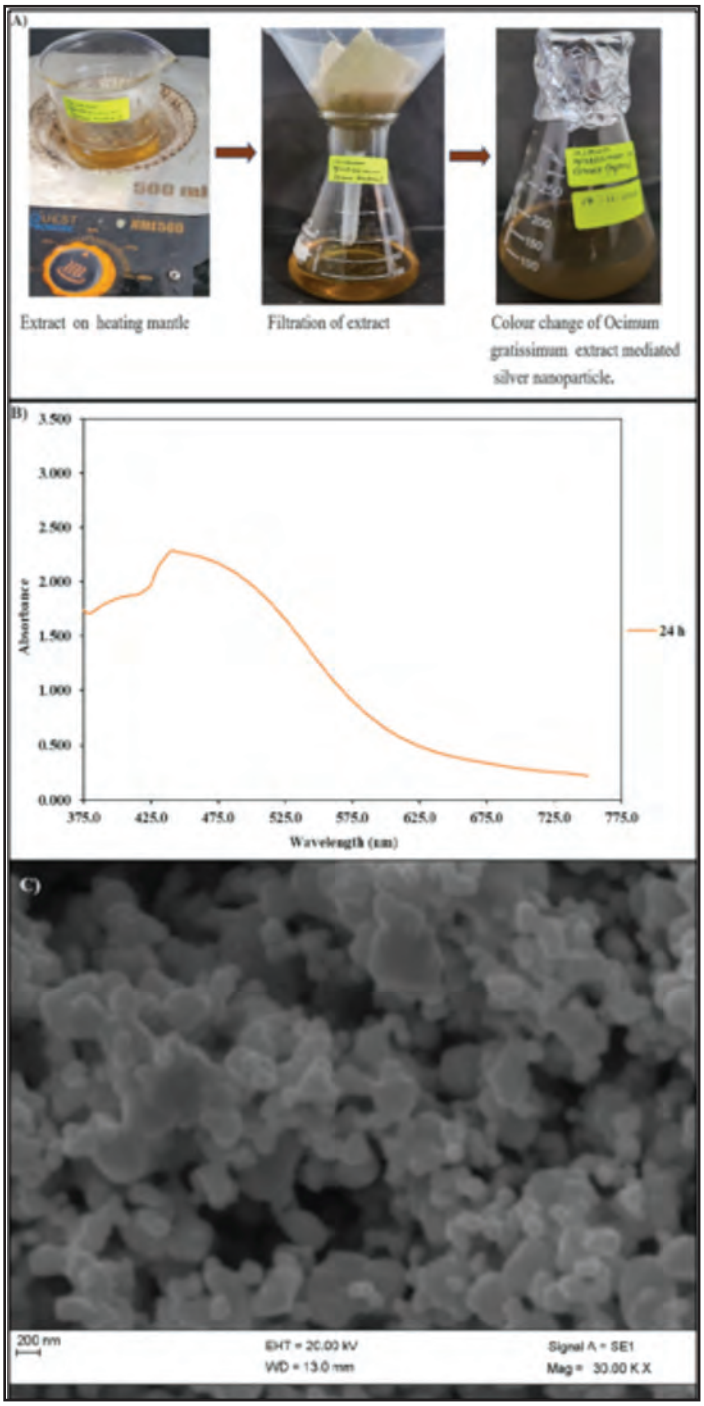

Fig. 1: A) Green synthesis of Ocimum gratissimum mediated AgNPs, B) UV spectra analysis of AgNPs, C) SEM analysis of green synthesized AgNPs.

wavelengths than smaller ones, which typically exhibit SPR peaks at shorter wavelengths. Figure 1C displays the SEM images of the AgNPs produced. The photographs demonstrate the existence of uniformly sized spherically shaped nanoparticles. The smooth surface of the nanoparticles suggests that there was no noticeable agglomeration or aggregation. The form and surface characteristics of each nanoparticle can be observed through SEM photos, which offer comprehensive insights into each one. The spherical morphology of the nanoparticles is compatible with the development of AgNPs throughout the production process, and is of excellent quality and consistency.

Antimicrobial Activity

The antimicrobial activity of the green-synthesized AgNPs was investigated using the agar well diffusion method against E. coli, *S. aureus*, and *Pseudomonas sp.* A zone of inhibition of 19 mm was noted for *E. coli* at a concentration of 100µg/mL, where the standard was 40 mm. In contrast, a

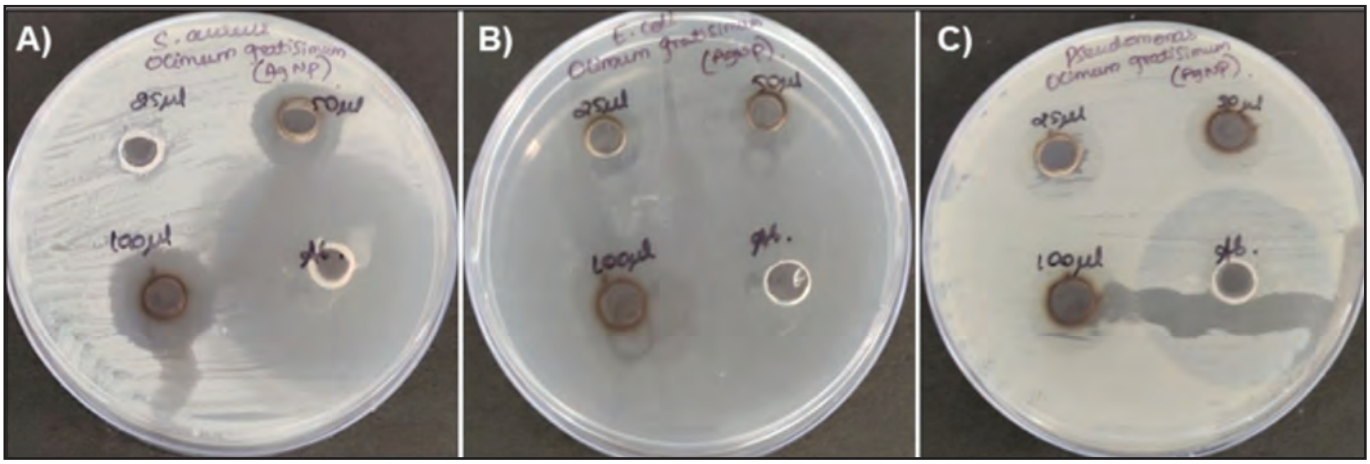

Fig. 2: Antibacterial activity of Ocimum gratissimum mediated AgNPs using agar well diffusion method.

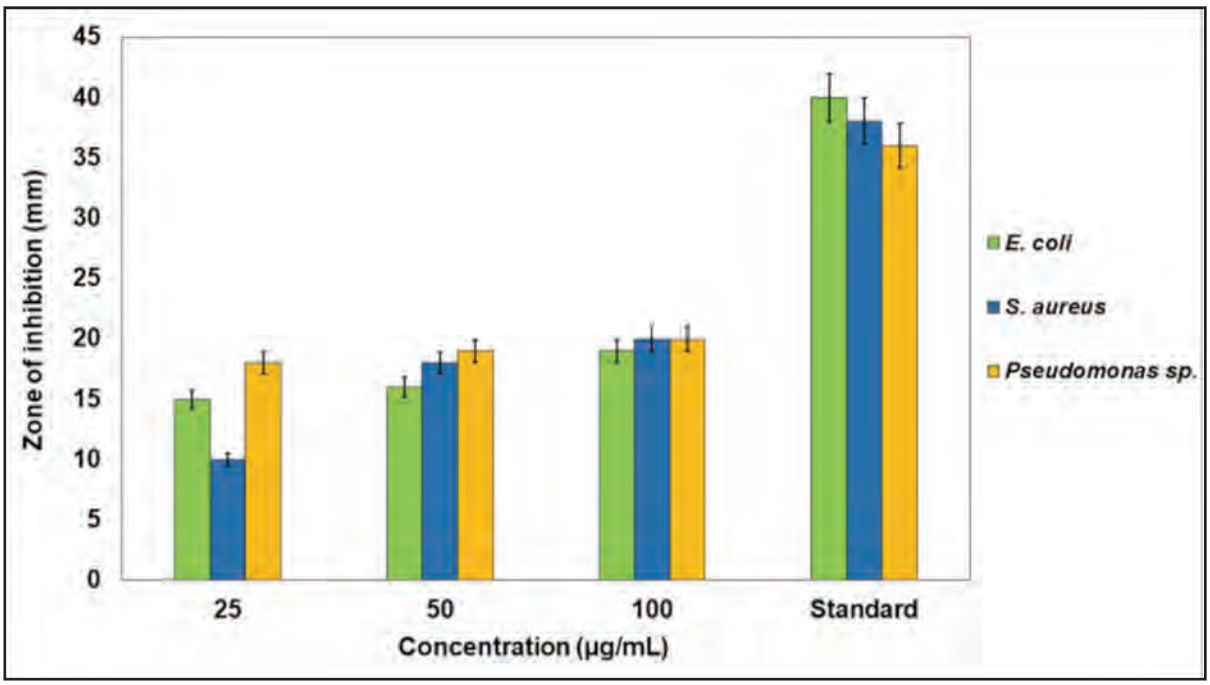

Fig. 3: Graphical representation of *Ocimum gratissimum* mediated AgNPs against *E. coli, for S. aureus* and *Pseudomonas sp.*

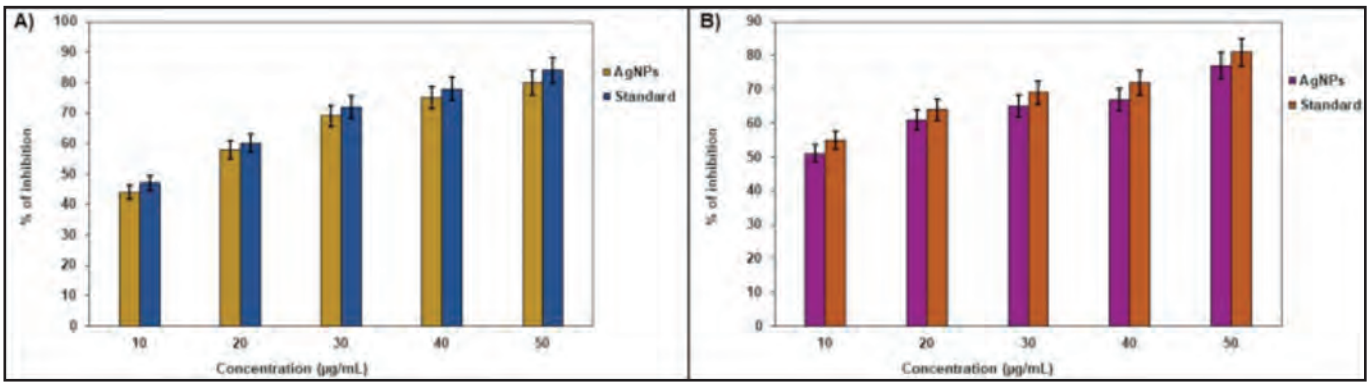

Fig. 4: Anti-inflammatory activity of *Ocimum gratissimum* mediated AgNPs using A) BSA assay and B) EA assay.

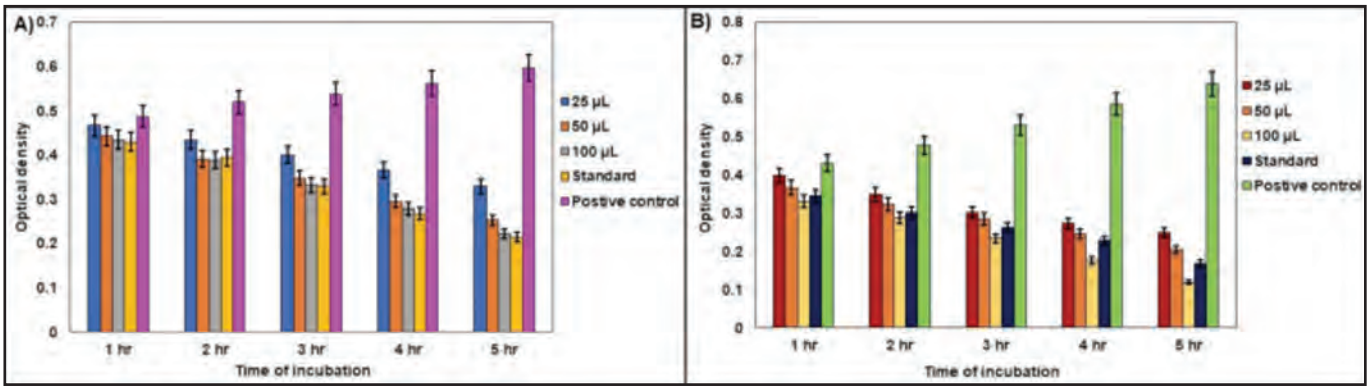

Fig. 5: Time kill curve analysis of the green synthesized AgNPs using *O. gratissimum* against A) *E. coli,* and B) *S. aureus*

20 mm zone of incubation was observed for *S. aureus* and *Pseudomonas sp* at the concentration of 100µg/mL. In comparison, the standard revealed 38 mm and 36 mm zones of incubation, as shown in Figures 2 and 3.

Anti-inflammatory Effect

The anti-inflammatory activity of green synthesized AgNPs using *O. gratissimum* showed 76% inhibition of protein denaturation was observed at 50 µL concentration in the BSA assay and 74% inhibition in the EA assay. The Anti-inflammatory activity was found as 80% for standard diclofenac sodium. The effects of Ocimum gratissimum mediated AgNPs were comparable to those of the standard - diclofenac sodium at almost all concentrations, as shown in Figures 4A and B.

Time Kill Assay for E. coli and S. aureus

A time-kill curve assay was performed in MHB media and the antibacterial activity of AgNPs was determined against *E. coli* and *S. aureus* using optical density values. The lower the optical density, the greater the antibacterial effect. The results showed that the tested bacteria were completely inhibited by AgNPs. At 5 hr, maximum growth inhibition with 0.2 optical density was seen at 100µL concentration for *E. coli. S. aureus* showed 0.1 optical density at 100 µL,concentration as represented in Figures 5A and B.

DISCUSSION

In this study, Ocimum Gratissimum (OG) enabled the green production of AgNPs. Bovine serum and egg albumin assays were used to evaluate the anti-inflammatory and antibacterial properties of green-synthesized AgNPs made using Ocimum Gratissimum (OG) extract by agar well diffusion and time-kill assay (for antimicrobial effect). In a similar study in our institution, the anti-inflammatory effects of combined herbal Ocimum Gratissimum and Ocimum Tenuiflorum extract-mediated AgNPs and ZnONPs were assessed by BSA and Egg albumin methods, and the results were (75% for the Green synthesized AgNPs and ZnONPs and 81% for Standard Diclofenac at 50 µl). In our study, it was noted as 76% and 81% for AgNPs and Diclofenac sodium). This reproducibility confirms the anti-inflammatory effects of Karunthulasi (OG)-mediated AgNPs. In an earlier study, it was also shown that AgNPs are slightly better than ZnO NPs as anti-inflammatory agents.

AgNPs inhibited TNF-α, COX-2, and MMP-3 expression and inhibited IL-1β, IL-6, and TNF-α) in macrophages. Therefore, it has anti-inflammatory and wound-healing effects.[24,25] Their study on the molecular mechanisms of *Ocimum Gratissimum* showed that it inhibits the lipoxygenase pathway, resulting in decreased synthesis of leukotrienes via the arachidonic acid pathway. It was also shown that the phytocompound OG has an affinity for inflammatory cytokines such as interleukin 1, interleukin 6, TNF-α, interleukin 8, and monocyte chemoattractant protein-1, and can modulate their effects. Thus, the synergistic anti-inflammatory effect of OG and AgNPs is highly plausible.

In this study, the antibacterial activity of OG-mediated AgNPs against *E. coli, S. Aureus*, and *Pseudomonas sp.* was investigated using the agar well diffusion method. The zone of inhibition was 42% (42 mm- Standard,17.5mm- OG), 50% (40mm and 20 mm), and 53% (38mm and 20 mm) for *E. coli, S. aureus*, and *Pseudomonas sp* at a maximum concentration of 100µl. Even at lower concentrations, the effects were almost the same for *Pseudomonas sp.*[26] In their study, only OG plant extracts showed antibacterial properties. In another study on AgNPs by Sharma et al., OG-mediated AgNPs were shown to have antibacterial effects against gram-negative bacteria. In this study, the effect against *Pseudomonas sp*, a gram-negative bacterium, was better, even at lower concentrations. In a previous study,[27] the antimicrobial activity of OG-mediated ZnONPs was studied against oral pathogens, and the zone of inhibition was only 9 mm at all concentrations, suggesting that OG-mediated ZnONPs may not be as effective as antimicrobials when compared with OG-mediated AgNPs.[28,29]

For the time-kill curve assay, the optical density readings were recorded every hour from to 1-5 hours for concentrations of 25, 50, and 100 µL of OG-mediated AgNPs with bacterial inoculum of *E. coli* and *S. aureus,* 5×10^5 CFU/mL in MHA medium. For OG-mediated AgNPs, the optical density of E. coli was almost the same as that of the standard antibiotic drugs until 5 h. For *S. aureus* the optical density was lower for OG -AgNPs for all concentrations at all durations, with the lowest value for 100 µL at 5 h. A lower optical density indicated a greater antimicrobial effect of the OG-mediated AgNPs.

CONCLUSION

OG-mediated AgNPs have potent antibacterial activity against E. coli, Pseudomonas sp, and S. aureus exhibiting a maximum zone of inhibition. It can be used as an adjunct in combination with standard antimicrobials, thereby reducing the dosage of antimicrobial drugs. The anti-inflammatory activity of AgNPs was evaluated using BSA and EA assays, which showed 76% inhibition compared with the standard. This indicates that further research needs to be conducted using In vitro and In vivo analyses.

CONFLICT OF INTEREST

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

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REFERENCES

1. Barillo DJ, Marx DE. Silver in medicine: A brief history BC 335 to present. Burns 2014; 40: S3-8.
2. Alexander JW. History of the medical use of silver. Surg Infect 2009; 10(3): 289-92.
3. Medici S, Peana M, Nurchi VM, Zoroddu MA. Medical uses of silver: history, myths, and scientific evidence. J Med Chem 2019; 62(13): 5923-43.
4. Liu J, He H, Xiao D, Yin S, Ji W, Jiang S, Luo D, Wang B, Liu Y. Recent advances of plasmonic nanoparticles and their applications. Materials. 2018; 11(10): 1833.
5. Anastasiadis SH, Chrissopoulou K, Stratakis E, Kavatzikidou P, Kaklamani G, Ranella A. How the physicochemical properties of manufactured nanomaterials affect their performance in dispersion and their applications in biomedicine: a review. Nanomaterials. 2022; 12(3): 552.
6. Nguyen NP, Dang NT, Doan L, Nguyen TT. Synthesis of silver nanoparticles: from conventional to 'modern' methods—a review. Processes 2023; 11(9): 2617.
7. Rónavári A, Igaz N, Adamecz DI, Szerencsés B, Molnar C, Kónya Z, Pfeiffer I, Kiricsi M. Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications. Molecules 2021; 26(4): 844.
8. Zahoor M, Nazir N, Iftikhar M, Naz S, Zekker I, Burlakovs J, Uddin F, Kamran AW, Kallistova A, Pimenov N, Ali Khan F. A review on silver nanoparticles: Classification, various methods of synthesis, and their potential roles in biomedical applications and water treatment. Water 2021; 13(16): 2216.
9. Rafique M, Sadaf I, Rafique MS, Tahir MB. A review on green synthesis of silver nanoparticles and their applications. Artif Cells Nanomed Biotechnol 2017; 45(7): 1272-91.
10. Shanmugam R, Subramaniam R, Kathirason SG, Ali D, Balusamy SR, Gurusamy A, Arunachalam K, Sellami H. Curcumin-chitosan nanocomposite formulation containing Pongamia pinnata-mediated silver nanoparticles, wound pathogen control, and anti-inflammatory potential. Biomed Res Int 2021; 2021.
11. Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Adv 2019; 9(5): 2673-702.
12. Yakoup AY, Kamel AG, Elbermawy Y, Abdelsattar AS, El-Shibiny A. Characterization, antibacterial, and cytotoxic activities of silver nanoparticles using the whole biofilm layer as a macromolecule in biosynthesis. Sci Rep 2024; 14(1): 364.
13. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. Phcog Rev 2017; 11(22): 57.
14. Morones-Ramirez JR, Winkler JA, Spina CS, Collins JJ. Silver enhances antibiotic activity against gram-negative bacteria. Sci Transl Med 2013; 5(190): 190ra81-.
15. Singh R, Shedbalkar UU, Wadhwani SA, Chopade BA. Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. Appl Microbiol Biotechnol 2015; 99: 4579-93.
16. Ugbogu OC, Emmanuel O, Agi GO, Ibe C, Ekweogu CN, Ude VC, Uche ME, Nnanna RO, Ugbogu EA. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (Ocimum gratissimum L.). Heliyon 2021; 7(11).
17. Imtiaz T, Priyadharshini R, Rajeshkumar S, Sinduja P. Green synthesis and Characterization of silver nanoparticles synthesized using piper longum and its antioxidant activity. J Pharm Res Int 2021; 33(51A): 342-52.
18. Das P, Ghosal K, Jana NK, Mukherjee A, Basak P. Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. Mater Chem Phys 2019; 228: 310-7.
19. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, Radu S. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front microbiol 2018; 9: 379304.
20. Weinstein MP. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. (No Title). 2018.
21. Zainin NS, Lau KY, Zakaria M, Son R, Razis AA, Rukayadi Y. Antibacterial activity of Boesenbergia rotunda (L.) Mansf. A. extract against Escherichia coli. Int Food Res J 2013; 20(6): 3319.
22. Kamiloğlu Beştepe SE, Sarı G, Özdal T, Çapanoğlu Güven E. Guidelines for cell viability assays. Food Front.2020;1(3).
23. Varghese RM, Kumar A, Shanmugam R. Comparative anti-inflammatory activity of silver and zinc oxide nanoparticles synthesized using Ocimum tenuiflorum and Ocimum gratissimum herbal formulations. Cureus 2024; 16(1).
24. Mikhailova EO. Silver nanoparticles: mechanism of action and probable bio-application. Journal of functional biomaterials 2020; 11(4): 84.
25. Tyavambiza C, Elbagory AM, Madiehe AM, Meyer M, Meyer S. The antimicrobial and anti-inflammatory effects of silver nanoparticles synthesised from Cotyledon orbiculata aqueous extract. Nanomaterials 2021; 11(5): 1343.
26. Olaoye IF, Oso BJ, Aberuagba A. Molecular mechanisms of anti-inflammatory activities of the extracts of Ocimum gratissimum and Thymus vulgaris. Avicenna J Med Biotechnol 2021; 13(4): 207.
27. Chanthaboury M, Choonharuangdej S, Shrestha B, Srithavaj T. Antimicrobial properties of Ocimum species: An in vitro study. J Int Soc Prev Community Dent 2022; 12(6): 596-602.
28. Anandachockalingam A, Shanmugam R, Ryntathiang I, Jothinathan MK, Anandachockalingam III A. Green Synthesis of Silver Nanoparticles Using Zingiber officinale and Ocimum gratissimum Formulation for Its Anti-inflammatory and Antidiabetic Activity: An In Vitro Study. Cureus 2024; 16(4).
29. Pfeifle A, Thulasi Raman SN, Lansdell C, Zhang W, Tamming L, Cecillon J, Laryea E, Patel D, Wu J, Gravel C, Frahm G. DNA lipid nanoparticle vaccine targeting outer surface protein C affords protection against homologous Borrelia burgdorferi needle challenge in mice. Front immunol 2023; 14: 1020134.