

Anti-Inflammatory and Antimicrobial Activity of Silver Nanoparticles Synthesized Using *Piper Longum*

Obuli Ganesh Kishore S¹, R Priyadharshini^{1*}, S Rajeshkumar², Palati Sinduja¹

¹Department of Pathology, Saveetha Dental College & Hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

²Department of Pharmacology, Saveetha Dental College & Hospitals,
Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

ABSTRACT

Background: *Piper longum* commonly known as long pepper is a traditional medicinal plant that has been used for ages. It was most used to treat respiratory infections, bronchitis, cholera, etc. and could aid in appetite and digestion. Nanoparticles have been employed in the field of medicine due to their ability to target specific cells without damaging the adjacent cells. Nanoparticles were employed as an antimicrobial in bandages whose medicinal properties play a role in wound dressings and antiseptic creams. To comprehend the antimicrobial and anti-inflammatory property of *Piper longum*, silver nanoparticles were synthesised, and the effects were correlated with their ability to inhibit various microbes in the human body.

Aim: The present study aimed to analyse the antimicrobial and anti-inflammatory activity of silver nanoparticle particles synthesized using *Piper longum* extract.

Materials and methods: Plant extract-based silver nanoparticles were tested for its anti-inflammatory activity by protein denaturation assay. The standard anti-inflammatory used was diclofenac sodium. To assess the antimicrobial activity, the prepared extract was inoculated in different culture plates containing various microorganisms. The results obtained were collected and statistically analyzed in SPSS software and graphs were obtained.

Results: The silver nanoparticles synthesised using *Piper longum* showed highest absorbance value at a concentration of 10 microliter (102 nm) when subjected to albumin denaturation assay to check for its anti-inflammatory activity. The maximum percentage of inhibition recorded was 81.1% at 20 microliter concentrations. The zone of inhibition against *C. albicans* was found to be 8mm, 9mm, 11mm zones at 25, 50, and 100µL and was higher than the standard antifungal agents used. The ANOVA *p* value was found to be less than 0.05 which indicated statistically significant values.

Conclusion: Silver nanoparticles synthesized using *Piper longum* extract can act as a potential antifungal agent and anti-inflammatory activity. However, the anti-inflammatory activity of the extract varied with the different concentrations of the extract.

Key words: Anti-inflammatory, antimicrobial, albumin denaturation assay, the zone of inhibition, absorbance, percentage of inhibition, Innovative technique

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Corresponding author: R Priyadharshini
e-mail ✉: priyadharshini.sdc@saveetha.com
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INTRODUCTION

Prolonged usage of steroidal and non-steroidal anti-inflammatory drugs are known to be associated with peptic ulcer formation. The search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects should be produced. The uses of medicinal plants for the development of the new drug molecule against bacterial infections show a bright future [1]. A wide variety of medicinal plants used

traditionally have not yet been systematically investigated against various microbial pathogens [2].

Piper longum (pippali) also called as Javanese from the *piperaceae* family. *Piper longum* commonly referred to as "long pepper" in India is seen within the tropical and subtropical regions of the planet throughout the Indian subcontinent, Sri Lanka, Middle Eastern countries, and America [3,4]. Piperine is an alkaloid in long pepper for its pungency. It is a rejuvenator and relieves inflammation and pain. It dispels the discomfort in the intestine [5]. Even though there are modern medicines available, herbal medicines have often retained popularity for historical and cultural reasons. Since the usage of those herbal medicines has increased, the problems regarding their

safety, quality, and efficacy in industrialized and developing countries have cropped up [6]. Although there are numerous indications for its use, controlled trials are needed to work out its efficacy [7].

The fruit of long pepper is utilized in palsy, gout, and lumbago. The fruits have a bitter, hot, sharp taste and are tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac, and digestive. They need a pungent pepper-like taste and produce salivation and numbness of the mouth. The fruits and roots are attributed with numerous medicinal uses and should be used for diseases of tracts, cough, bronchitis, asthma etc, as counter-irritant and analgesic when applied locally for pain in muscles and inflammation, as snuff in coma, drowsiness and internally as a carminative [8]. Besides fruits, the roots and thicker parts of the stem are cut and dried and used as a crucial drug within the Ayurvedic and Unani systems.

Natural products with anti-inflammatory activity have been used for a long time as a folk remedy for inflammatory conditions such as fevers, pain, migraine, and arthritis. As the inflammatory basis becomes clearer, there arises a need for various natural food products rich in antioxidants to counteract the diseases arising due to the same [9]. The highest anti-inflammatory potential was observed in chili pepper. Among the selected plants, the plants that improved the secreted cytokine profile were allspice, basil, bay leaves, black pepper, licorice, nutmeg, oregano, sage and thyme. The compounds apigenin, capsaicin, chrysin, diosmetin, kâmpferol, luteolin, naringenin, quercetin and resveratrol moderately reduced IL-6 and TNF-alpha secretion [10].

Medicinal plants with antimicrobial, antioxidant, and anti-inflammatory properties have played an important role in the wound healing process. Poly Herbalism results in cheaper medication by reducing the duration of therapy or individual cost for anti-inflammatory and antimicrobial medications. The incidences of new and relapsing infectious disease and antibiotic resistance have greatly increased the susceptibility of delayed healing [11]. A combinatorial synthesis approach can be applied to synthesize the compound which can mimic the natural component present in these medicinal plants with better efficacy [12]. The spices that we Indians use to cook daily have been used for ages for adding flavor and also for the house-hold treatment of infectious diseases. Our team has extensive knowledge and research experience that has translated into high quality publications [13-32]. The aim of the current study is to analyse the Anti-inflammatory and Antimicrobial activity of silver nanoparticles synthesized using *Piper longum*.

MATERIALS AND METHODS

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Reagents and chemical

BSA (Bovine serum albumin) was used as a reagent for the assay. Bovine serum albumin makes up approximately 60% of all proteins in animal serum. It is

commonly used in cell culture, particularly when protein supplementation is necessary, and the other components are unwanted.

Preparation of plant extract

The samples were collected by using a randomized sampling method. The plant extract was purchased readymade to hasten the study. 0.5g of *Piper longum* extract was added to 100ml of distilled water and was boiled for 5 minutes at 50 degrees Celsius. The solution was filtered. 1.16g of AgNO₃ was added to the extract and was kept in the shaker at 750rpm for half a day. After the complete dissolution of silver. A sample of extracts with different readings in the spectrophotometer were taken in different time intervals. A spectrophotometer is an optical device that can determine the concentration of a compound or particles in a solution or suspension.

Anti-inflammatory activity

The anti-inflammatory activity for silver nanoparticles was tested using an albumin denaturation assay. 0.05 mL of Solanum torvum gel of various fixation (10µL, 20µL, 30µL, 40µL, 50µL) was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. Micro pipetting the solutions showed minor errors which was an inconvenience. These samples were incubated at room temperature for 20 minutes and then heated at 55 °C in a water bath for 30 minutes. The samples were cooled, and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing the following equation.

The absorbance of control - Absorbance of sample × 100/
Absorbance of control

Antimicrobial activity

The agar well diffusion method was used to determine the antimicrobial activity of silver nanoparticles using *Piper longum*. Different concentrations of compounds were tested against *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus mutans*. The fresh suspension of microbes was dispersed on the surface of agar plates. Different concentrations of nanoparticles (50, 100, and 150 microliters) were incorporated into the wells and the plates were incubated at 37 degrees Celsius for 24 hours. The antibiotics and antifungal agents were used as a positive control. Zone of inhibition was recorded in each plate. The study was validated by guides and experts in nano research. Only the zone of growth inhibition was measured while the other criterias of organism with colour change and other activity are excluded.

Statistical analysis

The values obtained from antioxidant and cytotoxic activity assay are entered in excel sheets and correlation analysis was done with IBM SPSS software version 23.

The ANOVA p value was found to be less than 0.05 which indicated statistically significant values.

RESULTS

Antifungal activity

The nano preparation of *Piper longum* plant extract

mediated nano formulation showed various percentage of inhibition at different concentration (10µL-50µL) (Table and Figure 1). The nano preparation of *Piper longum* plant extract inhibited *S. mutans*, *C. albicans*, *E. faecalis*, and *S. aureus* at different concentrations (25µL, 50µL, and 100µL)(Figures 2 and Figure 3).

Table 1: The table represents the zone of inhibition of different pathogens at concentrations 25, 50 and 100µL and the zone of inhibition of the standard antibiotic on different pathogens.

Pathogen	25µL	50µL	100µL	Ab
<i>C. albicans</i>	8mm	9mm	11mm	8mm
<i>S. aureus</i>	8mm	8mm	11mm	23mm
<i>E. faecalis</i>	8mm	9mm	7mm	40mm
<i>S. mutans</i>	14mm	13mm	12mm	36mm

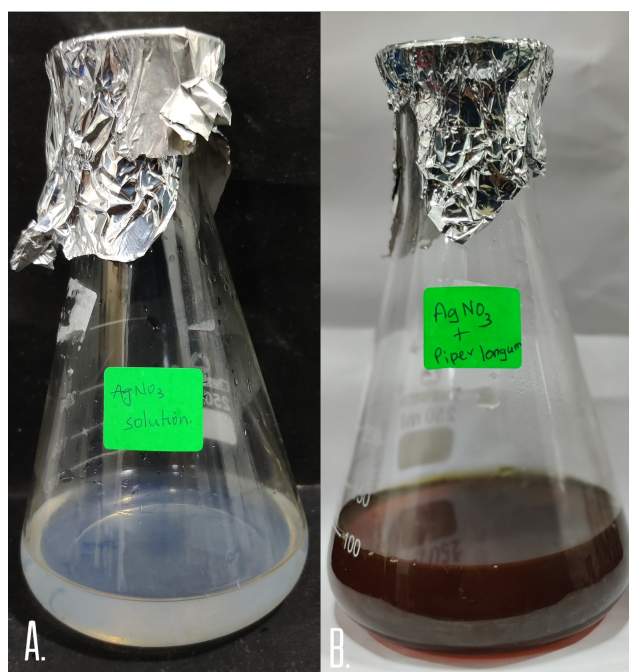


Figure 1: *Piper longum* mediated silver nanoparticles preparations. A - after dissolving AgNO₃ in distilled water. B - after the plant extract was mixed with AgNO₃ solution. The characteristic color and property of the prepared extract are observed. There was also a characteristic color change observed after the filtration of the extract.

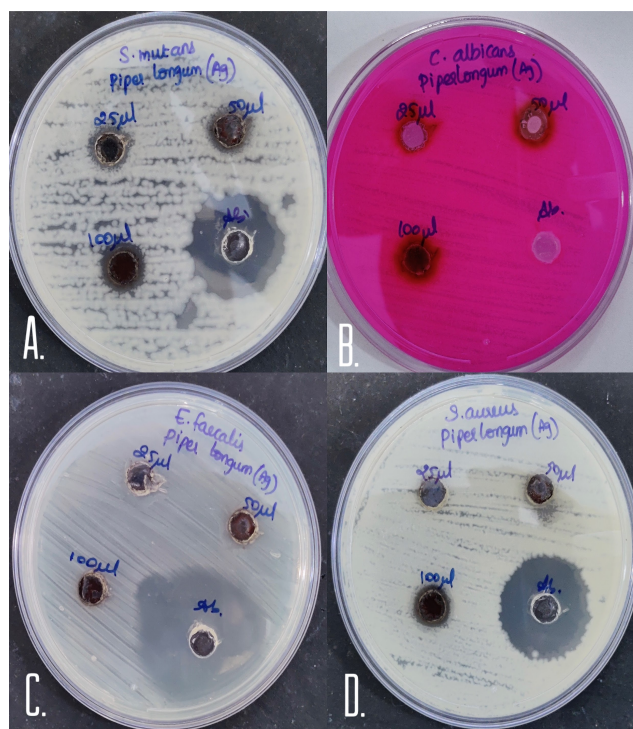


Figure 2: Antimicrobial activity observed in agar plates containing different microorganisms. In plate A, the zone of inhibition of *S. mutans* was observed at different concentrations (25µL, 50µL, and 100µL). In plate B, the zone of inhibition of *C. albicans* at different concentrations was observed. In plate C, the zone of inhibition of *E. faecalis* was observed at different concentrations. In plate D, the zone of inhibition of *S. aureus* was observed at different concentrations.

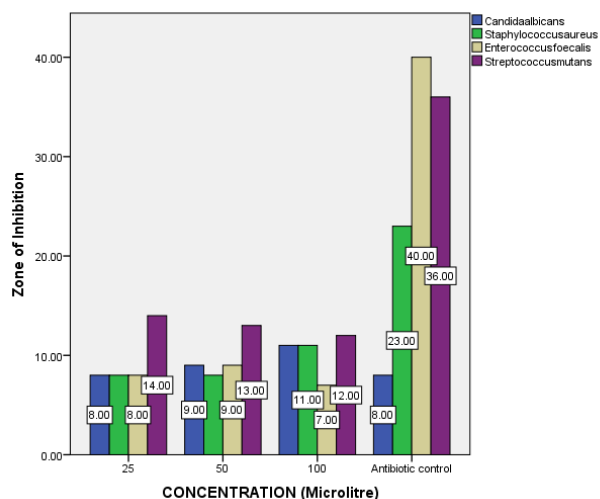


Figure 3: The graph represents the antimicrobial activity of the extract over different microorganisms. X axis showed concentration at varying microlitre. The zone of inhibition was measured as diameter. Y axis represents zone of inhibition of varying microorganisms. *C. albicans* (blue), *S. aureus* (green), *E. faecalis* (yellow), and *S. mutans* (purple). *C. albicans* showed an increased zone of inhibition.

Anti-Inflammatory

The anti-inflammatory activity is shown in Figures 4 and Figure 5, Table 2.

Table 2: The table is the comparison of the control and the percentage of inhibition with increase in concentration (μL).

Concentration (Microlitre)	Control	Percentage of inhibition
10	65.6	79.9
20	55.4	81.1
30	40.8	80.7
40	34.9	80.9
50	26.4	79.1

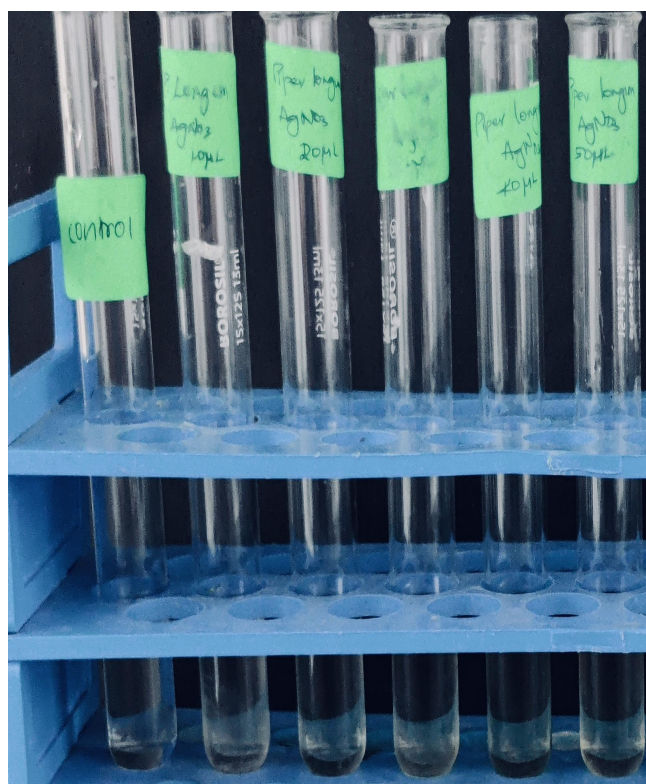


Figure 4: Anti-inflammatory activity assessed using albumin denaturation assay of the reaction mixture at different concentrations compared to a positive control diclofenac sodium.

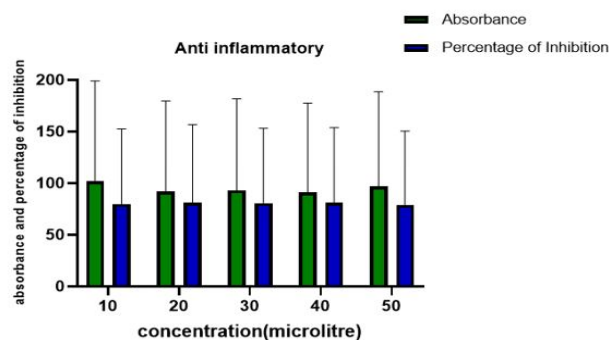


Figure 5: The graph represents the anti inflammatory activity of extract and control with the X axis representing concentration at varying microlitre. Y axis represents the absorbance and percentage of inhibition showing negative correlation with increase in concentration ($r=-1$) with 26mm at 50microlitre concentration.

DISCUSSION

The study showed that silver nanoparticles synthesized using *Piper longum* extract can act as an anti-inflammatory agent. It was observed from the spectra that the extract at 660 nm had the highest value of absorbance at a concentration of 10 μL which was found to be 0.102, which proved the denaturation of albumin was significant. In a similar study conducted, the zinc oxide nanoparticles synthesized using grape seed extract showed the highest absorbance value at 50μL concentration [12,33]. Hence, agents that can prevent

protein denaturation can be used as an anti-inflammatory agent. The inhibition of protein denaturation by the prepared extract was maximum at 20 μ L concentration which was found to be 81.1%. From another similar research conducted, the maximum inhibition of protein denaturation was observed at 20 μ L concentration which was found to be 79%. The study was on coriander oleoresin-mediated selenium nanoparticles [34]. By comparing with the control which was diclofenac sodium, it showed a 0.087 absorbance value and 81.9% inhibition of protein denaturation at 10 μ L concentration. The highest percentage of inhibition of the extract prepared showed a maximum value of 89% at 50 μ L concentration [35]. From a study conducted, we may understand that silver when compared with any anti-inflammatory agent can yield potent anti-inflammatory activity [36]. Basically the more the protein is denatured the more there will be inflammation. Hence the extent of inflammation can be reduced by inhibiting protein denaturation. From a study conducted, L Theanine mediated silver nanoparticles were close to being apt as an anti-inflammatory agent as it had only a slight decrease compared to the control [37]. At 10 μ L concentration, the prepared extract showed an absorbance value of 0.102 nm and 79.9% inhibition, at 20 μ L concentration, it showed 0.092 nm absorbance value and 81.1% inhibition, at 30 μ L the absorbance value was 0.093 nm and the percentage of inhibition was 80.7%. At 40 μ L and 50 μ L concentrations, the absorbance values were 0.091nm and 0.097nm respectively and the percentage of inhibition was 80.9% and 79.1% respectively. The absorbance value of the control was 0.087nm and the percentage of inhibition was 81.9%.

The optimized silver nanoparticles were tested for antimicrobial activities in *S.mutans*, *S.aureus*, *E. faecalis*, and *C. albicans*. The zone of inhibition was observed for each of the organisms at 25, 50, and 100 μ L concentrations. The growth of all cultures in normal conditions showed all the phases of growth, but when they were treated with the synthesized silver nanoparticles there was a reduction in the growth phase. From a study conducted by Soumya Et al. the bacterial cultures showed a bacteriostatic effect on adding the synthesized selenium nanoparticles [37,38]. By comparing the results obtained with a study conducted by Swapna Et al. the silver nanoparticles confirmed antibacterial activity by forming a zone of inhibition against *S. aureus*, *Lactobacillus species*, *S. mutans* [39]. The antibacterial activity is mainly due to the penetration of the cations of the nanoparticles penetrating the cell membrane and killing them.

The agar plate containing *S. mutans* culture showed 14mm, 13mm, and 12mm zones of inhibition at 25, 50, and 100 μ L concentrations respectively. Whereas the same organism showed a zone of inhibition of 36mm with a standard antibiotic. Similarly, *S. aureus* showed 8, 8, 11mm zones of inhibition at various concentrations whereas the standard antibiotic showed 23mm inhibition. *E. faecalis* showed 8mm, 7mm, and 11mm zones of inhibition at 25, 50, and 100 μ L concentrations,

whereas the standard antibiotic showed a 40mm zone of inhibition. When the fungi *C. albicans* was cultured and the zone of inhibition for the prepared extract was assessed, it showed 8mm, 9mm, 11mm zones at 25, 50, and 100 μ L concentrations which were higher than the standard antifungal. The standard antifungal showed only an 8mm zone of inhibition. Hence the optimized nanoparticles can be a potent antifungal agent. The antimicrobial activity of silver nanoparticles synthesized using *Piper longum* plant extract can be used as an antimicrobial agent after controlling and regulating the morphology and particle size of synthesized nanoparticles.

In a previous study conducted by Satheesha et al. *S. aureus* was the most sensitive bacteria followed by *E. faecalis* and *S. mutans*. The gram-positive organisms showed a maximum zone of inhibition at 100 μ L concentration which showed a diameter of 42mm [40]. In the study conducted by Nafeesa et al. the alcoholic extract of tulsi leaves mediated silver nanoparticles were assessed to observe the antifungal activity. The plant extracts mediated Ag nanoparticles showed immense antifungal potential and can be used in the management of fungal infections [41]. Silver nanoparticles were found to be potent inhibitors of *C.albicans* biofilm formation from a study conducted [42]. The mechanism of fungal growth inhibition was not due to the penetration of the cell membrane, it was due to the release of silver ions that infiltrated into the cells leading to the formation of organic compounds present in the cell wall and cytoplasm. The silver nanoparticles hence have proved to have a cytotoxic effect on the fungi *C.albicans*.

The limitation of this study is that during the micropipette of solutions, concentration errors were obtained which were difficult to rectify. Prepared extract was not tested in different concentrations. Different species of *Piper longum* plant were not tested for antimicrobial and anti-inflammatory activities. Comparing the anti-inflammatory effect with different standards other than diclofenac sodium was not done. With further advancements in this study, increased concentrations of the same extract could be used to test different activities, which could lead to the production and manufacturing of natural product-based medicines.

CONCLUSION

Piper longum plant has better anti-inflammatory and antifungal activity as compared to the standard drugs examined. Further development and processing of the extract can lead to the development of a potent anti-inflammatory and antifungal agent. The present study paves way for creating new pathways in the field of natural medicine and can potentially improve the health of the individual by providing natural and safer methods.

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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