Pharmacogn. Res., 2024; 16(4):943-948.
https://www.phcogres.com

Original Article

Controlling of Oral Pathogens and Anti-Inflammatory Activity of Copper Oxide Nanoparticles Synthesized Using *Cymbopogon citratus* and *Zingiber officinale*

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ABSTRACT

Introduction: Green chemistry is considered a preferable alternative to hazardous chemical compounds when it comes to producing non-toxic nanomaterials. Copper Oxide Nanoparticles (CuONPs) are well-known for its antimicrobial, anti-carcinogenic and anti-inflammatory properties. Lemongrass is essential for immunological health and infection prevention. Ginger is known for its antibacterial properties and it is thought to reduce nausea and improve absorption by facilitating the passage of food from the stomach to the small intestine. **Objectives:** The primary aim of the study is to evaluate the antioxidant, anti-inflammatory and antimicrobial properties of the prepared copper oxide nanoparticles. **Materials and Methods:** The free radical scavenging ability of the prepared CuONPs was investigated using DPPH and Hydrogen peroxide radical scavenging assay. The anti-inflammatory capability of synthesized CuONPs was examined using Bovine serum albumin and Egg albumin denaturation assay. The bactericidal and bacteriostatic effect of the prepared CuONPs was analysed with time kill curve assay. **Results:** The result shows that the prepared CuONPs possess significant antioxidant and anti-inflammatory activity at the highest concentrations of 50 µL. The synthesized copper oxide nanoparticles are found to possess excellent bactericidal activity at the 100 µL concentrations compared to standard amoxyrite against the tested oral pathogens. **Conclusion:** The prepared nanoparticles are found to possess strong antioxidant; anti-inflammatory and bactericidal effect and the activity is concentration dependent.

Keywords: Lemongrass, Ginger, Copper oxide nanoparticles, Oral pathogens.

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Received: 04-07-2024;
Revised: 24-07-2024;
Accepted: 04-09-2024.

INTRODUCTION

The natural environment contains nanoparticles, which are extremely small particles generated by human activity and often enclosed in an interfacial layer. They form particulate dispersions and are solid particles with diameters ranging from 10 to 100 nanometers.[1] Nanotechnology is the practical application of nanoscale innovation utilizing chemical, physical and biological techniques on a scale ranging from atoms to submicron dimensions.[2] The green methodology, as an alternative to the traditional physical and chemical methodologies, completely removes the possibility of producing undesirable or dangerous by-products. A new and intriguing method in the field of nanotechnology is the biological production of nanoparticles. It is simple to scale up for large-scale synthesis, requires little high energy or high temperature and is also economical to employ plants for the biosynthesis of nanoparticles.[3]

Lemongrass (*Cymbopogon citratus*) leaves are employed in medicinal applications for their ability to combat infections. Lemongrass prevents the growth of the bacteria and yeast and it is a great pain reliever and fever reducer. Lemongrass contains antioxidants and also a rich source of flavonoids.[4] Ginger (*Zingiber officinale*) has antibacterial properties and is also good at halting growth of the bacteria. It helps to settle the vomiting and also the nausea caused by the chemotherapy. Ginger is an anti-inflammatory and helpful in treating the symptoms of osteoarthritis. Bioactive compounds found in Ginger have the potential to decelerate the progression of certain types of cancer.[5]

DOI: 10.5530/pres.16.4.108

Metal nanoparticles have been biosynthesized using a variety of sources, including natural products, bacteria, fungi, yeast and enzymes. Copper oxide nanoparticles are mainly antimicrobial agents and also have great antiviral effects. It attracted attention mostly by antimicrobial and bactericidal properties.[6] Copper Oxide Nanoparticles (CuONPs) have great biological properties and also have antibacterial property against drug-resistant bacteria. Copper nanoparticles are found to possess anti-inflammatory and antioxidant activity.[7,8]

The chemical approach is simple and economical. The use of toxic materials has been linked to adverse effects on human and biological health, according to many researchers. Green manufacturing of synthetic materials or chemicals is currently essential to improving humans and maintaining the natural world. Copper oxide nanoparticle synthesis using green synthesis is the best method for creating nanostructured materials because it reduces the amount of toxic material that is harmful to human health and the environment.[9,10] In this research, copper oxide nanoparticles were produced by utilizing an aqueous formulation prepared from lemongrass leaves and dry ginger. The objective was to access these prepared CuONPs for their anti-inflammatory, antioxidant and antimicrobial abilities.

MATERIALS AND METHODS

Preparation of Plant Formulation

Fresh leaves of Lemongrass were obtained from the Nanoherbal Garden present in Saveetha Dental College, Chennai. The leaves were properly double-cleansed with Mili-Q water and then they were exposed to the sun for five days to dry. The dried leaves were powdered and crushed. Dry Ginger powder was commercially collected from the local store present in Thiruverkadu, Chennai. 1 g of the lemongrass leaf powder and 1 g of dry ginger was precisely weighed and added to 100 mL of distilled water and it was boiled at 50ºC for 15 min to make it as a solution. This enables the phytochemicals in the powder to become active and Whatman filter paper No: 1 was utilized to filter the formulation.

Biosynthesis of Copper oxide nanoparticles

A precisely measured volume of 50 mL of the boiled formulation was mixed with an equal amount of 30 mM copper sulfate, maintaining a consistent ratio of 50:50. This mixture solution was left to stand at Room Temperature (RT) on a stirrer for duration of 48 hr. Subsequently, to eliminate any remaining debris and untreated components, the resulting solution was subjected to centrifugation at 8000 RPM for 10 min, after that pellet was preserved for future use at a low temperature. The pellet was dried using a hot air oven to make it as a powder form. From the powder of synthesized nanoparticles, 100 mg of CuONPs was mixed in 10 mL of distilled water and used for conducting studies.

Anti-inflammatory Activity

Bovine Serum Albumin Denaturation Assay (BSA assay)

The assay was carried out based on a previous article conducted by Yazhlini P *et al.*, 2022 to evaluate the anti-inflammatory activity of the prepared Lemongrass and Ginger mediated CuONPs using BSA assay.[11]

Egg Albumin Denaturation Assay (EA assay)

The assay was carried out based on a previous article conducted by Ammulu MA *et al.*, 2021 to investigate the anti-inflammatory ability of the synthesized CuONPs using EA assay.[12]

Antioxidant Activity

DPPH Radical Scavenging Assay (DPPH assay)

The assay was carried out based on a previous article conducted by Xie J and Schaich KM, 2014 to investigate the antioxidant activity of the synthesized CuONPs was examined using DPPH assay.[13]

Hydrogen Peroxide Radical Scavenging Assay (H_2O_2 assay)

The assay was carried out based on a previous article conducted by Ganapathy *et al.*, 2014 to investigate the antioxidant activity of the synthesized copper oxide nanoparticles using H_2O_2 assay.[14]

Time-Kill Curve Assay

The assay was carried out based on a previous article conducted by Tharani M *et al.*, 2023 to investigate the time-kill kinetics of the synthesized copper oxide nanoparticles using time-kill curve assay.[15]

RESULTS

Visual observation

The CuONPs were synthesized using the green synthesis method by utilizing lemongrass and dry ginger as reducing and stabilizing agents. As displayed in Figure 1 (a) and (b), the bioactive compounds found in lemongrass and dry ginger exhibit a reducing effect, as evidenced by the change in colour from light brown to dark brown.

Anti-inflammatory Activity

The anti-inflammatory capability of the CuONPs produced using Lemongrass and dry Ginger was evaluated using BSA and EA assays. Figure 2 (a) shows the anti-denaturation effect of the prepared CuONPs on bovine serum protein at five different concentrations compared to the standard value and the synthesized nanoparticles show 42% inhibition at 10 µL concentration, 55% inhibition at 20 µL, 65% inhibition at 30 µL, 70% inhibition at 40 µL and 75% inhibition at 50 µL. Figure 2 (b)

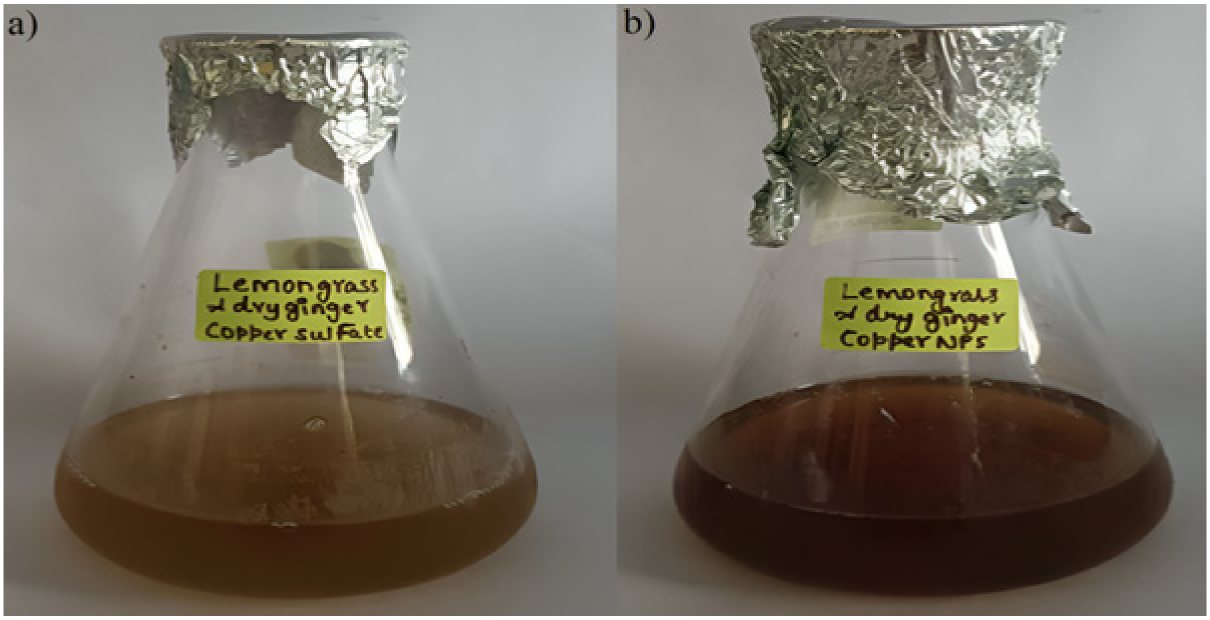

Figure 1: Visual observation of CuONPs synthesized using Lemongrass and Dry ginger (a) Initial colour change (b) Final colour change.

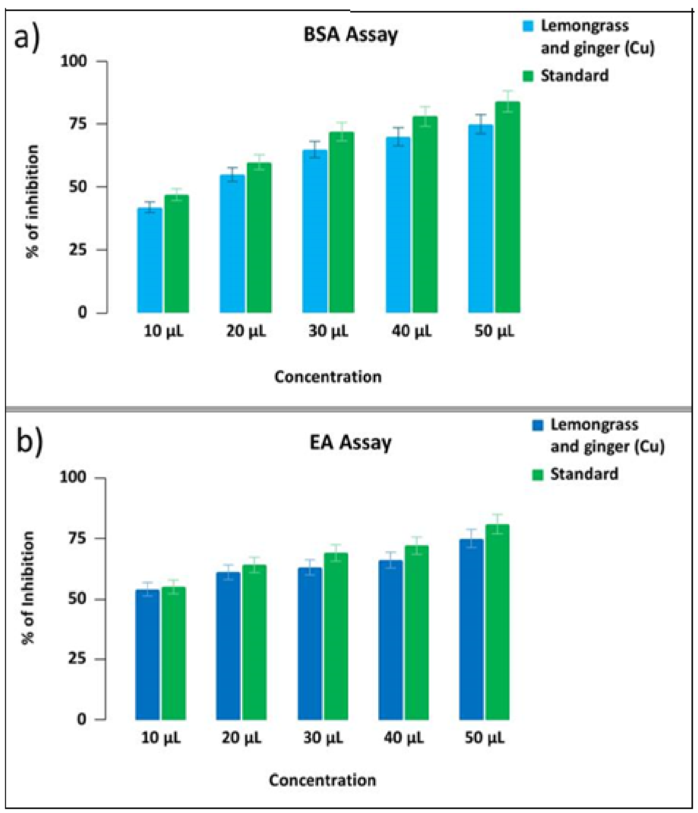

Figure 2: Graph displaying the anti-inflammatory activity of the synthesized copper oxide nanoparticles (a) Bovine serum albumin denaturation assay (b) Egg albumin denaturation assay.

shows the anti-denaturation effect of the prepared CuONPs on egg albumin protein at the same five different concentrations and the prepared nanoparticles show 54% inhibition at 10 µL, 61% inhibition at 20 µL, 63% inhibition at 30 µL, 66% inhibition at 40 µL and 75% inhibition at 50 µL. The results of both assays show that the synthesized copper oxide nanoparticles display potent anti-inflammatory capability by preventing the denaturation of both albumin proteins.

Antioxidant Activity

The antioxidant activity of the CuONPs synthesized using Lemongrass and dry Ginger was evaluated using DPPH and

H_2O_2 assays. Figure 3 (a) displays the antioxidant activity of the prepared nanoparticles against DPPH free radicals at five different concentrations compared to the standard value and the synthesized nanoparticles show 63.83% inhibition at 10 µL concentration, 71.72% inhibition at 20 µL, 84.29% inhibition at 30 µL, 87.36% inhibition at 40 µL and 92.1% inhibition at 50 µL. Figure 3 (b) shows the antioxidant activity of the prepared nanoparticles against hydroxyl free radicals at five different concentrations and the nanoparticles show 51.27% inhibition at 10 µL, 55.34% inhibition at 20 µL, 63.76% inhibition at 30 µL, 72.57% inhibition at 40 µL and 86.23% inhibition at 50 µL. The result of both assays shows that the synthesized copper oxide nanoparticles display excellent antioxidant activity by scavenging both free radicals.

Time Kill Curve Assay

The results of the kill curve assay show that the prepared CuONPs show a reduction in the number of viable cells for the tested oral pathogens. The graphs of the time-kill curve assay display that the prepared nanoparticles show the maximum bactericidal activity on *S. mutans* (Figure 4a) and *E. coli* (Figure 4b) and on *S. aureus* (Figure 4c), the synthesized nanoparticles show both slight bactericidal and bacteriostatic activity. The results display that bactericidal and bacteriostatic activity of the nanoparticles is gradually taking place over time. The produced nanoparticles at 100 µL concentration show maximum bactericidal activity when compared to the standard amoxyrite on all the three tested organisms.

DISCUSSION

Copper oxide nanoparticles prepared using the green synthesis method are the biocompatible and economical method for the synthesizing of nanoparticles compared to the chemical synthesis which are more toxic comparatively. The bioactive compounds present in the Lemongrass and Ginger which are used in our study as a reducing agent will increase the therapeutic effect of the prepared nanoparticles. In one previous study, the Copper nanoparticles prepared using *Ocimum sanctum* showed 81.3% inhibition in the DPPH method,[16] and in another study, the CuONPs prepared using *Cissus arnotiana* showed the highest percentage of inhibition at the higher concentration in the DPPH method.[17] These studies display that Lemongrass and dry ginger-mediated CuONPs showing excellent antioxidant activity correlates with the previous findings.

Blue tea-mediated copper nanoparticles were examined for anti-inflammatory activity using a BSA assay and the results displays that the prepared NPs possess significant anti-inflammatory activity.[18] Another study shows that the CuNPs prepared using *Sarcostemma acidum* exhibited 86.30% inhibition at the highest concentration of 100 µL in the EA assay while in the BSA assay, it showed 88.45% inhibition.[19] These two studies show that copper nanoparticles have strong anti-inflammatory activity and they synchronize with our current findings.

The previous study carried out using copper nanoparticles was examined for its bactericidal effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the result shows

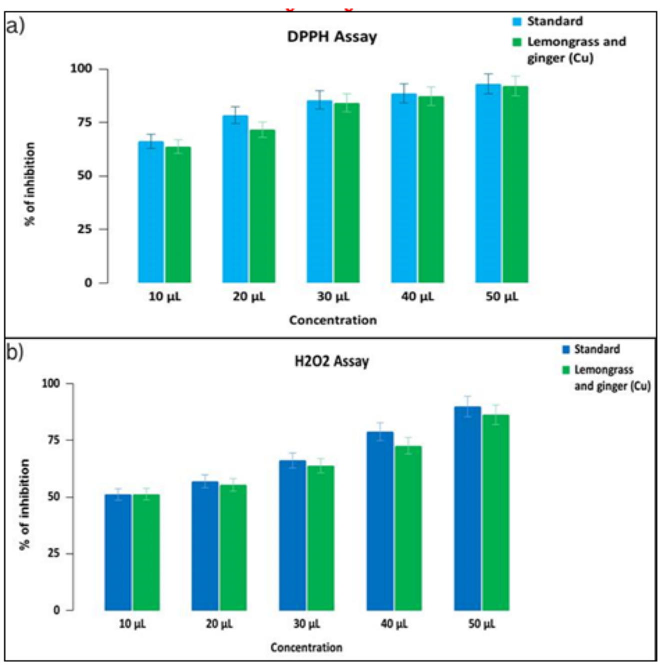

Figure 3: Graphs displaying the antioxidant activity of the synthesized copper oxide nanoparticles (a) DPPH radical scavenging assay (b) Hydrogen peroxide radical scavenging assay.

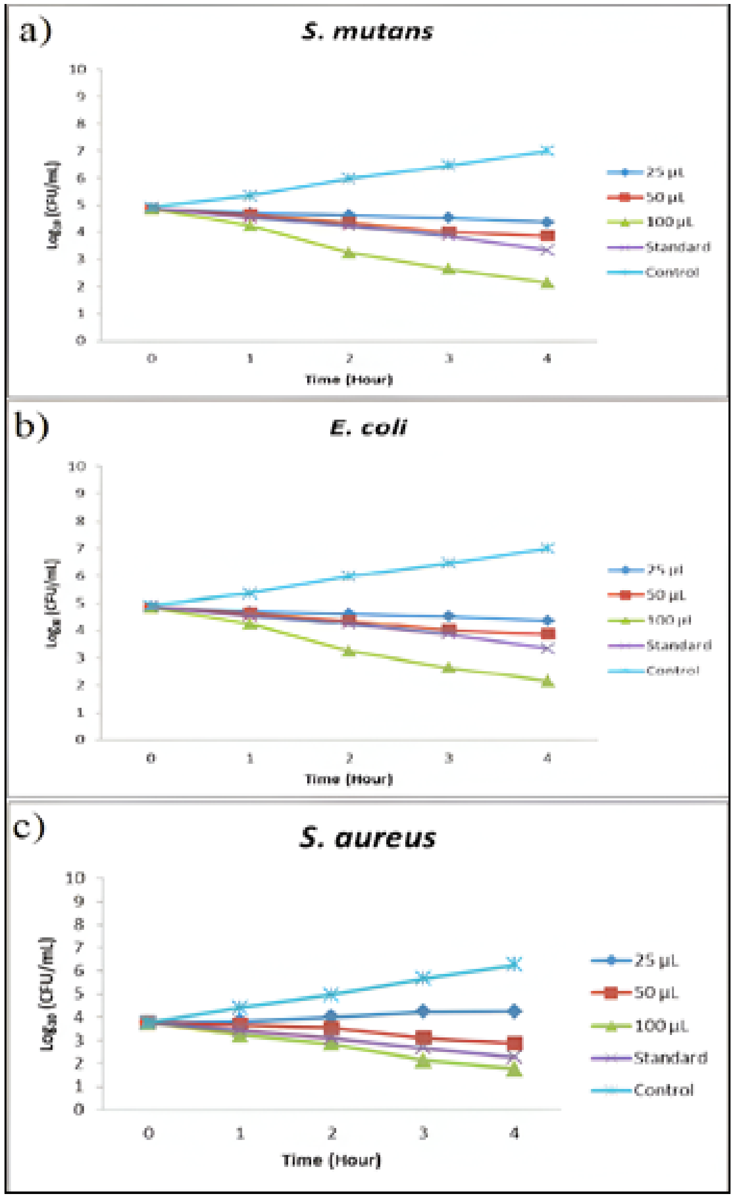

Figure 4: Graphs showing the time kill kinetics analysis of the prepared Copper oxide nanoparticles against oral pathogens (a) *S. mutans* (b) *E. coli* (c) *S. aureus.*

that at the end of the fourth hour, the prepared nanoparticles displayed maximum reduction in the cell density in both the tested organisms.[20] Isoquercetin and Cassinopin-capped copper nanoparticles displayed the maximum bactericidal effect against the biofilm-forming methicillin-resistant *Staphylococcus aureus*.[21] These findings display the potential bactericidal effect of copper oxide nanoparticles in a time-kill curve assay. The results of our study show that the copper oxide nanoparticles synthesized using Lemongrass and Ginger have a great bactericidal and bacteriostatic effect on the tested oral pathogens and they also possess significant antioxidant and anti-inflammatory activity.

CONCLUSION

The current study exhibits that the prepared CuONPs have potent antioxidant and anti-inflammatory activity and it has excellent bactericidal activity. The synthesized nanoparticle shows activity in a concentration-dependent manner i.e. the highest concentration shows the maximum activity. As the synthesized nanoparticles show greater bactericidal and bacteriostatic activity against oral pathogens, they can be further utilized in the dental field by using the nanoparticles in dental fillings, coating the nanoparticles in dental implants and other dental materials. However further studies are needed to examine other therapeutic ability of the prepared nanoparticles.

ACKNOWLEDGEMENT

We would like to thank Saveetha Institute of Medical and Technical Sciences for support.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ABBREVIATIONS

CuONPs: Copper Oxide Nanoparticles; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **H_2O_2:** Hydrogen Peroxide; **BSA:** Bovine Serum Albumin; **EA:** Egg Albumin; **RT:** Room Temperature; **RPM:** Revolutions Per Minute; **MRSA:** Methicillin-Resistant Staphylococcus Aureus.

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Cite this article: Shanmugam R, Ravikumar R, Kumar AS, Anandan J, Loganathan A, Jain K. Controlling of Oral Pathogens and Anti-Inflammatory Activity of Copper Oxide Nanoparticles Synthesized Using *Cymbopogon citratus* and *Zingiber officinale*. Pharmacog Res. 2024;16(4):943-8.