

# Antimicrobial and cytotoxic effect of lemon grass and ginger formulation assisted copper-oxide nanoparticles

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## ABSTRACT

**Background:** Copper nanoparticles are widely used as antioxidant, antimicrobial, anticancer, anti-inflammatory, antihepatotoxic agents. Being more biocompatible, eliminates the risk for toxicity, ginger has been shown to help reduce blood sugar levels and help regulate insulin response in people. Lemongrass tea is more effective and helps to maintain optimum levels of insulin and improve the glucose tolerance in the body and also helps in preventing the growth of some bacteria and yeast.

**Aim:** The aim of the study is to identify the antimicrobial and cytotoxic effect of lemon grass and ginger formulation assisted copper-oxide nanoparticles

**Method:** Anti-microbial assay is done by testing the sample with certain main microorganisms and for the Cytotoxic effect, Lethal test is done using brine shrimps.

**Results:** Copper nanoparticles synthesized using lemon grass and ginger extract can act as a potential cytotoxic agent and antimicrobial activity. However, the antimicrobial activity of the extract varied with the different bacterial samples. The cytotoxicity of nano-materials and nanocomposites tested against brine shrimps showed minimal lethality. Copper nanoparticles are previously reported to show potent antibacterial and to show cytotoxic activities in-vivo/in-vitro and our results are in accordance with their results.

**Conclusion:** For the first time it is concluded that lemon-grass and ginger mediated Copper nanoparticles are potent therapeutic agents to be used in biomedical applications both in-vivo/in-vitro. Biologically synthesized copper-oxide nanoparticles show exhibitory diverse therapeutic potential which gains more importance currently.

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## INTRODUCTION

Nanotechnology involves science, engineering and innovation that considers matter at atomic, molecular or supramolecular levels to produce nanometric materials and nanosystems with further developed properties, such as high surface-to-volume ratios and high dispersion in solution. Nanomaterials are small solid particles having a dimension of 1-100 nanometers. Nanomaterials show promise in antibacterial therapy because of their improved and distinct physicochemical properties, including very small dimensions, enormous surface area contrasted with their mass, and higher reactivity(1). Nanoparticles are also well known for focusing drug delivery and checking specific microorganism development. Therefore, nanoproducts are widely used in different industrial, medical, and dentistry sectors(2). As nanostructured, very few for example gold, palladium, silver, and platinum are synthesized from nature(3).

## KEYWORDS:

Antimicrobial effect, cytotoxic effect, lemon grass, ginger formulation, copper-oxide, nanoparticles

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Many processes have been created to synthesize nanoparticles for various applications.

Among them green synthesis is acquiring significance as a result of their immense applications in the field of physics, chemistry, biology, and medicine. In green synthesis nanoparticles are produced by utilizing plants or microorganisms(4). Biological agents are used as reducers which are secondary metabolites to synthesize nanoparticles from plants. Plant extracted nanomaterial is being used as a medication in biomedical industries. They are essentially reliable, low in cost, nontoxic, stable in nature and eco friendly(5). Some nanoparticles including gold, titanium, silver, and zinc have effectively shown bacterial action against a large number of pathogenic organic entities. Depending upon their morphological and physical characteristics, as well as their composition, MNPs from plants with medicinal worth can exhibit improved antibacterial, antifungal and antibiofilm activities, hence constituting an extremely encouraging method for fighting antimicrobial resistance(6).

The natural biological methodology of metal or metal oxide nanoparticle synthesis focuses on the utilization of bacteria, fungi, algae, yeast and plant extracts, as reducing agents for the synthesis of nanoparticles which support biocompatibility and enormous scale production(7). Antibiotic treatment of oral biofilms is inadequate, frequently prompting persistent oral diseases and constrained tooth extraction or implant removal due to the improvement of antibiotic resistance(8). Oral biofilms contain a complex polymicrobial community in which oral streptococci are initial colonizers(9). In this study, the copper/copper oxide nanoparticles were synthesized from CuSO<sub>4</sub> solution using aqueous extract of lemongrass leaf and ginger as a reducing agent for evaluating its antimicrobial and cytotoxic properties.

## MATERIALS AND METHODS

### Antibacterial Activity

Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Bacillus*, and *E.coli*. Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar was prepared and sterilized for 15 minutes at 121 °C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentrations (25µL, 50 µL ,100 µL) were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 °C. After the incubation time the zones of inhibition were measured.

### Cytotoxic activity

#### Brine Shrimp Lethality Assay

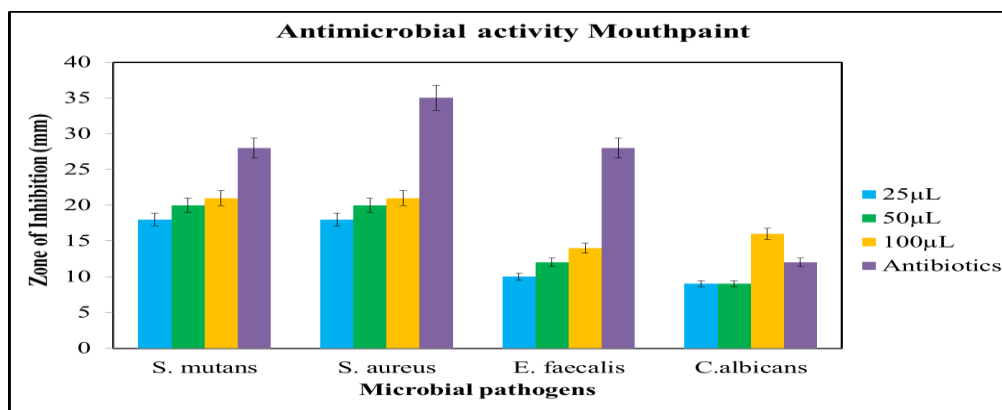
##### Salt water preparation

2g of iodine free salt was weighed and dissolved in 200 ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10 µL,20 µL,40 µL,80 µL and control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100

## RESULT



**Graph 1:** displays the antibacterial effectiveness of copper-oxide nanoparticles augmented with lemongrass and ginger extract against various infections. The highest zone of inhibition was shown by *Streptococcus aureus* at a concentration of 100 liters (21 mm), *Staphylococcus mutans* at a concentration of 100 liters (14 mm), *Enterococcus faecalis* at a concentration of 100 liters (16 mm), and *candida albicans* at a concentration of 100 liters (22 mm). This demonstrated that the antibacterial activity increased with the concentration of Copper-Oxide nanoparticles combined with lemongrass and ginger formulation.

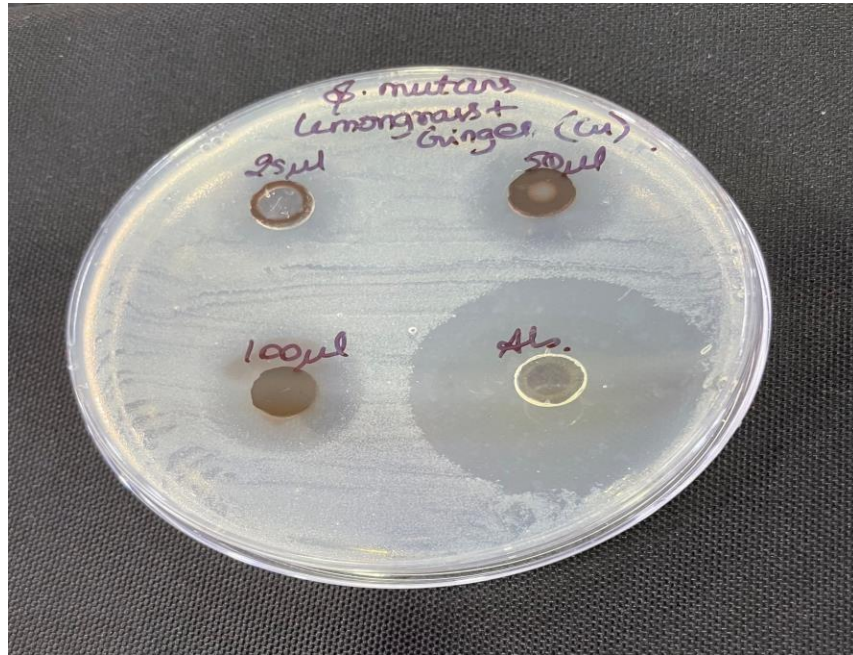


Figure 1: Antimicrobial Activity of Copper-oxide nanoparticles formulated with Lemongrass and Ginger extract against *Streptococcus mutans*.

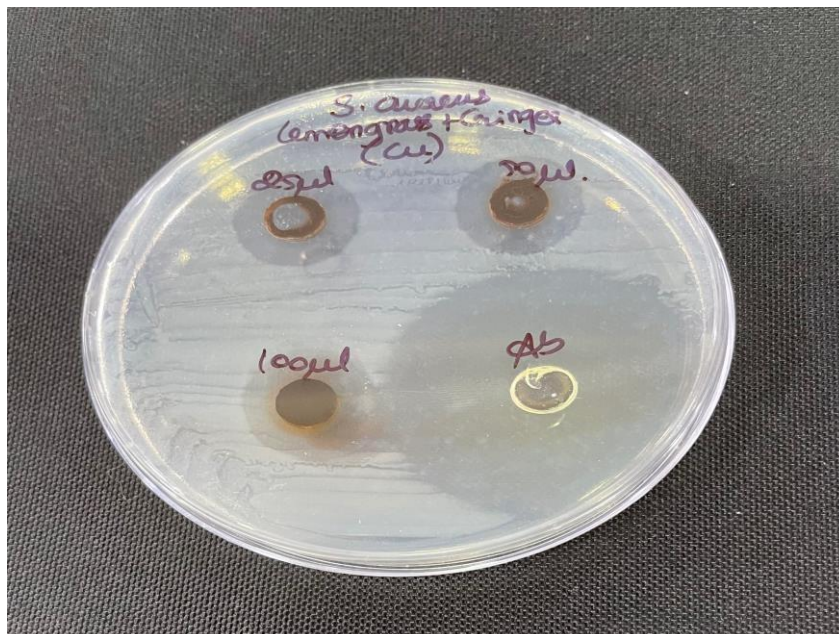


Figure 2: Antimicrobial Activity of Copper-oxide nanoparticles formulated with Lemongrass and Ginger extract against *Staphylococcus aureus*.



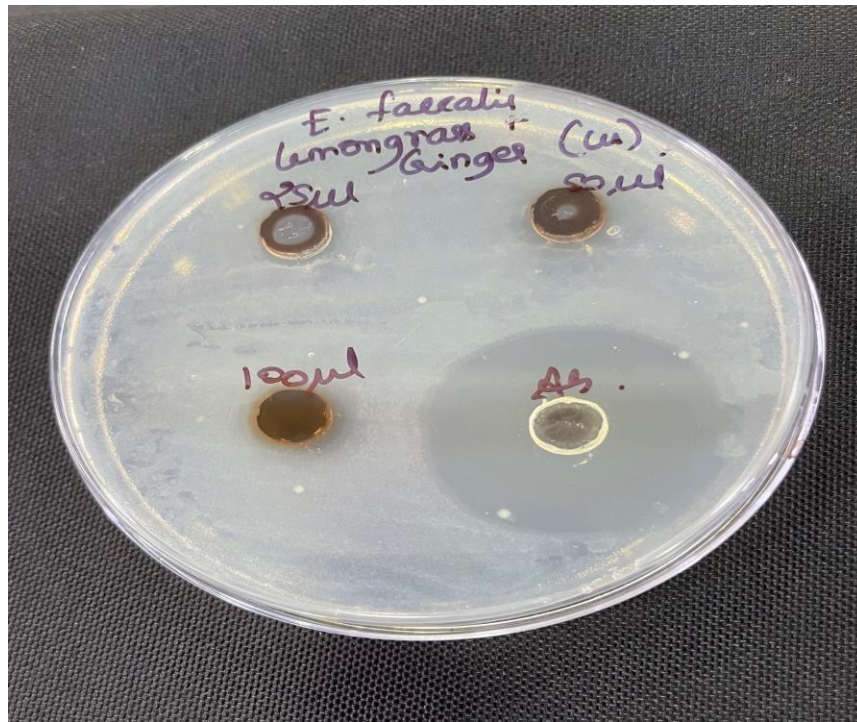


Figure 3: Antimicrobial Activity of Copper-oxide nanoparticles formulated with Lemongrass and Ginger extract against *Enterococcus faecalis*.

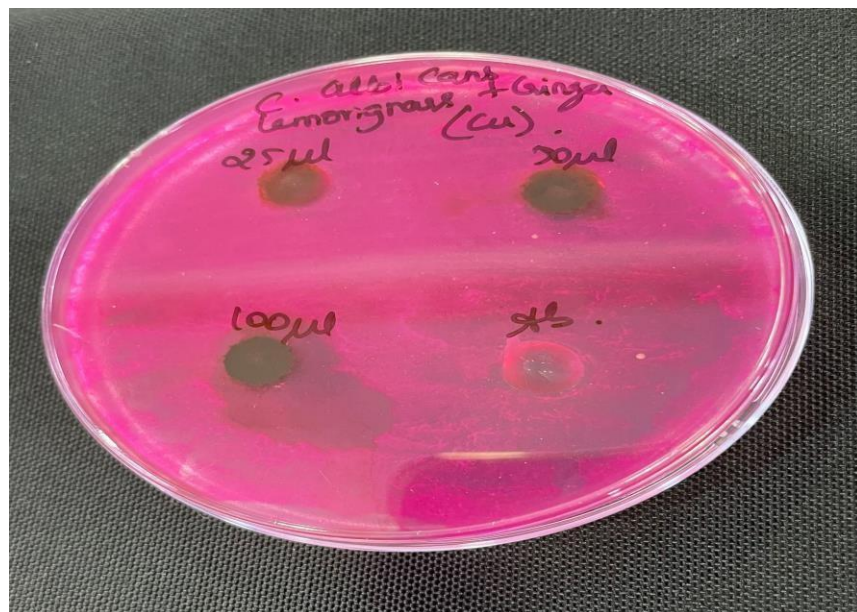
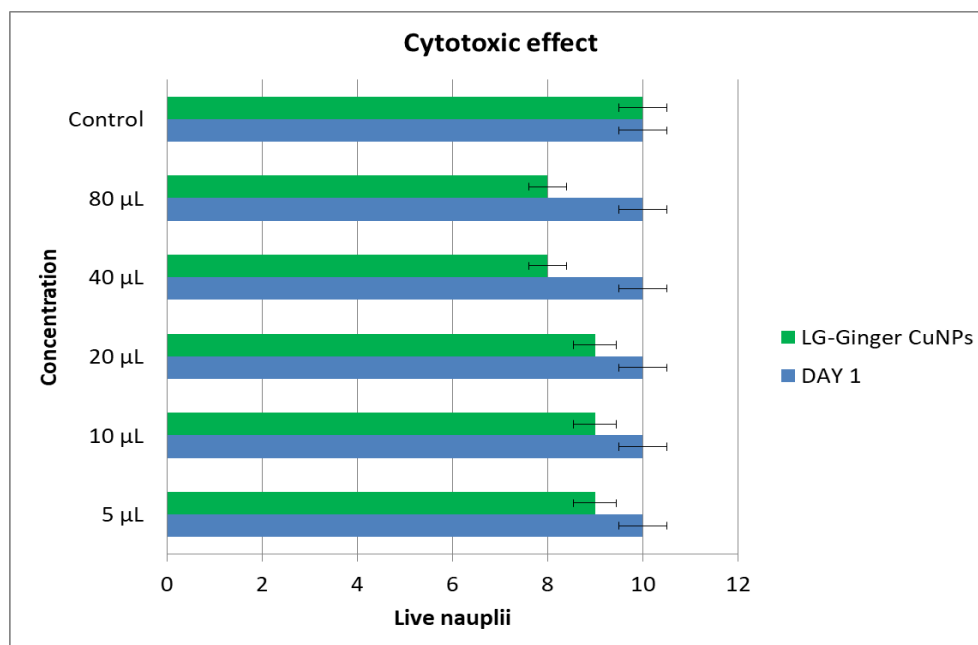


Figure 4: Antimicrobial Activity of Copper-oxide nanoparticles formulated with Lemongrass and Ginger extract against *Candida albicans*.

### Cytotoxic effect

Graph-2: Cytotoxic effect of Lemongrass and ginger mediated Copper-oxide nanoparticles at varying concentration ( $\mu$ l)



**Graph 2:** depicts the cytotoxic effectiveness of copper-oxide nanoparticles augmented with lemongrass and ginger extract against the Brine Shrimp at different concentrations for 24 hours. When compared to the control, 5µl, 10µl and 20µl show high cytotoxic activity when compared to 40µl and 80µl. This demonstrates that a potential cytotoxic effect is present when lemongrass and ginger mediated copper-nanoparticles are used to check the lethal assay.

## DISCUSSION

In the previous study, the antibacterial activity was tested against both Gram-positive and Gram-negative organisms. Stem bark extract exhibited good antibacterial activity against the tested organisms. The stem bark extract showed maximum activity against *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and the leaf extract showed maximum activity against *Escherichia coli*, *Alcaligenes faecalis* and *Pseudomonas aeruginosa*(10). Lemon grass has wonderful antioxidant, antimicrobial, and anti-inflammatory activities. Phytocompounds present in lemon grass also showed antidiabetic, hypolipidemic, anticancer, gastroprotective, and wound-healing properties(11).

The study conducted by Saha et al, found that Cu NPs of lemon grass exhibited good antibacterial potential against gram-positive and gram-negative bacterial strains. The antibacterial activity of lemon grass depends upon the concentration of silver nanoparticles(12). Stem bark extract showed maximum activity against *Enterobacter aerogenes* (21.5 mm) whereas in the present study, *Candidiasis albicans* showed maximum zone of inhibition for anticariogenic properties(13). In the study conducted by udar et al, the ginger ethanolic extract possesses antitumorogenic, antioxidant and hypoglycaemic properties, hepato-renal protective effect(14). Lemon grass can be developed as a chemotherapeutic agent in breast cancer treatment. In the previous study conducted by goel et al found that lemon grass extract showed maximum zone of inhibition against *Staphylococcus aureus*(15). The standard antibiotic showed the maximum zone of inhibition compared with both the plants. It was clearly observed that ethanolic extract of both the plants were having good antimicrobial

activity towards *Staphylococcus aureus*(8).

Antioxidant activity in DPPH from (27.58 -- 226.28 µg/mL) and in ABTS (14.17 -- 117.62 µg/mL). Highest antioxidant activity was observed in GT and lowest was observed in rose tea (RT). Heatmap was made for catechin visualization in green herbal teas (GHT). Principal Component Analysis (PCA) showed the variation of amino acids in all the herbal tea samples which was found in the range from 0.82 to 2.86%(16)

In the present study, only four oral pathogens were tested for antimicrobial activity. In future studies, more oral pathogens can be tested . In the present study, *s.aureus* and *s.mutants* has a greater zone of inhibition at all the concentrations and hence new antimicrobial drugs can be developed from the copper nanoparticles of lemon grass and ginger formulation

## CONCLUSION

From the above study, it's concluded that the copper nanoparticles of lemon grass might represent a replacement antimicrobial source with stable, biologically active components which will establish a scientific base for the utilization in modern medicine. Use of herbal medicine is increasing worldwide. Medicinal plants are safe with no side effects and they possess effective action against bacteria and other microorganisms. Based on the results recorded in the present study, it is concluded that lemongrass has a potential antimicrobial activity on human oral pathogenic microorganisms. Hence the present study findings provide a wonderful scientific base for some of the medicinal claims of lemon grass and ginger.

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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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Informed Consent- None declared

## Authorship contribution

AMP compiled the manuscript RKS conducted the study TL designed the study AJ Performed Proof reading of the Manuscript

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