Original Article

Cytotoxicity, Antimicrobial, Anti-inflammatory and Antioxidant Activity of Camellia Sinensis and Citrus Mediated Copper Oxide Nanoparticle—An *In vitro* Study

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ABSTRACT

Aim: Several applications of copper oxide nanoparticles (CuONPs) have been documented in various fields, including healthcare, dentistry, medication delivery, tissue and cancer imaging, biolabeling, and biosensing. Therefore, this study aimed to synthesize CuONPs using the plant extracts of Camellia Sinesis (CS) and citrus limon (CL). The nanoparticles were then evaluated for their cytotoxicity, antibacterial, anti-inflammatory, and antioxidant activities. **Materials and Methods:** CuONPs were prepared using CS and CL through the green synthesis method. The Zone of Inhibition (ZOI) test was used to assess the antibacterial activity against strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans*. The albumin denaturation assay was used to assess the substances' anti-inflammatory activity. The cytotoxicity was determined by conducting the brine shrimp lethality test. Additionally, the antioxidant nature was tested using the 1,1-diphenyl-2-picryl hydrazyl method. **Results:** CuONPs mediated by CS and CL were successfully synthesized. The nanoparticles demonstrated significant antimicrobial activity against the bacteria being studied, specifically *S. aureus*. The cytotoxic effect was observed to be the least when the concentrations were below 20 µL. A potent antioxidant effect, characterized by its maximum absorbance at 517 nm, was observed at a concentration of 50 µL. A significant anti-inflammatory effect was noted for all tested concentrations. **Conclusion:** The use of CS- and CL-mediated CuONPs demonstrates a favorable antimicrobial effect with reduced cytotoxicity, as well as improved anti-inflammatory and antioxidant effects at higher concentrations.

Keywords: *Anti-inflammatory, antimicrobial, camellia sinensis, citrus limon, copper oxide, CuONPs. green synthesis, cytotoxicity, nanoparticles*

Introduction

Nanoparticles have become popular due to their novel properties, such as a high specific surface area that results in increased reactivity.[1] The conventional methods used for synthesizing nanoparticles have several drawbacks, including high energy consumption, the release of toxic chemicals, and the requirement for complex equipment and synthesis conditions. There is a growing interest in green synthesis as an alternative and cost-effective technique for nanoparticles.[2]

Copper is both a cost-effective metal and an abundant metal. Copper oxide exhibits a variety of potential physical characteristics and has antimicrobial properties, along with low toxicity. These qualities make it a promising candidate for medical and dental applications.[3] Obtaining

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copper oxide nanoparticles (CuONPs) directly from copper salts is challenging due to the nature of copper, which belongs to the group of light transition metals. the group of light transition metals. To regulate particle size, the use of capping agents such as surfactants is necessary. Dental cements, restorative materials, adhesives, resins, irrigating solutions, obturations, orthodontic archwires and brackets, implant surface coatings, and the process of bone regeneration might all be enhanced by copper nanoparticles.[4] CuONPs exhibit distinctive crystal morphologies and possess an exceptionally large surface area, contributing to their antibacterial characteristics.[5] When combined with other metals, ceramics, and polymers, they easily mix and bind, displaying their physicochemical stability.[6] In their study, Amiri *et al.* stated the potential bactericidal activity of CuONPs and their effectiveness as a control agent for preventing dental caries.[7]

Copper ions are frequently reduced using reducing agents derived from plant extracts as a part of the green synthesis process for CuONPs. The specific plant or plant component utilized in the production process is crucial because it directly influences the characteristics of the resultant nanoparticles.

People worldwide have long valued the cultivated evergreen shrub, Camellia Sinesis (CS), for its curative and restorative properties.[8] It is the most widely consumed beverage globally.[9] The tea plant contains several beneficial substances, including polyphenols, flavonoids, and catechins, which are responsible for its beneficial properties. The antibacterial, antioxidant, anti-allergic, and anti-inflammatory properties can be attributed to flavonoids. Water-soluble compounds known as tea catechins have a significant antibacterial effect, primarily on Gram-positive bacteria. The emergence of numerous drug-resistant diseases has captured the attention of pharmacologists, who are now searching for new molecules with robust antibacterial properties and novel mechanisms of action.[10]

One of the most significant taxonomic components of the Rutaceae family is the genus Citrus. In common parlance, the fruits produced by species in this genus are commonly referred to as "citrus."[11] Citrus fruits are well known for their excellent nutritional, therapeutic, and cosmetic properties. Citrus is a therapeutic plant that possesses a variety of pharmacological qualities. Citric acid, phenolic compounds, flavonoids, carotenoids, and ascorbic acid are among the bioactive components that are abundant in it.[12] The primary flavanone found in lemons is hesperidin. It has been shown to affect capillary resistance, vascular permeability, and possess analgesic and anti-inflammatory activities (Fuster, 1997).[13]

In this study, the researchers investigated the cytotoxic, antimicrobial, anti-inflammatory, and antioxidant effects of nanoparticles infused with CS and citrus limon (CL). These effects were studied in relation to the extensive medical background. The safety and biocompatibility of the synthesized nanoparticles were also investigated to assess their suitability for incorporation into over-the-counter mouthwashes for orthodontic patients. This is done to reduce plaque accumulation around brackets and bands, in addition to routine oral hygiene practices.

Materials and Methods

Plants extract formulation and characterization of nanoparticles

CuONPs were prepared by combining equal parts of CL extract and CS. CS and CL were separately pulverized into a coarse powder. Then, 2 g of each was added individually to 100 mL of distilled water for dispersion. The extract was subsequently filtered using filter paper and then refrigerated at 4°C. Prior to filtration, it was boiled at 60°C in a heat mantle [Figure 1]. Afterward, 0.016 g of copper sulfate and 90 mL of distilled water were used to treat the CS and CL. The extract was placed in an orbital shaker and spun at 120 rpm for the entire night. A twin-beam UV spectrophotometer that operates at wavelengths between 250 and 650 nm was used to check on the formation of nanoparticles hourly. The centrifuged nanoparticle solution changed color from blue to brown after being spun at 8000 rpm for 10 min. The pellets were kept, and the supernatant was thrown away[14] [Figure 2].

Figure 1: Camellia sinensis and Citrus limon boiling extract

Figure 2: Camellia sinensis and Citrus limon nanoparticles synthesis

Antimicrobial activity

The antimicrobial activity of the corresponding nanoparticles was tested against four organisms.

The test organisms used in this study were the synthetic strains of *Enterococcus faecalis* ATCC 29212, *Streptococcus mutans* ATCC 25175, and *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC *10231*.

Agar well diffusion test

For this experiment, Candida was grown on Sabouraud agar and bacteria were grown on sterile Mueller Hinton Agar. The colonies were spread out over the prepared agar plates to determine the zone of inhibition. The media was added to the plates after they had been sterilized and given time to solidify. The test organisms were then swabbed after the wells were cut with a 9 mm sterile polystyrene tip. Different concentrations of nanoparticles (25, 50, and 100 µL) were loaded. A positive control was present in the fourth well and consisted of fluconazole for fungus and amoxicillin for bacteria. The plates were incubated for 24 h at 37°C. Following the incubation period, the zones of inhibition were measured[15] [Figure 3].

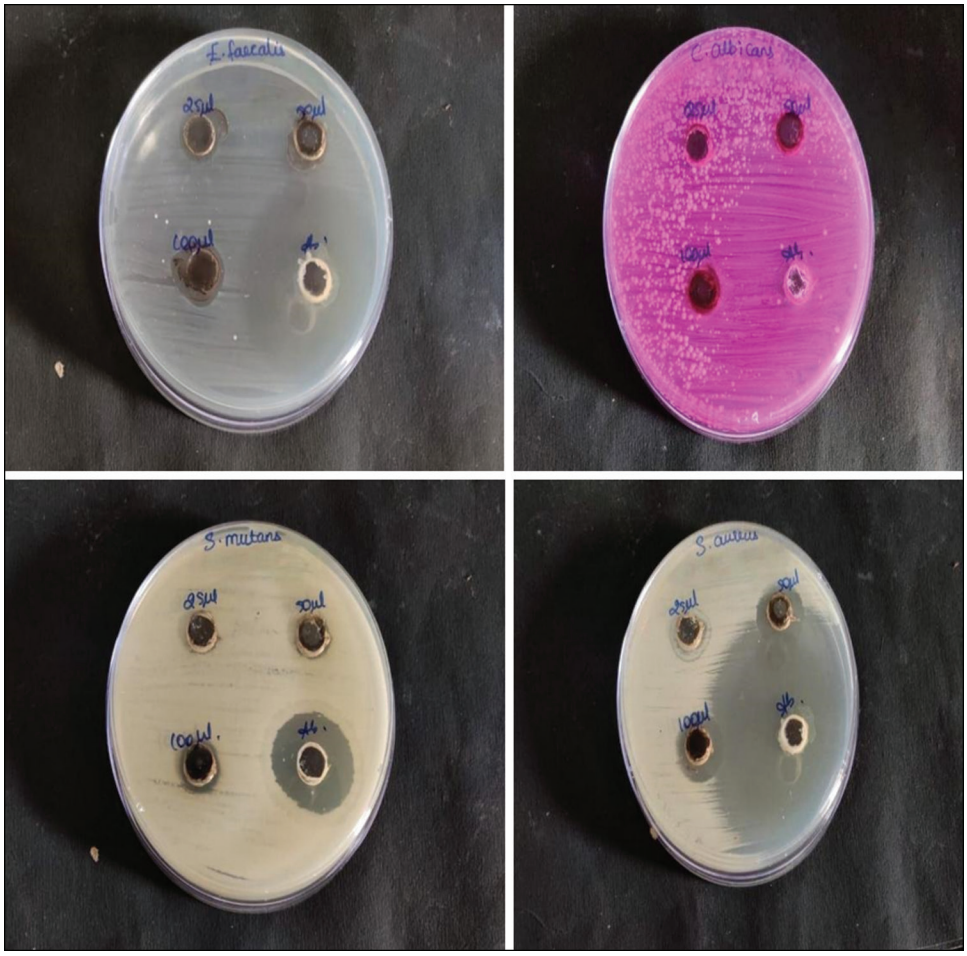

Figure 3: Zone of inhibition of *S. mutans, S. aureus, E. faecalis,* and *C. albicans* against CS- and CL-mediated CuONPs

Cytotoxicity testing

Saltwater preparation

About 2 g of iodine-free salt were dissolved in 200 mL of purified water. 6-well ELISA plates received 10–12 mL of saline water. Ten hatched nauplii (5, 10, 20, 40, 80, and control) were thereafter introduced gradually to each well. The right concentrations of the nanoparticles were subsequently added. For 24 h, the plates were incubated. Then, using a microscope, they were counted. This underwent another review by a different researcher to confirm accuracy. Formula: (number of live nauplii/number of dead nauplii + number of living nauplii) * 100 was used to calculate the number[16] [Figure 4].

Anti-inflammatory activity

Albumin denaturation assay

Muzushima and Kabayashi's modified version of the standard approach was used to assess *Solanum torvum* gel's anti-inflammatory capabilities (Das *et al*. 2019).[17] Different volumes of *Solanum torvum* gel (10, 20, 30, 40, and 50 mL) were mixed with 0.05 mL of bovine serum albumin (1% aqueous solution). A small amount of 1N hydrochloric acid was added to the mixture to achieve the pH of 6.3. These samples were incubated for 20 min at room temperature followed by a 30-min period of heating at 55°C in a water bath. Using a spectrophotometer, the samples' absorbance at 660 nm was measured after cooling. The benchmark used was diclofenac sodium, and the control was DMSO. Using the following equation, the percentage of protein denaturation was calculated

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

Egg albumin denaturation assay

About 2.8 mL of pH-6.3 phosphate-buffered saline and 0.2 mL of egg albumin from hen eggs were combined to create a 5-mL solution. Syzygiumcaryophyllatum preparations were prepared at various concentrations (10, 20, 30, 40, and 50 µL). The positive control was sodium diclofenac. For 15 min, the mixture was heated in a water bath at 37°C. The samples were cooled to room temperature before the absorbance at 660 nm was measured.

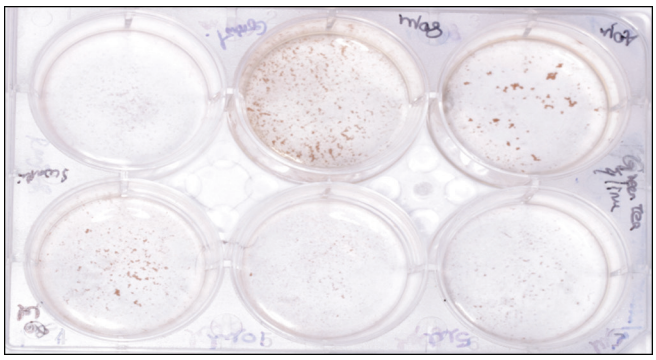

Figure 4: Cytotoxicity activity of CS- and CL-infused CuONPs

Antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) method

The antioxidant capacity of Copper-induced CS and CL nanoparticles was evaluated using the DPPH assay. For a 30-min incubation period, CuONPs were mixed with varying concentrations (10–50 g/mL) of CS and CL extract, 1 mL of 0.1 mM DPPH in methanol, and 450 mL of 50 mM Tris HCl buffer (pH 7.4). Subsequently, it was discovered that the absorbance at 517 nm was attributable to the decrease in the quantity of DPPH free radicals. As a control, butylated hydroxytoluene (BHT) was utilized. The percentage of inhibition was determined using the following equation:

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

Results

Antimicrobial activity

By assessing the zone of inhibition against *S. mutans, S. aureus, E. faecalis, and C. albicans,* the antibacterial activity of the synthesized CS- and CL-produced CuONPs was assessed. There was significant antibacterial action observed against the tested pathogens, although it was not comparable to the zone of inhibition caused by the antibiotic. The zone of inhibition increased as the nanoparticle concentration increased. *S. aureus* was reported to have the largest zone of inhibition [Figure 5].

Cytotoxicity

By examining the death and growth inhibition of the nauplii fish at increasing concentrations, the cytotoxicity of the obtained nanoparticles was investigated. At concentrations greater than 20 µL, a very minimal cytotoxic effect was observed, as shown in Figure 6.

Antioxidant activity

By comparing it to a control substance, BHT, the antioxidant activity was measured and assessed using the DPPH assay. The absorbance at 517 nm was determined using the DPPH assay. The percentage of inhibition at various doses (10–50 µL) is shown in Figure 7. The proportion of inhibition exhibited an incremental pattern with concentration, whereas the activity was found to increase with the dosage volumes.

Anti-inflammatory activity

Data on the anti-inflammatory activity were collected using the Bovine serum assay and albumin

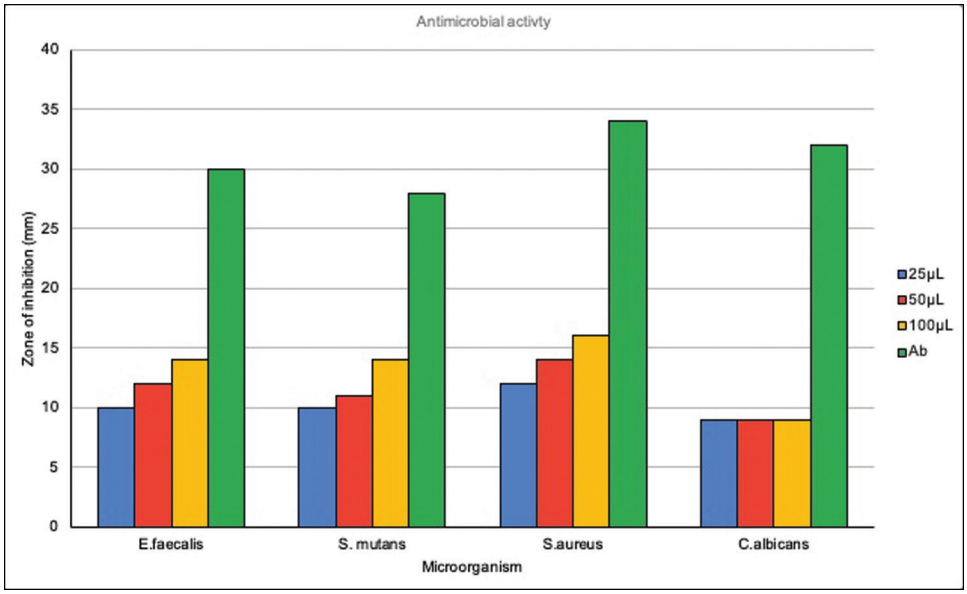

Figure 5: Antimicrobial activity of CS and CL CuONPs

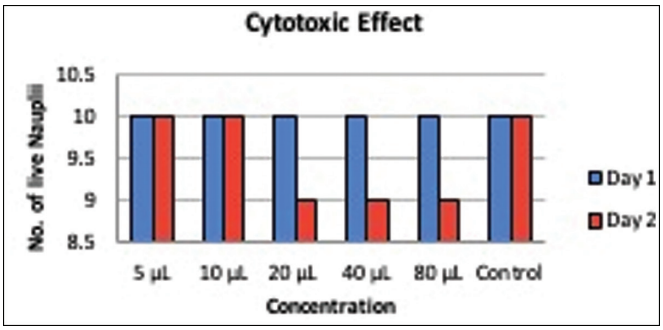

Figure 6: Results of the cytotoxicity tests

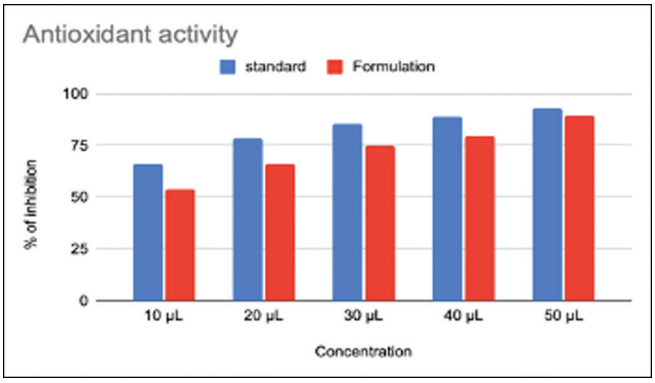

Figure 7: Percentage of inhibition at various doses

denaturation assay. Figures 8 and 9 demonstrate the anti-inflammatory efficacy of CuONPs. The highest efficacy was observed at 50 µL and there was a gradual incremental trend with increasing concentration. Its action increases as the concentration increases.

Discussion

The present study focused on synthesizing CuONPs using the extracts from CS and CL. The NPs synthesized using this method exhibited significant antimicrobial activity against the bacterial and fungal strains investigated in the study. The tested bacterial strains were common oral microbes. The concentration of the prepared nanoparticles was found to have a direct impact on the level of antioxidant and anti-inflammatory activity. The CuONPs synthesized in this study exhibited a minimal cytotoxic effect at concentrations below 20 µL.

A study conducted by Amiri *et al.*[18] investigated the green synthesis of copper nanoparticles using CL. The study also involved the characterization and evaluation of the nanoparticles' antibacterial activity. The nanoparticle in this study was characterized by its blue color in the formulated solution, which changed to brown after centrifugation. The highest antibacterial activity was found against *S. Aureus*, which aligns with the results of the present study.[18] Similarly, Rajeshkumar and Rinitha,[14] Rajeshkumar *et al.*,[19] and Padma *et al.*[20] used similar methods for the green synthesis of CuONPs, but from different sources, namely *Persea americana* seed, *Cissus arnottiana* plant, and *Punica granatum*, respectively.

The CuONPs obtained in the studies by Padma *et al.*[20] and Rajeshkumar *et al.*[19] exhibited notable antimicrobial activity against *Streptococcus* species, along with strong antioxidant properties. These findings were very similar to the findings of the current studies. In a study, Jadhav *et al.*[21] synthesized CuONPs

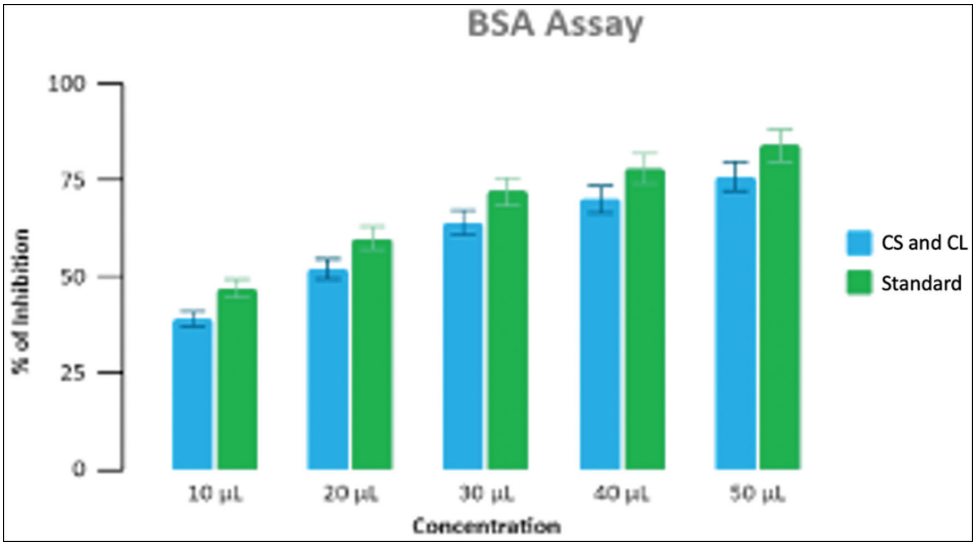

Figure 8: Depicts anti-inflammatory activity assessed with BSA assay

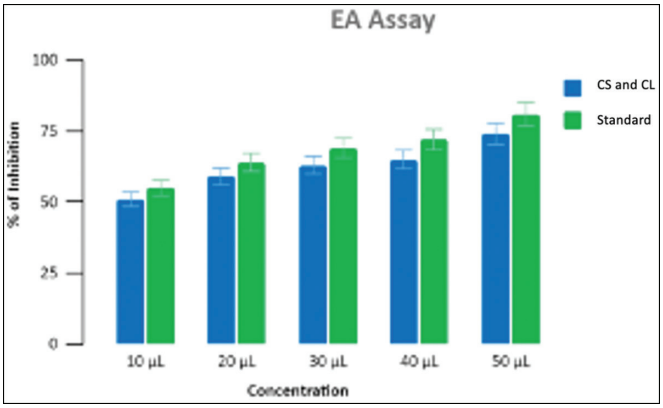

Figure 9: % of inhibition based on nanoparticle concentration

using the chemical method and examined their antimicrobial activity against *S. aureus*. It was reported that the antimicrobial activity was dose-dependent and increased with an increase in concentration, which is similar to the findings of the present study.[21] In their study, Argueta *et al*.[22] found a significant bactericidal effect of CuONPs against *S. aureus* and *S. mutans*. They also observed an improvement in the shear bond strength of the CuONPs modified orthodontic adhesive.

Ren *et al*. reported a study on the characterization of CuONPs using a chemical synthesis method for antimicrobial applications. The study found that the polymers incorporating CuONPs showed improved bactericidal properties against *S. aureus*.[23]

Based on the *in vitro* activity, the concentration of 50 µL displayed the highest percentage of inhibition, indicating enhanced anti-inflammatory activity. The EA assay revealed a significant anti-inflammatory action, with the highest efficacy observed at concentrations of 50 µL. According to the DPPH assay, the newly synthesized nanoparticle exhibited a higher absorbance level at 517 nm, approximately 85%. The radical scavenging ability of the CuONP displayed the highest percentage of inhibition at a concentration of 40 µg/mL, according to Rajeshkumar *et al*.'s[24] investigation. However, in our study, we observed that the concentration exhibiting the maximum antioxidant activity was 50 µg/mL.[14,20,24] In a study on the biogenesis of CuONPs using CS extract, Lei *et al*.[25] proposed the absence of any cytotoxicity in the nanoparticle obtained, even at elevated doses. The analysis of the DPPH findings revealed an increase in antioxidant activity that was dose-dependent manner. The enhanced antioxidant performance may be attributed to the interaction between copper nanoparticles and DPPH, involving the exchange of electrons and hydrogen ions.[25,26]

Successful outcomes obtained in investigations by the following authors can be used to support the choice of utilizing CS and CL for the green synthesis of the CuONPs. In their study on the antimicrobial activity of the citrus plant, Farhana *et al*.[27] stated that the presence of bioactive compounds indicates the presence of metabolic toxins or broad-spectrum antibiotic compounds. In their report, Hamilton–Miller *et al*.[28] discussed the antimicrobial properties of CS. They found that the polyphenol fractions of CS contain catechin fractions, which have the ability to inhibit the growth of various bacterial species.[28] In a study by Yusof *et al*., it was found that considerable inhibition of both bacteria and fungi was produced by the production of zinc oxide nanoparticles using chitosan.[3,29,30] The

synergetic effects of these plant extracts have not been demonstrated in any previous investigation. Therefore, an effort has been made to investigate them. Combining CS with CL will certainly have positive effects, including enhanced anti-inflammatory and antioxidant effects at increased concentrations. Further studies will focus on using these NPs in dental applications.

Limitations

This study was conducted *in vitro*, and further *in vivo* experiments need to be carried out. The cost of preparing CuONPs from natural sources should be taken into consideration. Further studies need to be carried out to assess the biocompatibility and determine its suitability for incorporation into various dentifrices and composites.

Conclusion

CS- and CL-generated CuONPs have good antimicrobial effects and less cytotoxic characteristics and better anti-inflammatory and antioxidant capabilities at concentrations higher than 50 µL.

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Conflict of interest

There are no conflicts of interest.

Authors contributions

Not applicable.

Ethical policy and institutional review board statement

Not applicable.

Patient declaration of consent

Not applicable.

Data availability statement

The manuscript contains each and every dataset created or examined throughout this investigation.

References

1. Chang YN, Zhang M, Xia L, Zhang J, Xing G. The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials (Basel) 2012;5:2850-71.
2. Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, *et al*. Green synthesis of nanoparticles: Current developments and limitations. Environ Technol Innovation 2022;26:102336.
3. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 2008;4:707-16.
4. Applerot G, Lellouche J, Lipovsky A, Nitzan Y, Lubart R, Gedanken A, *et al*. Understanding the antibacterial mechanism of CuO nanoparticles: Revealing the route of induced oxidative stress. Small 2012;8:3326-37.
5. Ingle AP, Duran N, Rai M. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: A review. Appl Microbiol Biotechnol 2014;98:1001-9.
6. Xu VW, Nizami MZI, Yin IX, Yu OY, Lung CYK, Chu CH. Application of copper nanoparticles in dentistry. Nanomaterials (Basel) 2022;12:805.
7. Aljedaani RO, Kosa SA, Abdel Salam M. Ecofriendly green synthesis of copper (II) oxide nanoparticles using leaves (Molokhaia) extract and their application for the environmental remediation of direct violet dye via advanced oxidation process. Molecules 2023;28:16.
8. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)—A review. Food Res Int 2009;42:529-35.
9. Popkin BM, D'Anci KE, Rosenberg IH. Water, hydration, and health. Nutr Rev 2010;68:439-58.
10. Bansal S, Choudhary S, Sharma M, Kumar SS, Lohan S, Bhardwaj V, *et al*. Tea: A native source of antimicrobial agents. Food Res Int 2013;53:568-84.
11. Klimek-Szczykutowicz M, Szopa A, Ekiert H. (Lemon) Phenomenon—A review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants (Basel) 2020;9:119.
12. Saini RK, Ranjit A, Sharma K, Prasad P, Shang X, Gowda KGM, *et al*. Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. Antioxidants (Basel) 2022;11:239.
13. Galati EM, Monforte MT, Kirjavainen S, Forestieri AM, Trovato A, Tripodo MM. Biological effects of hesperidin, a citrus flavonoid (Note I): Anti-inflammatory and analgesic activity. Farmaco 1994;40:709-12.
14. Rajeshkumar S, Rinitha G. Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel *Persea americana* seeds. OpenNano 2018;3:18-27.
15. Perumal VK. Antimicrobial activity of gotukola leaves and neem leaves—A comparative *in vitro* study. J-AIM 2015;3:11-15.
16. Nagalingam M, Rajeshkumar S, Balu SK, Tharani M, Arunachalam K. Anticancer and antioxidant activity of *Morinda citrifolia* leaf mediated selenium nanoparticles. J Nanomater 2022;2022:1-7.
17. Das P, Ghosal K, Jana NK, Mukherjee AA, 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.
18. Amiri M, Etemadifar Z, Daneshkazemi A, Nateghi M. Antimicrobial effect of copper oxide nanoparticles on some oral bacteria and candida species. J Dent Biomater 2017;4:347-52.
19. Rajeshkumar S, Menon S, Venkat Kumar S, Tambuwala MM, Bakshi HA, Mehta M, *et al*. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract. J Photochem Photobiol B 2019;197:111531.
20. Padma P, Banu S, Kumari S. Studies on green synthesis of copper nanoparticles using *Punica granatum*. Annu Res Rev Biol 2018;23:1-10.
21. Jadhav S, Gaikwad S, Nimse M, Rajbhoj A. Copper oxide nanoparticles: Synthesis, characterization and their antibacterial activity. J Clust Sci 2011;22:121-9.

22. Argueta-Figueroa L, Scougall-Vilchis RJ, Morales-Luckie RA, Olea-Mejía OF. An evaluation of the antibacterial properties and shear bond strength of copper nanoparticles as a nanofiller in orthodontic adhesive. Aust Orthodo J 2015;31:42-8.
23. Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents 2009;33:587-90.
24. Rajeshkumar S, Menon S, Venkat Kumar S, Ponnanikajamideen M, Ali D, Arunachalam K. Anti-inflammatory and antimicrobial potential of *Cissus quadrangularis*-assisted copper oxide nanoparticles. J Nanomater 2021;2021.
25. Dou L, Zhang X, Zangeneh MM, Zhang Y. Efficient biogenesis of Cu2O nanoparticles using extract of *Camellia sinensis* leaf: Evaluation of catalytic, cytotoxicity, antioxidant, and anti-human ovarian cancer properties. Bioorg Chem 2021;106:104468.
26. Gültekin DD, Güngör AA, Önem H, Babagil A, Nadaroğlu H. Synthesis of copper nanoparticles using a different method: Determination of its antioxidant and antimicrobial activity. JOTCSA 2017;3:623-36.
27. Bhuiyan FR, Hasan M, Imran AS, Ahmed SR, Shanzana P, Marufatuzzahan. Antimicrobial activity screening for three Citrus pulp extracts and phytochemical constituency profiling. J Pharmacogn Phytochem 2019;8:157-161.
28. Hamilton-Miller JM. Antimicrobial properties of tea (*Camellia sinensis* L). Antimicrob Agents Chemother 1995;39:2375-7.
29. Majeed AA, Al-Aubydi MA. Assessment the modulation effect of using green synthesis ZnO NPs against multidrug resistant *Klebsiella pneumoniae* isolated from respiratory tract infection. Iraqi J Sci 2019;26:1221-31.
30. Yusof NAA, Zain NM, Pauzi N. Synthesis of ZnO nanoparticles with chitosan as stabilizing agent and their antibacterial properties against Gram-positive and Gram-negative bacteria. Int J Biol Macromol 2019;124:1132-36.