

ANTICARIOGENIC ACTIVITY OF COPPER NANOPARTICLES SYNTHESIZED USING RED TEA: AN IN VITRO STUDY

Running Title: Anticariogenic activity of red tea mediated copper nanoparticles

ABSTRACT:

Background: Nanotechnology is rapidly growing in various fields of science like medicinal, agricultural and physical and material sciences. Copper nanoparticles are particularly attractive because of copper's high natural abundance and low cost and the practical and straightforward multiple ways of preparing copper based nanomaterials.

Aim: To assess the anticariogenic activity of copper nanoparticles synthesized using red tea.

Materials and Methods: The red tea powder was acquired. An aqueous extract was prepared and mixed with copper sulphate for copper nanoparticles formation and centrifuged for 10 minutes. The extract was then placed in the well cultured agar plates against *Candida albicans*, *Streptococcus mutans*, *S. aureus* and *E. Faecalis* and incubated for 24 hours. The zones of inhibition were then calculated.

Results: Against *S. aureus*, 50 µl showed 20 mm of zone of inhibition, 100 µl showed 16 mm of zone of inhibition and 150 µl showed 20 mm of zone of inhibition. 19 mm of zone of inhibition were noted against the antibiotic. Against *C. albicans*, 50 µl showed 12 mm of zone of inhibition, 100 µl showed 13 mm of zone of inhibition and 150 µl showed 12 mm of zone of inhibition. 10 mm of zone of inhibition were noted against the antibiotic. Against *S. mutans*, 50 µl showed 17 mm of zone of inhibition, 100 µl showed 14 mm of zone of inhibition and 150 µl showed 19 mm of zone of inhibition. 35 mm of zone of inhibition were noted against the antibiotic. Against *E. faecalis*, 50 µl showed 12 mm of zone of inhibition, 100 µl showed 15 mm of zone of inhibition and 150 µl showed 19 mm of zone of inhibition. 35 mm of zone of inhibition were noted against the antibiotic.

Conclusion: The anticariogenic activity of copper nanoparticles synthesized using red tea were studied and their wider zones of inhibition were suggestive of good anticariogenic

activity. We conclude that copper nanoparticles synthesized using red tea can be used against *S. aureus*, *S. mutans*, *C. albicans* and *E. faecalis* as an anticariogenic agent.

Keywords: Anticariogenic; Copper nanoparticles; *Streptococcus mutans*; Red tea; Innovative; Green synthesis.

INTRODUCTION:

In this modern period, nanoscience and nanotechnology are growing branches of science that deal with various processes like fabrication and characterization of different nano metals and non-metal of different sizes, shapes and compositions (1–8). Among the different nanoparticles present, copper nanoparticles (CuNPs) are mostly used as they are simple to produce by reducing copper ions that are present in the aqueous solution of copper sulphate. Further it has various other uses like an antibiotic and an antifungal agent and thus showing antimicrobial activity in treating wounds (9). Nanoparticles are the particles with size ranging from 1 nm to 100 nm providing solutions to environmental and technological challenges and applied in almost all the fields. The copper nanoparticles because of their unique physical and chemical properties, low cost preparation and less toxic nature have been a great interest to researchers and have become an active area in the academic field and most importantly in the field of nanoscience and technology (10). Nanotechnology is mainly used to produce and process products eco-friendly and to minimize the use of hazardous environments (11). Nanoparticles containing antioxidant and antimicrobial properties are considered as a new trend of medicinal and therapeutic agents and even in the prevention of deterioration of food and pathogenic microorganisms (12).

Copper, a transition metal, is the most frequently occurring element to be integrated into essential biochemical pathways (13). The nanoparticles of metals or metal oxides that are obtained are often combined with nanocomposites. It exhibits various new characteristics and properties that a single material does not have. Copper nanoparticles have gained attention in the last 2 decades because of their simple nature and the property of exhibiting a range of potentially useful physical properties depending upon their size, shape and composition (14). Strong water in copper vessels purifies water by killing some species and strains of bacteria and efficiently destroys bacteria thereby having

bactericidal properties (15). Moreover, copper is an inexpensive antimicrobial agent when compared to other agents like gold and silver. It has antioxidant properties and longer shelf life when compared to other organic antimicrobial agents(16) (17) (18) (19). The presence of these unique physical, chemical and biological properties are due to their highly unusual crystal morphology and high surface area –volume ratio (20).

Aspalathus linearis, commonly known as rooibos or red tea, is a shrub native to the Cederberg region in the Western Cape Province of South Africa (21,22). It is frequently used to make a mild tasting tisane rich in polyphenol antioxidants but with no caffeine and very little tannins; it is also claimed to cure insomnia, allergies, and nervous breakdown as well as improve the appetite (23,24). Our team has extensive knowledge and research experience that has translated into high quality publications.(25–37),(38–42) (43) (44). Recent scientific endeavors suggest that rooibos may confer various antioxidant-associated health benefits including antimutagenic, anticarcinogenic, anti-inflammatory, and antiviral properties and antiatherosclerotic effects (24,45,46). In this context, this study aims to assess the anticariogenic effect of copper nanoparticles synthesized using red tea.

MATERIALS AND METHODS:

Preparation of the Extract

In a beaker, 1 g of freshly acquired red tea powder was added to 100 ml of distilled water. It was mixed well and boiled for 5-10 minutes at 60-70 °C (Figure 1). The solution was then filtered using filter paper. The filtered extract was collected and stored.

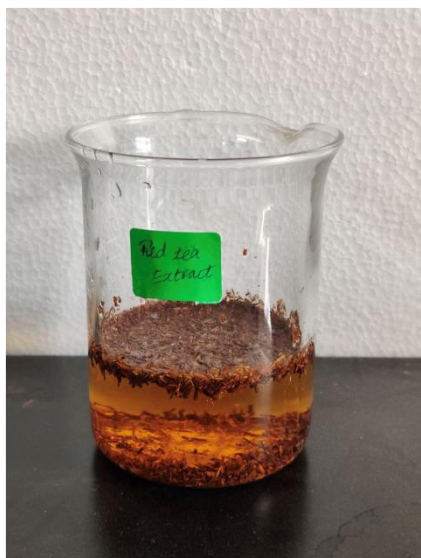


Figure 1: Red tea powder mixed in distilled water

Synthesis of Nanoparticles

20mM of CuSO_4 was added to the 20 ml of distilled water and kept in a magnetic stirrer for nanoparticle synthesis. The colour change was observed. Reading was noted every 2 hours. The solution of copper nanoparticles was centrifuged at 8000 rpm for 10 minutes. Then, the copper nanoparticles were collected and stored (Figure 2).

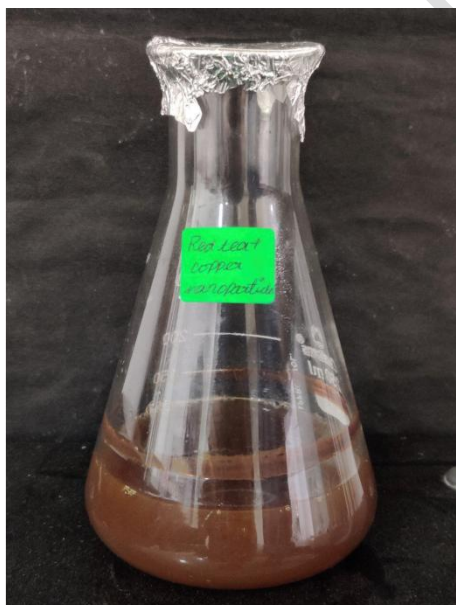


Figure 2: Red tea mediated copper nanoparticles

Anticariogenic activity

Agar well diffusion method was used to determine the anticariogenic activity of synthesised red tea mediated copper nanoparticles. Different concentrations of copper nanoparticles were tested against *C. albicans*, *S. mutans*, *S. aureus* and *E. faecalis*. Different concentrations of copper nanoparticles (50 µl, 100 µl, 150 µl) were incorporated into the prepared wells and the plates were incubated at 37 °C for 24 hours to study its effect. Antibiotic (Amoxicillin) was used as positive control and the zones of inhibition were recorded.

RESULTS:

Zone of inhibition using different concentrations of red tea mediated copper nanoparticles shows anticariogenic activity against *C. albicans* (Figure 3), *S. mutans* (Figure 4), *S. aureus* (Figure 5) and *E. faecalis* (Figure 6). Against *S. aureus*, 50 µl showed 20 mm of zone of inhibition, 100 µl showed 16 mm of zone of inhibition and 150 µl showed 20 mm of zone of inhibition. 19 mm of zone of inhibition were noted against the antibiotic. Against *C. Albicans*, 50 µl showed 12 mm of zone of inhibition, 100 µl showed 13 mm of zone of inhibition and 150 µl showed 12 mm of zone of inhibition. 10 mm of zone of inhibition were noted against the antibiotic. Against *S. mutans*, 50 µl showed 17 mm of zone of inhibition, 100 µl showed 14 mm of zone of inhibition and 150 µl showed 19 mm of zone of inhibition. 35 mm of zone of inhibition were noted against the antibiotic. Against *E. faecalis*, 50 µl showed 12 mm of zone of inhibition, 100 µl showed 15 mm of zone of inhibition and 150 µl showed 19 mm of zone of inhibition. 35 mm of zone of inhibition were noted against the antibiotic. (Table 1)

Zone of inhibition by disk-diffusion method shows anticariogenic activity in different concentrations of red tea mediated copper nanoparticles (Figure 7). Zones of inhibition obtained for different microorganisms at various concentrations of polyherbal extract were compared using ANOVA test. The results obtained for anticariogenic activity against *C. albicans*, *S. mutans*, *S. aureus* and *E. faecalis* was found to be statistically significant with the p value of <0.05 (Table 2 and Table 3).



Figure 3: Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *C. albicans*

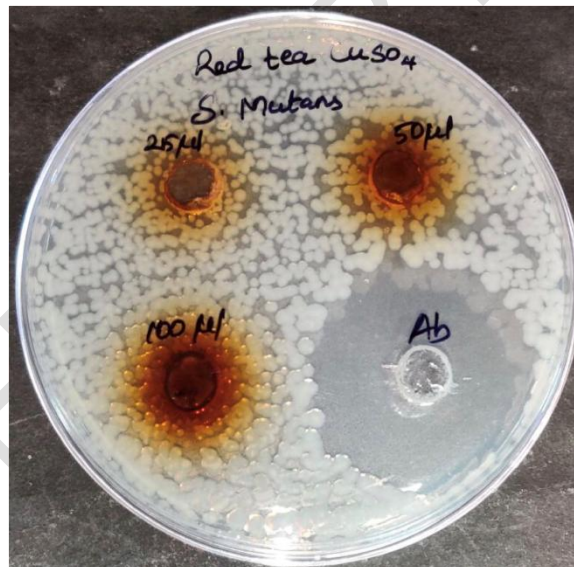


Figure 4: Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. mutans*.



Figure 5: Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. aureus*.

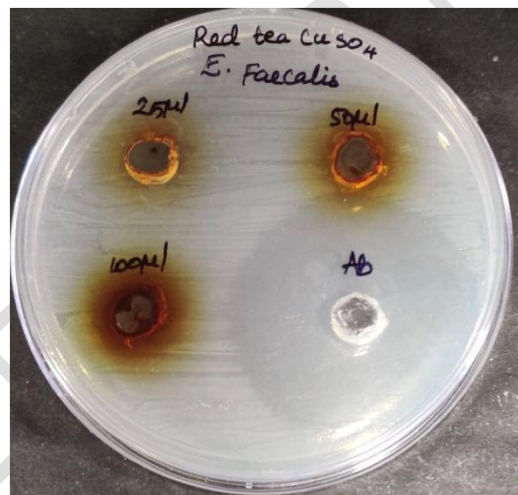


Figure 6: Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *E. faecalis*.

Concentration (micro litres)	<i>S. aureus</i>	<i>C. albicans</i>	<i>S. mutans</i>	<i>E. faecalis</i>
50 µl	14	12	17	12
100 µl	16	13	14	15

150 μ l	20	12	19	19
Antibiotic	19	10	35	35

Table 1: Zone of inhibition using different concentrations of red tea mediated copper nanoparticles against *C. albicans*, *S. mutans*, *S. aureus* and *E. faecalis*.

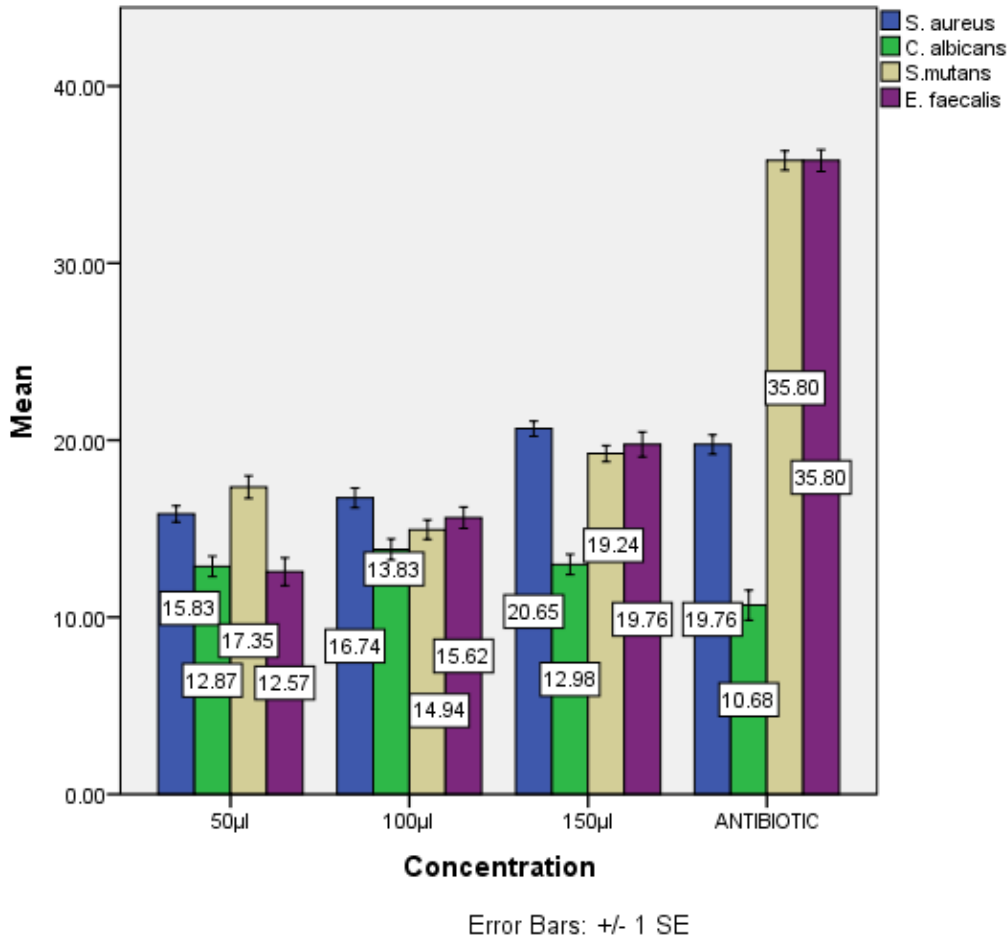


Figure 7: Bar graph representing the anticariogenic activity of red tea mediated copper nanoparticles at varying concentrations along with the positive control (amoxicillin). The concentration was plotted on the X axis and the zone of inhibition was plotted on the Y axis. Here, blue represents the *S. aureus*, green represents the *C. albicans*, brown represents the *S. mutans* and violet represents the *E. faecalis*. At 50 μ l and 100 μ l, the antimicrobial activity against *S. aureus* was found to be statistically significant when compared to the standard ($p < 0.05$). At 100 μ l, the antimicrobial activity against *S. mutans* was found to be statistically significant when compared to the standard ($p < 0.05$). At 50 μ l, 100 μ l and 150 μ l, the antimicrobial activity against *C. albicans* was found to be statistically significant when

compared to the standard ($p < 0.05$). At 50 μl , 100 μl and 150 μl , the antimicrobial activity against *E. faecalis* was found to be statistically significant when compared to the standard ($p < 0.05$) (one way ANOVA followed by post hoc analysis).

Table 2: ANOVA test for anticariogenic activity

		Sum of Squares	df	Mean Square	F	Sig.
<i>S. aureus</i>	Between Groups	48.481	3	16.160	21.703	.000*
	Within Groups	5.957	8	.745		
	Total	54.438	11			
<i>C. albicans</i>	Between Groups	16.235	3	5.412	4.148	.048*
	Within Groups	10.437	8	1.305		
	Total	26.672	11			
<i>S. mutans</i>	Between Groups	807.817	3	269.272	296.509	.000*
	Within Groups	7.265	8	.908		
	Total	815.082	11			
<i>E. faecalis</i>	Between Groups	961.249	3	320.416	230.841	.000*

	Within Groups	11.104	8	1.388		
	Total	972.354	11			

*(p<0.05)

Table 3: Post Hoc analysis for anticariogenic activity

Dependent variable	Concentration (I)	Concentration (J)	Significance	
<i>S. aureus</i>	50µl	100µl	.595	
	100µl	150µl	.002*	
	150µl	50µl	.001*	
	ANTIBIOTIC	50µl	100µl	.002*
		100µl	150µl	.011*
		150µl	50µl	.608
<i>C. albicans</i>	50µl	100µl	.736	
	100µl	150µl	.798	
	150µl	50µl	.999	
	ANTIBIOTIC	50µl	100µl	.167
		100µl	150µl	.039*
		150µl	50µl	.142
<i>S. mutans</i>	50µl	100µl	.058*	
	100µl	150µl	.002*	

	150µl	50µl	.148
	ANTIBIOTIC	50µl	.000*
		100µl	.000*
		150µl	.000*
<i>E. faecalis</i>	50µl	100µl	.053*
	100µl	150µl	.011*
	150µl	50µl	.000*
	ANTIBIOTIC	50µl	.000*
		100µl	.000*
		150µl	.000*

*(p<0.05)

DISCUSSION:

Dental caries have become a serious oral health problem for older adults. This is partially due to the increasing longevity of the population and the increases in tooth retention within this age group (47). Copper ions have been reported to have an antibacterial effect both in vitro (48–50) and in vivo (51). Copper reduces the number of bacteria on tooth surfaces. The suggested mode of action of copper is the limitation of bacterial growth and the inhibition of glycolysis, leading to a decrease in acid production (52,53). Copper has also been found to interfere with glucan formation by glucosyltransferase. Such a process may contribute to reducing plaque accumulation (54). Copper is traditionally a well-known antimicrobial material. However, it remains unclear as to the precise mechanism by which copper nanoparticles exert their antimicrobial activity. As with silver, it is thought that copper partly elicits its antimicrobial activity by combining with the –SH groups of key enzymes(55). Yoon et al. (2007) (56) demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and *Bacillus subtilis*.

A study by Ruparelia et al. (57) investigated antimicrobial properties of silver and copper nanoparticles on *E. coli*, *B. subtilis* and *Staphylococcus aureus* (*S. aureus*). Results of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and disk diffusion test were taken in that study. The study revealed that the copper nanoparticles were more efficient compared to the silver particles against *B. subtilis* which is usually recommended to flow from to more affinity of the copper nanoparticles to surface amines and carboxyl groups of *B. subtilis*.

The antibacterial activity of copper nanoparticles was assessed in liquid also as solid growth media(58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) ((68,69) (70) (71). On solid media, the antibacterial characterization of the prepared NPs was measured by colony forming unit (CFU). In liquid media, the antibacterial behaviour of copper nanoparticles was studied by determination of the optical density (OD). The results demonstrated that the antibacterial efficacy of copper nanoparticles relied on the concentration of the nanoparticles; low concentrations just led to a delay within the lag phase, showing the micro nutritional role of copper for bacteria. In contrast, at higher concentrations, they showed bacterial growth inhibition (72).

The medicinal properties of red tea have now drawn extensive attention. Studies on the antioxidant, antimutagenic, anti-allergenic, vasodilatory and dermatological effects of rooibos tea have been done (73). Some studies evaluated rooibos against certain microorganisms, such as *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans* and *Candida albicans* (74–77)

A study by Britz et al., (78) assessed the antimicrobial activity of rooibos on food spoilage organisms and potential pathogens. the study stated that the rooibos clearly had an inhibitory effect on the growth of microbes such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans* and *Saccharomyces cerevisiae*. Similarly, in our study red tea mediated copper nanoparticles showed an inhibitory effect against *C. albicans*, *S. mutans*, *S. aureus* and *E. faecalis*. However, these findings need to be confirmed with further clinical trials.

CONCLUSION:

Within the limitations, the present study suggests that the red tea mediated copper nanoparticles showed anticariogenic activity against *S. mutans*, *C. albicans*, *E. faecalis* and *S. aureus* and therefore can be used for clinical application.

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