Journal of Pharmaceutical Research International

33(61A): 297-307, 2021; Article no.JPRI.77930
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Anticariogenic Activity of Copper Nanoparticles Synthesized Using Red Tea: An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i61A35589

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77930

Original Research Article

Received 20 October 2021
Accepted 24 December 2021
Published 28 December 2021

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

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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.

1. 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. The presence of these unique physical, chemical and biological properties are due to their highly unusual crystal morphology and high surface area –volume ratio [16].

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 [17,18]. 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 [19,20]. Our team has extensive knowledge and research experience that has translated into high quality publications [21–40]. Recent scientific endeavors suggest that rooibos may confer various antioxidant-associated health benefits including antimutagenic, anticarcinogenic, anti-inflammatory, and antiviral properties and antiatherosclerotic effects [20,41,42]. In this context, this study aims to assess the anticariogenic effect of copper nanoparticles synthesized using red tea.

2. MATERIALS AND METHODS

2.1 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 ℃ (Fig. 1). The solution was then filtered using filter paper. The filtered extract was collected and stored.

2.2 Synthesis of Nanoparticles

20mM of CuSO4 was added to the 20 ml of distilled water and kept in a magnetic stirrer for nanoparticle synthesis. The color 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 (Fig. 2).

2.3 Anticariogenic Activity

Agar well diffusion method was used to determine the anticariogenic activity of synthesized 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 ℃ for 24 hours to study its effect. Antibiotics (Amoxicillin) was used as positive control against *S. mutans, S. aureus* and *E. faecalis* and Fluconazole was used as the positive control against *C. albicans* and their zones of inhibition were recorded.

3. RESULTS

Zone of inhibition using different concentrations of red tea mediated copper nanoparticles shows anticariogenic activity against *C. albicans* (Fig. 3), *S. mutans* (Fig. 4), *S. aureus* (Fig. 5) and *E. faecalis* (Fig. 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 fluconazole. 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).

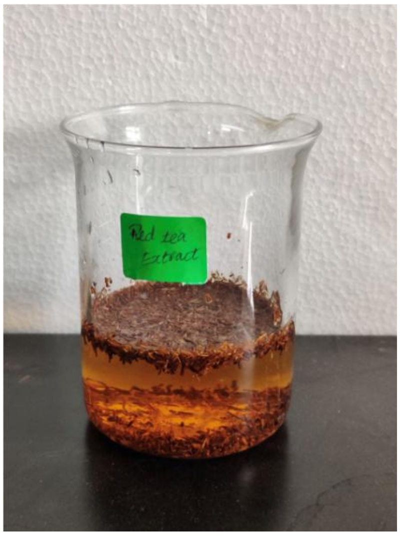

Fig. 1. Red tea powder mixed in distilled water

Zone of inhibition by disk-diffusion method shows anticariogenic activity in different concentrations of red tea mediated copper nanoparticles (Fig. 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).

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

Concentration (micro litres)	*S. aureus*	*C. albicans*	*S. mutans*	*E. faecalis*
50 µl	14	12	17	12
100 µl	16	13	14	15
150 µl	20	12	19	19
Antibiotic	19	10	35	35

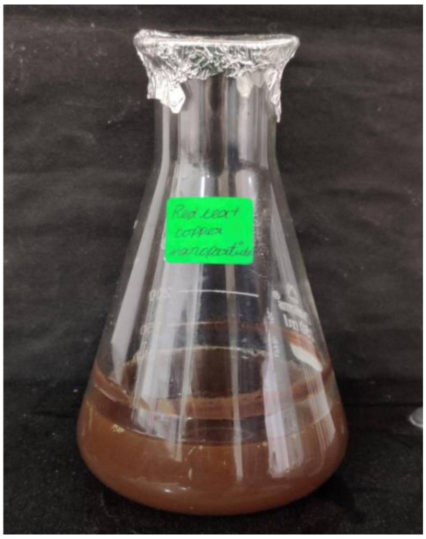

Fig. 2. Red tea mediated copper nanoparticles

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

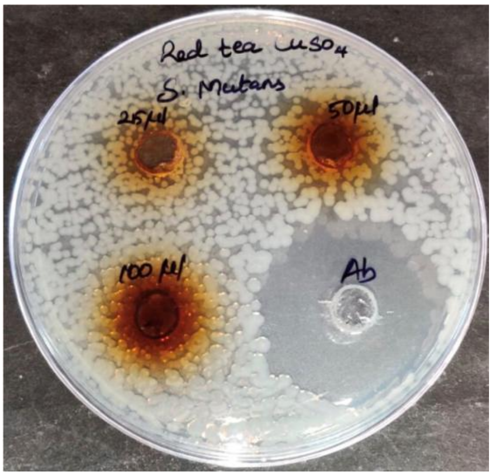

Fig. 4. Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. mutans*

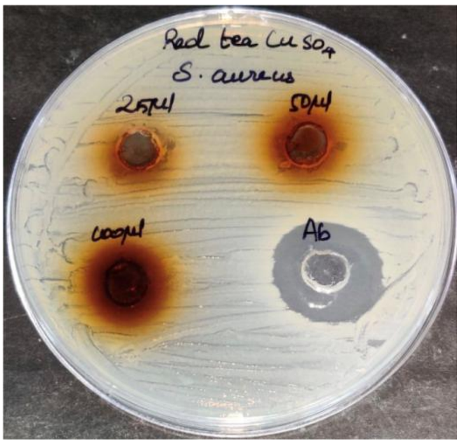

Fig. 5. Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *S. aureus*

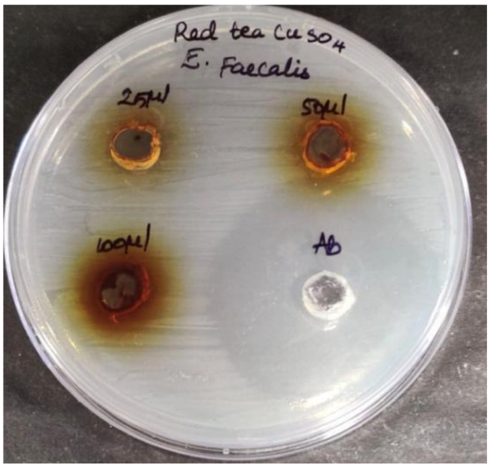

Fig. 6. Zone of inhibition of red tea medicated copper nanoparticles by disk diffusion method showing anticariogenic activity against *E. faecalis*

4. 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 increase in the tooth retention in this age group [43]. Copper ions have been reported to have an antibacterial effect both in vitro [44–46] and in vivo [47]. Copper reduces the number of bacteria on tooth surfaces. The suggested mode of the action of copper is the limitation of bacterial growth and the inhibition of glycolysis, leading to a decrease in acid production [48,49]. Copper has also been found to interfere with glucan formation by glucosyltransferase. Such a process may contribute to reducing plaque accumulation [50]. 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 [51] Yoon et al. [52] demonstrated superior antimicrobial activity with copper nanoparticles against *E. coli* and *Bacillus subtilis*.

A study by Ruparelia et al. [53] 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*.

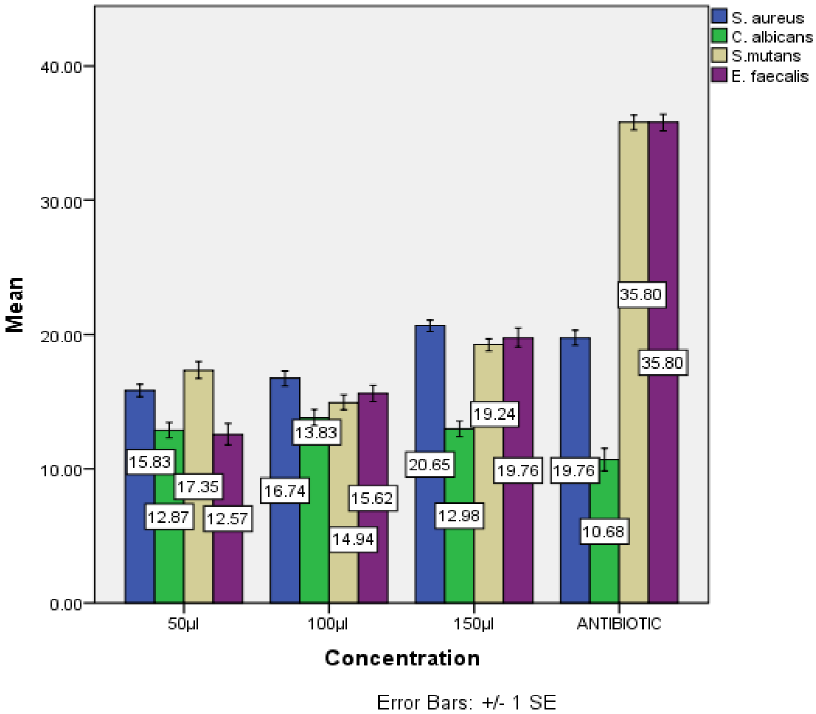

Fig. 7. Bar graph representing the anticariogenic activity of red tea mediated copper nanoparticles at varying concentrations along with the positive control (amoxicillin). The concentration was plotted on the X axis and the zone of inhibition was plotted on the Y axis. Here, blue represents the *S. aureus*, green represents the *C. albicans*, brown represents the *S. mutans* and violet represents the *E. faecalis*. At 50 µl and 100 µl, the antimicrobial activity against *S. aureus* was found to be statistically significant when compared to the standard ($p<0.05$). At 100 µl, the antimicrobial activity against *S. mutans* was found to be statistically significant when compared to the standard ($p<0.05$). At 50 µl, 100 µl and 150 µl, the antifungal 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.
S. aureus	Between Groups	48.481	3	16.160	21.703	.000*
	Within Groups	5.957	8	.745		
	Total	54.438	11			
C. albicans	Between Groups	16.235	3	5.412	4.148	.048*
	Within Groups	10.437	8	1.305		
	Total	26.672	11			
S. mutans	Between Groups	807.817	3	269.272	296.509	.000*
	Within Groups	7.265	8	.908		
	Total	815.082	11			
E. faecalis	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
S. aureus	50µl	100µl	.595
	100µl	150µl	.002*
	150µl	50µl	.001*
	ANTIBIOTIC	50µl	.002*
		100µl	.011*
		150µl	.608
C. albicans	50µl	100µl	.736
	100µl	150µl	.798
	150µl	50µl	.999
	ANTIFUNGAL	50µl	.167
		100µl	.039*
		150µl	.142
S. mutans	50µl	100µl	.058*
	100µl	150µl	.002*
	150µl	50µl	.148
	ANTIBIOTIC	50µl	.000*
		100µl	.000*
		150µl	.000*
E. faecalis	50µl	100µl	.053*
	100µl	150µl	.011*
	150µl	50µl	.000*
	ANTIBIOTIC	50µl	.000*
		100µl	.000*
		150µl	.000*

(p<0.05)

The antibacterial activity of copper nanoparticles was assessed in liquid also as solid growth media. On solid media, the antibacterial characterization of the prepared NPs was measured by colony forming unit (CFU). In liquid media, the antibacterial behavior 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 [54].

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 [55]. Some studies evaluated rooibos against certain microorganisms, such as *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans* and *Candida albicans* [56–59].

A study by Britz et al. [60] 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.

Further research on isolating different microorganisms may be undertaken and can be incorporated into existing anti-cariogenic herbal compositions to improve their efficacy. Promising results regarding the anti cariogenic effect of red tea mediated copper nanoparticles can be further validated with future in vivo studies to find the safe and effective concentration for clinical usage.

5. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to acknowledge the help and support rendered by the Department of Periodontics, Department of Nano Biomedicine, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for the constant assistance with the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Devi RS, Shruthi Devi R, Jeevitha M, Preetha S, Rajeshkumar S. Free radical scavenging activity of copper nanoparticles synthesized from dried ginger [Internet]. Journal of Pharmaceutical Research International. 2020;1–7. Available:http://dx.doi.org/10.9734/jpri/2020/v32i1930703
2. Sagana M, Rajasekar A, Rajeshkumar S. Antifungal activity of grape seed extract mediated zinc oxide nanoparticles - an in vitro study. Plant Cell Biotechnology and Molecular Biology. 2020;21(29-30):14–20.
3. Yuvashree CS, Rajasekar, Rajeshkumar S. Cytotoxic effect of titanium dioxide nanoparticles synthesized using grape seed extract: an in vitro study. Plant Cell Biotechnology And Molecular Biology. 2020;21(31-32):120–6.
4. Shivani N, Rajasekar A, Rajeshkumar S. Antifungal activity of grape seed extract mediated titanium oxide nanoparticles against *Candida albicans*: An in vitro study. Plant Cell Biotechnology and Molecular Biology. 2020;21(35-36):8–15.
5. Devi BV, Rajasekar A, Rajeshkumar S. Antiinflammatory activity of zinc oxide nanoparticles synthesized using grape seed extract: an in vitro study. Plant Cell Biotechnology and Molecular Biology. 2020;21(33-34):6–16.
6. Pereira WD, Rajasekar A, Rajeshkumar S. Green synthesis of selenium nanoparticles (senps) using aqueous extract of clove and cinnamon. Plant Cell Biotechnology and Molecular Biology. 2020;21(29-30):85–91.
7. PRANATI, Rajasekar A, Rajeshkumar S. Anti inflammatory and cytotoxic effect of clove and cinnamon herbal formulation. Plant Cell Biotechnology and Molecular Biology. 2020;21(29-30):69–77.
8. Anjum AS, Rajasekar, Rajeshkumar S. Synthesis and characterization of grape seed mediated titanium dioxide nanoparticles: An in vitro study. Plant Cell Biotechnology And Molecular Biology. 2020;21(33-34):17–23.
9. Ashtaputrey SD, Ashtaputrey PD, Rathod G. Eco-friendly green synthesis and characterization of silver nanoparticles derived from *Murraya koenigii* leaves extract [Internet]. Asian Journal of Chemistry. 2017;29:1966–8. Available:http://dx.doi.org/10.14233/ajchem.2017.20680
10. Padma P, Banu S, Kumari S. Studies on green synthesis of copper nanoparticles using *Punica granatum* [Internet]. Annual Research & Review in Biology. 2018;23:1–10. Available:http://dx.doi.org/10.9734/arrb/2018/38894
11. Stepankov MS, Zemlyanova MA, Zaitseva NV, Ignatova AM, Nikolaeva AE. Features of bioaccumulation and toxic effects of copper (II) oxide nanoparticles under repeated oral exposure in rats. Pharm Nanotechnol [Internet]; 2021. Available:http://dx.doi.org/10.2174/2211738509666210728163901
12. El-Refai AA, Ghoniem GA, El-Khateeb AY, Hassaan MM. Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents [Internet]. Journal of Nanostructure in Chemistry. 2018;8:71–81. Available:http://dx.doi.org/10.1007/s40097-018-0255-8
13. Robkhob P, Ghosh S, Bellare J, Jamdade D, Tang I-M, Thongmee S. Effect of silver doping on antidiabetic and antioxidant potential of ZnO nanorods. J Trace Elem Med Biol. 2020;58:126448.
14. Dobrucka R. Antioxidant and catalytic activity of biosynthesized CuO

nanoparticles using extract of *Galeopsidis herba* [Internet]. Journal of Inorganic and Organometallic Polymers and Materials. 2018;28:812–9.
Available:http://dx.doi.org/10.1007/s10904-017-0750-2

15. Das PE, Abu-Yousef IA, Majdalawieh AF, Narasimhan S, Poltronieri P. Green synthesis of encapsulated copper nanoparticles using a hydroalcoholic extract of leaves and assessment of their antioxidant and antimicrobial activities. Molecules [Internet]. 2020;25(3).
Available:http://dx.doi.org/10.3390/molecules25030555
16. Asemani M, Anarjan N. Green synthesis of copper oxide nanoparticles using *Juglans regia* leaf extract and assessment of their physico-chemical and biological properties [Internet]. Green Processing and Synthesis. 2019;8:557–67.
Available:http://dx.doi.org/10.1515/gps-2019-0025
17. McKay DL, Blumberg JB. A review of the bioactivity of South African herbal teas: rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*). Phytother Res. 2007;21(1):1–16.
18. Marnewick JL. Antioxidant properties of rooibos (*Aspalathus linearis*) – In vitro and in vivo evidence [Internet]. Systems Biology of Free Radicals and Antioxidants. 2014;4083–108.
Available: http://dx.doi.org/10.1007/978-3-642-30018-9_164
19. Morton JF. Rooibos tea, aspalathus linearis, a caffeine-less, low-tannin beverage [Internet]. Economic Botany. 1983;37:164–73.
Available:http://dx.doi.org/10.1007/bf02858780
20. Joubert E, Gelderblom WCA, Louw A, de Beer D. South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides*—A review [Internet]. Journal of Ethnopharmacology. 2008;119: 376–412.
Available:http://dx.doi.org/10.1016/j.jep.2008.06.014
21. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018;89(10): 1241–8.
22. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020;43(7):729–30.
23. Faleh AA, Sukumaran A, PNS. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol Macromol. 2018;120 (Pt A):876–85.
24. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. Cochrane Database Syst Rev. 2018;11: CD011423.
25. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardio-vascular disease. Hypertens Res. 2020;43(8):851–3.
26. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol. 2019;16(12):935–6.
27. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. Comput Commun. 2019;148: 176–84.
28. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing relationship between patient and doctor in public dental health using particle memetic multivariable logistic regression analysis approach (MLRA2). J Med Syst. 2018;42(10):183.
29. Varghese SS, Ramesh A, Veeraiyan DN. Blended module-based teaching in biostatistics and research methodology: A retrospective study with postgraduate dental students. J Dent Educ. 2019;83(4): 445–50.
30. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, characterization and biological applications of biosynthesized silver nanoparticles with chitosan-fucoidan coating. Molecules [Internet]. 2018;23(6).
Available:http://dx.doi.org/10.3390/molecules23061429
31. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of Different Concentrations of Three Root Canal Sealers on Human

Mesenchymal Stem Cells. Biomolecules [Internet]. 2018;8(3).
Available:http://dx.doi.org/10.3390/biom8030068

32. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from cuttlefish bone: Isolation, characterizations, and applications. Biotechnol Bioprocess Eng. 2018;23(4):383–93.
33. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. Measurement. 2019;135:672–7.
34. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of vertical root fractures by cone-beam computed tomography in root-filled teeth with confirmation by direct visualization: A systematic review and meta-analysis. J Endod. 2021;47(8):1198–214.
35. Ramani RH, Tilakaratne P, Sukumaran WM, Ramasubramanian G, Krishnan A, RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. Oral Dis [Internet]; 2021.
Available:http://dx.doi.org/10.1111/odi.13937
36. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. Oral Dis [Internet]; 2021.
Available:http://dx.doi.org/10.1111/odi.13798
37. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021;105390.
38. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol. 2021 Jun 14;105375.
39. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humaniz Comput [Internet]; 2018.
Available: https://doi.org/10.1007/s12652-018-1166-8
40. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 knowledge and perceptions among dental specialists: A cross-sectional online questionnaire survey. Risk Manag Healthc Policy. 2021;14:2851–61.
41. Marnewick J, Joubert E, Joseph S, Swanevelder S, Swart P, Gelderblom W. Inhibition of tumor promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), unique South African herbal teas [Internet]. Cancer Letters. 2005;224:193–202.
Available:http://dx.doi.org/10.1016/j.canlet.2004.11.014
42. Marnewick JL, van der Westhuizen FH, Joubert E, Swanevelder S, Swart P, Gelderblom WCA. Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin B1 in rat liver. Food Chem Toxicol. 2009;47(1):220–9.
43. Saunders RH Jr, Meyerowitz C. Dental caries in older adults. Dent Clin North Am. 2005 Apr;49(2):293–308.
44. Bundy KJ, Butler MF, Hochman RF. An investigation of the bacteriostatic properties of pure metals. J Biomed Mater Res. 1980;14(5):653–63.
45. Duguid R. Copper-inhibition of the growth of oral streptococci and actinomyces. Biomaterials. 1983;4(3):225–7.
46. Morrier JJ, Suchett-Kaye G, Nguyen D, Rocca JP, Blanc-Benon J, Barsotti O. Antimicrobial activity of amalgams, alloys and their elements and phases. Dent Mater. 1998;14(2):150–7.
47. Foley J, Blackwell A. In vivo cariostatic effect of black copper cement on carious dentine. Caries Res. 2003;37(4):254–60.
48. Rosalen PL, Bowen WH, Pearson SK. Effect of copper co-crystallized with sugar on caries development in desalivated rats [Internet]. Caries Research. 1996;30:367–72.
Available:http://dx.doi.org/10.1159/000262344
49. Oppermann RV, Rølla G, Johansen JR, Assev S. Thiol groups and reduced acidogenicity of dental plaque in the presence of metal ions in vivo. Scand J Dent Res. 1980;88(5):389–96.
50. Giertsen E, Scheie AA, Rölla G. Inhibition of plaque formation and plaque acidogenicity by zinc and chlorhexidine combinations [Internet]. European Journal of Oral Sciences. 1988;96:541–50.

Available:http://dx.doi.org/10.1111/j.1600-0722.1988.tb01594.x
51. Allaker RP. The use of antimicrobial nanoparticles to control oral infections [Internet]. Nano-Antimicrobials. 2012;395–425.
Available: http://dx.doi.org/10.1007/978-3-642-24428-5_14
52. Yoon KY, Byeon JH, Park JH, Hwang J. Susceptibility constants of *Escherichia coli* and Bacillus subtilis to silver and copper nanoparticles [Internet]. Science of the Total Environment. 2007;373:572–5.
Available:http://dx.doi.org/10.1016/j.scitotenv.2006.11.007
53. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 2008;4(3):707–16.
54. Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed A, Yawar W, et al. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli [Internet]. Annals of Microbiology. 2010;60:75–80.
Available:http://dx.doi.org/10.1007/s13213-010-0015-6
55. Hübsch Z, Van Vuuren SF, Van Zyl RL. Can rooibos (*Aspalathus linearis*) tea have an effect on conventional antimicrobial therapies? [Internet]. South African Journal of Botany. 2014;93:148–56.
Available:http://dx.doi.org/10.1016/j.sajb.2014.04.004
56. Nakano M, Itoh Y, Mizuno T, Nakashima H. Polysaccharide from *Aspalathus linearis* with strong anti-HIV activity. Biosci Biotechnol Biochem. 1997;61(2):267–71.
57. Schepers S. Antimicrobial activity of rooibos tea (*Aspalathus linearis*) on food spoilage organisms and potential pathogens. 2001;224.
58. Almajano MP, Pilar Almajano M, Carbó R, Angel López Jiménez J, Gordon MH. Antioxidant and antimicrobial activities of tea infusions [Internet]. Food Chemistry. 2008;108:55–63.
Available:http://dx.doi.org/10.1016/j.foodchem.2007.10.040
59. Coetzee G, Marx IJ, Pengilly M, Bushula VS, Joubert E, Bloom M. Effect of rooibos and honeybush tea extracts against *Botrytis cinerea* [Internet]. South African Journal of Enology and Viticulture. 2016;29.
Available:http://dx.doi.org/10.21548/29-1-1449
60. Britz TJ, Witthuhn RC, Joubert E. Antimicrobial activity of rooibos tea (*Aspalathus linearis*) on food spoilage organisms and potential pathogens. Master of Science in Food Science; 2001.

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