Preparation of mouthwash using Red tea and its antimicrobial and antioxidant activity

Running title : Red tea mediated mouthwash and its antimicrobial and antioxidant activity

ABSTRACT

Background

Poor oral hygiene provides an optimum environment for bacterial biofilms formation leading to the development of periodontal diseases. Currently, researchers focus on developing oral hygiene products such as mouthwash that contain available natural products with superior biocompatibility and a low cost.

Aim

The aim of the study was to prepare mouthwash using red tea and to evaluate its antimicrobial and antioxidant properties.

Materials and Methods

This study was performed as an in vitro study under a laboratory setting. Mouthwash was prepared using red tea in the lab. Subsequently the formulation was tested for its antimicrobial and antioxidant activity. Antimicrobial activity was conducted through agar well diffusion method. Antioxidant activity of the herbal mouthwash was tested using the DPPH assay at various concentrations. The obtained values were compared with that of the standard. The data was tabulated represented by the means of bar graphs

Results

There was an increase in the zone of inhibition of bacteria with an increase in concentration of mouthwash. DPPH assay for antioxidant activity revealed that at all concentrations the standard had more antioxidant activity compared to the herbal mouthwash. Throughout from 10µl to 50µl the antioxidant activity of the standard was greater than the prepared red tea mouthwash.

Conclusion

UNDER PEER REVIEW

Within the limitations of this study, Red tea mediated mouthwash was found to possess antioxidant and anti-inflammatory properties at varied concentrations.

Keywords

Mouthwash, Herbal, Anti-microbial, Anti-inflammatory, Innovative, Green synthesis

INTRODUCTION

Red tea originates from the leaves and stems of the indigenous South African plant rooibos(1) . It is used in clinical conditions such as nervous tension,dermatitis and indigestive problems(2).In recent years, antioxidative activity was also attributed to Red tea due to its flavonoid content. Oxidation of red tea leaves produces a reddish-brown colour of rooibos(3).As a fresh leaf, rooibos contains a high content of ascorbic acid which is lost when made into tea. Caffeine is absent in red tea and has low tannin levels(4). Components of Red tea include flavanols, polyphenols, flavanones, dihydrochalcones, aspalathin etc(4,5).

Antibiotic resistance is an emerging global issue which is of concern. The etiological factors behind the prevalence of such situations is overuse of antibiotics throughout therapeutic treatment of infection(6). Modern day chemotherapy can have a wide range of adverse effects from allergy to immunosuppression(7). Natural herbal products and its extensive parts were proven to reduce side effects caused by chemical drugs, therefore consequently they are preferred as an alternative remedy(8)(9–15)

The physiological and pathological changes in the human body depend on the free radical and reactive oxygen species interactions to maintain the normal cellular activities(16) . The free radicals when not balanced have deleterious effects on the organs of the body and can be the trigger for rheumatoid arthritis, stroke, liver diseases and various cancers(17). In aerobic organisms the imbalance between the reactive oxygen species generation and therefore the antioxidants level results in oxidative stress and cellular degeneration(18). Oxidative stress is involved in the pathogenesis of many diseases besides periodontitis for in the pathogenesis of periodontal diseases, the increased polymorph neutrophils count and activity cause a high rate of

UNDER PEER REVIEW

reactive oxygen species release(19). This results in increased oxidative stress in periodontal tissues. Antioxidants are required by periodontal tissues to stop tissue damage caused by reactive oxygen species(20,21). Antioxidants prevent the damage caused by formation of free radicals. Studies using different plant products have been tried in the past with beneficial therapeutic effects and less side effects.

Mouthwash solutions usually encompass antimicrobial activity that ensures their add elimination of harmful periodontal bacteria, which aids in preventing future cavity , gingivitis and periodontitis(22)(16). Mouthwash solutions are usually less effective against bacterial biofilms when compared to planktonic forms(23). Additionally, common mouthwash solutions were shown to possess variable antibacterial activity against bacteria in their biofilm state counting on their major active components. Poor oral hygiene provides an optimum environment for bacterial biofilms formation, thereby making the bacteria in the oral cavity less susceptible to mouthwash or any antiseptic formulation(24). Therefore, proper oral hygiene is essential in avoiding biofilms formation. Commercially available mouthwashes and antiseptic formulations are usually prepared using chemical components(25). However, the cytotoxicity of the chemical components of mouthwash solutions may cause side effects due to their direct contact with oral tissues(26)(27).

Our team has extensive knowledge and research experience that has translated into high quality publications(28–40),(41–45) (46) (47). The aim of this study was to prepare a herbal mouthwash using red tea and study its antimicrobial and antioxidant properties. The study also focuses on developing oral hygiene products such as mouthwash that contain available natural products with superior biocompatibility and a low cost of development.

UNDER PEER REVIEW

MATERIALS AND METHODS

This study was performed in the Blue Lab of Saveetha Dental College and Hospitals, Chennai. The study was performed during January 2021-February 2021.

Preparation of mouthwash : The powdered red tea were measured to 10g were taken and made up to a solution of 100mL using distilled water. The solution was boiled, cooled down and filtered to obtain the mouthwash. The concentrated mixture was then subjected to filtration and then collected in a beaker. Different concentrations of mouthwash was prepared by diluting the concentrated mixture with distilled water.

Antimicrobial activity of Aspalathus linearis: Antimicrobial activity of Aspalathus linearis were tested against *streptococcus mutans, Candida albicans, enterococcus faecalis, staphylococcus aureus*. Antimicrobial activity was conducted through agar well diffusion method which was tested against *Streptococcus mutans, Streptococcus aureus, Candida albicans and Enterococcus faecalis*. The organisms were grown in the nutrient broth overnight to attain the colony forming unit of ~10^5. 100µl of each bacterial culture was spread on the Luria-Bertani agar plates. Agar wells were made with the help of sterilized cork borers and loaded with different concentrations of extract and plates were incubated for 24 hours at 37^{o}C and diameters of zone of inhibition were recorded in millimetres. Different concentrations of the mouthwash (25µl, 50µl and 100µl) were incorporated into the wells and the plates were incubated at 37°C for 24 hours. Standard Chlorhexidine mouthwash was used as positive control. Zone of inhibition was recorded in each plate.

Antioxidant Activity of Aspalathus linearis: The DPPH assay was used to test the antioxidant activity of red tea herbal formulation. A test tube rack is arranged with five test tubes, each marked with a label signifying the various concentrations of extract from 10 to 50 µl Each test tube was loaded with DPPH (2,2diphenyl-1-picryl-hydrazyl-hydrate), ethanol and the extract. The DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in Ethanol. This free radical is reduced in the presence of an antioxidant molecule , giving rise to colourless ethanol solution. The rate of its activity is tested using a UV spectrophotometer. Later the reduction in quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Butylated hydroxytoluene was employed as the control. Percentage of inhibition was determined using the following equation:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}]}{} \times 100$$

UNDER PEER REVIEW

Absorbance of control

Statistical Analysis : The obtained data was sorted in MS Excel and the results were depicted in graphs and tabulations. ANOVA analysis performed for the various concentrations of the Red tea mouthwash's antimicrobial and antioxidant activity

RESULTS

The finding of the present study suggested that mouthwash prepared using red tea could be a potential source of antioxidants and could have greater importance as therapeutically agent in preventing or slowing oxidative stress related degenerative diseases. It has been observed that the antibacterial effect of the prepared red tea mouthwash was almost similar against all the organisms used in the study, with a maximum zone of inhibition against *Streptococcus mutans* and minimum zone of inhibition, against *Candida albicans*.

There was an increase in the zone of inhibition with an increase in concentration of mouthwash (Figure 1). The zone of inhibition for the prepared extract against *Streptococcus mutans* at the concentrations 25µl, 50µl and 100µl was found to be 20mm, 30mm and 25mm respectively. Similarly for *Candida albicans*, the zone of inhibition was found to be 10mm, 10mm and 15mm respectively. For *Streptococcus aureus*, the zones of inhibition values were 20mm, 21 mm and 25mm respectively. For *Enterococcus faecalis*, the zone of inhibition was found to be 10mm, 10mm and 22mm respectively . Antimicrobial activity of mouthwash is significantly lesser when compared to the Standard but increases as the concentration increases. It also shows that the antimicrobial activity of the red tea mouthwash is almost close to the standard at 100µl (Figure 2). The ANOVA test for Antimicrobial effect was found to be significant , which is $p<0.05$ (Table 1).

The results of the DPPH assay for antioxidant activity revealed that at all concentrations the standard had more antioxidant activity compared to the herbal formulation . Throughout from 10µl to 50µl the antioxidant activity of the standard was greater than the prepared herbal mouthwash. However at concentrations of 40-50µl, the antioxidant activity of the

UNDER PEER REVIEW

prepared mouthwash was similar to that of the standard mouthwash (Figure 3) . One way ANOVA followed by post hoc analysis performed, revealed that there was a concentration dependent increase in the antioxidant capacity of red tea mouthwash. Antioxidant capacity was least at 10µl concentration and significantly increased at concentrations of 40-50µl as observed in this study (Table 2).

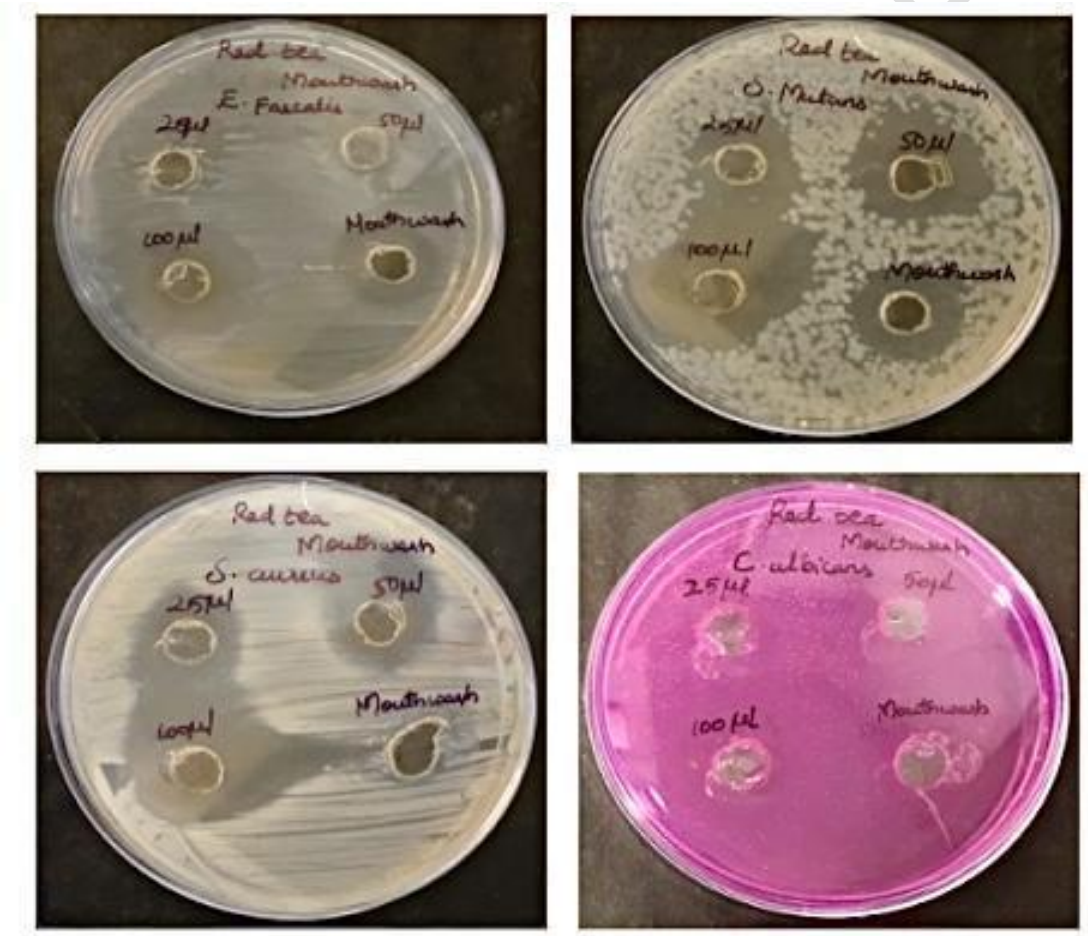

Figure 1 : Zone of inhibition of Red tea mouthwash against *Streptococcus mutans, Streptococcus aureus, Candida albicans and Enterococcus faecalis*

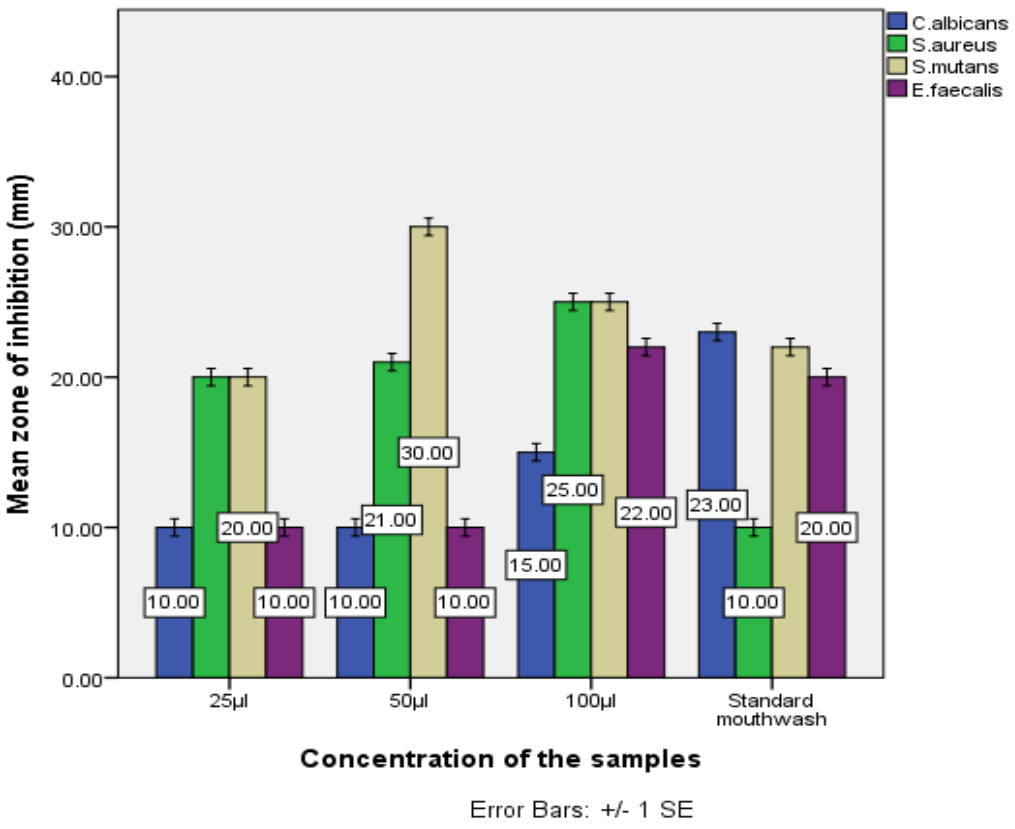

Figure 2 : Bar graph representing the Antimicrobial activity of Red tea mouthwash. X axis shows concentration of the samples and Y axis shows the mean value of zone of inhibition. Blue colour represents the Candida albicans, green colour represents staphylococcus aureus, yellow represents the enterococcus faecalis and purple represents Enterococcus faecalis. Zone of inhibition of the mouthwash is similar to that of the standard at a concentration of 100µl.

Table 1: Table depicts the one way ANOVA analysis performed for the various concentrations of the Red tea mouthwash's antimicrobial activity compared to that of standard mouthwash. The results obtained from ANOVA test were found to be statistically significant for streptococcus mutans, staphylococcus aureus, Candida albicans and enterococcus faecalis (p=0.00).

Dependent variable	**Concentration(I)**	**Concentration(J)**	**Significance (p<0.05)**
C.albicans	25µl	50µl	1.000
		100µl	0.001*
		Standard	0.000*
	50µl	100µl	0.001*
		Standard	0.000*
	100µl	Standard	0.000*
S.aureus	25µl	50µl	0.630
		100µl	0.001*
		Standard	0.000*
	50µl	100µl	0.005*
		Standard	0.000*

UNDER PEER REVIEW

	100µl	Standard	0.000*
S.mutans	25µl	50µl	0.000*
		100µl	0.001*
		Standard	0.144
	50µl	100µl	0.001*
		Standard	0.000*
	100µl	Standard	0.026*
E.faecalis	25µl	50µl	1.000
		100µl	0.000*
		Standard	0.000*
	50µl	100µl	0.000*
		Standard	0.000*
	100µl	Standard	0.144

*($p<0.05$)

UNDER PEER REVIEW

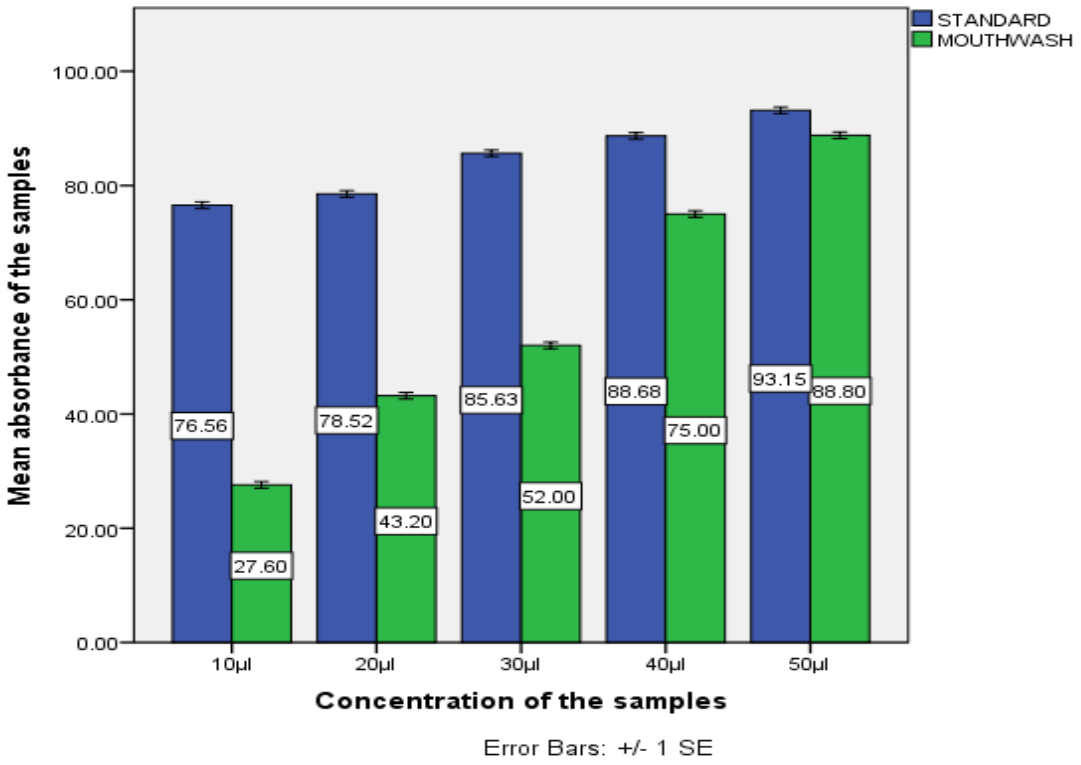

Figure 3: Graph depicts the antioxidant activity of Red tea mouthwash compared to the standard. The Y-axis depicts the mean absorbance values of the samples. The X-axis denotes the various concentrations. The blue bar depicts the standard while the green bar depicts the herbal mouthwash. Herbal formulation had significantly less antioxidant activity as compared to standard at all concentrations (p=0.00).

UNDER PEER

Table 2: Table depicts the one way ANOVA analysis performed for the various concentrations of the Red tea mouthwash's antioxidant activity as compared to that of standard. The results obtained from ANOVA test was found to be statistically significant for the standard and Red tea mouthwash (p=0.00).

Concentration (I)	**Concentration(J)**	**Significance (p<0.05)**
10µl	20µl	0.00*

	30µl	0.00*
	40µl	0.00*
	50µl	0.00*
20µl	30µl	0.00*
	40µl	0.00*
	50µl	0.00*
30µl	40µl	0.00*
	50µl	0.00*
40µl	50µl	0.00*

*($p<0.05$)

UNDER PEER REVIEW

DISCUSSION

The antimicrobial activity of the prepared mouthwash was attributed to the catechins and epicatechins released from them, pertaining to the changes in the membrane structure of microbes which causes cell death(48). Zone of inhibition was found to increase with increase in concentration of the mouthwash. Since both gram positive as well as gram negative bacteria are

found to be sensitive against red tea, it can be an efficient source for deriving those compounds(49).

Antioxidants are capable of protect cells from free radical damage, act as chemopreventive agents by inhibiting the generation of free radicals and play an important role in neutralizing oxidative stress, damage disk diffusion method is a well known method for the evaluation of free radical scavenging activity(50). Antioxidant activity was reported to be due to various phytocompounds such as phenols and flavonoids which are known for their antioxidant potential due to hydrogen donating properties of their hydroxyl groups(51). Previous studies on Red tea have shown that aqueous extracts and crude polyphenolic fractions of unfermented and fermented rooibos showed anti- and/or pro-oxidant activities, using a linoleic acid−Tween−buffer emulsion for lipid peroxidation and the deoxyribose degradation assay, based on a Fenton reaction model system containing FeCl3−EDTA and H2O2 for the generation of hydroxyl radicals(16)

In this study it was observed that although the antioxidant activity of the prepared mouthwash was not better than that of the standard, with increasing concentrations of the prepared mouthwash there is a change in the effectiveness of its antioxidant activity. The antioxidant activity of Red tea is contributed by the phenolic compounds and flavonoids present in the leaves of the plant predominantly.

CONCLUSION

Within the limitations of this study, the prepared red tea mouthwash was found to possess antioxidant and anti microbial properties at varied concentrations. This assay revealed that the extract can be incorporated in biomedical uses, thereby concluding that red tea mouthwash can be a pioneering step towards the shift to greener medicine. However further studies are necessary to examine underlying mechanisms of antioxidant effect and to isolate the active compounds responsible for these pharmacological activities.

UNDER PEER REVIEW

REFERENCES

1. Mezeske M. Red Tea. City Owl Press; 2018. 286 p.

2. Reinhard H, Zoller O. Pyrrolizidine alkaloids in tea, herbal tea and iced tea beverages-survey and transfer rates. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2021 Jul 8;1–20.

3. Song N-E, Kim MK, Lee K-G, Jang HW. Analysis of volatile compounds in rooibos tea (Aspalathus linearis) using different extraction methods and their relationship with human sensory perception. Food Res Int. 2021 Mar;141:109942.

4. Xiao X, Erukainure OL, Sanni O, Koorbanally NA, Islam MS. Phytochemical properties of black tea () and rooibos tea (); and their modulatory effects on key hyperglycaemic processes and oxidative stress. J Food Sci Technol. 2020 Dec;57(12):4345–54.

5. Mabuza N, Kinfe HH, Godeto TW, Ambushe AA. Estimated Contributions of Rooibos Tea to the Daily Manganese and Zinc Intakes Determined in Tea Leaves and Tea Infusions by Inductively Coupled Plasma-Mass Spectrometry. Biol Trace Elem Res. 2021 Mar;199(3):1145–52.

6. Barnes L. The Story of Rooibos Tea: Made in South Africa. 2018. 44 p.

7. South Africa. Department of Agriculture, Fruit and Fruit Technology Research Institute (South Africa). Control Strategy Against Fungal Diseases of Rooibos Tea. 1989.

8. Pickett V. Rooibos Tea: Health Benefits and Other Uses. 2012.

9. Sagana M, Rajasekar A, Rajeshkumar S. Antifungal activity of Grape Seed Extract Mediated Zinc Oxide Nanoparticles - An Invitro Study. Plant Cell Biotechnology and Molecular Biology. 2020 Aug 25;21(29-30):14–20.

10. 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 Aug 26;21(31-32):120–6.

UNDER PEER REVIEW

11. 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 Aug 26;21(35-36):8–15.

12. Devi Bv, Rajasekar A, Rajeshkumar S. Antiinflammatory Activity of Zinc Oxide Nanoparticles Synthesised Using Grape Seed Extract: An In Vitro Study. Plant Cell Biotechnology and Molecular Biology. 2020 Aug 26;21(33-34):6–16.

13. 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 Aug 25;21(29-30):85–91.

14. Pranati, Rajasekar A, Rajeshkumar S. Anti Inflammatory and Cytotoxic Effect of Clove and Cinnamon Herbal Formulation. Plant Cell Biotechnology and Molecular Biology. 2020 Aug 25;21(29-30):69–77.

15. 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 Aug 26;21(33-34):17–23.

16. King J. Antioxidant and Prooxidant Capacity of Rooibos Tea and Rooibos Tea Extracts. 2007. 158 p.

17. Nash LA. Rooibos Tea Flavonoids Increase Mineral Content in Human Osteoblast-like Cells. 2014.

18. Mabuza N. Fractionation and Speciation of Manganese and Zinc in Rooibos Tea Leaves and Tea Infusions. 2018. 101 p.

19. Areo OM, Njobeh PB. Risk assessment of heavy metals in rooibos (Aspalathus linearis) tea consumed in South Africa. Environ Sci Pollut Res Int [Internet]. 2021 Jun 18; Available from: http://dx.doi.org/10.1007/s11356-021-14968-2

20. Samodien S, Kock M de, Joubert E, Swanevelder S, Gelderblom WCA. Differential Cytotoxicity of Rooibos and Green Tea Extracts against Primary Rat Hepatocytes and

UNDER PEER REVIEW

Human Liver and Colon Cancer Cells - Causal Role of Major Flavonoids. Nutr Cancer. 2020 Sep 15;1–15.

21. Omolaoye TS, Windvogel SL, Du Plessis SS. The Effect of Rooibos (), Honeybush () and () on Testicular Insulin Signalling in Streptozotocin-Induced Diabetes in Wistar Rats. Diabetes Metab Syndr Obes. 2021 Mar 19;14:1267–80.

22. Preparation Of Tulasi, Aloe Vera And Turmeric Formulation And Its Antioxidant And Antimicrobial Activity [Internet]. Vol. 12, International Journal of Pharmaceutical Research. 2020. Available from: http://dx.doi.org/10.31838/ijpr/2020.sp1.439

23. Batalova V, Nabokova E, Reger A. Assessing antioxidant activity of tea extracts and some of chemicals using voltammetry and potentiometry [Internet]. 2016. Available from: http://dx.doi.org/10.1063/1.4964579

24. Kristanti RA, Punbusayakul N. Antioxidant and Antimicrobial activity of Commercial Green Tea In Chiang Rai [Internet]. Acta Horticulturae. 2009. p. 53–8. Available from: http://dx.doi.org/10.17660/actahortic.2009.837.4

25. Darmajana DA, Hadiansyah F, Desnilasari D. The antioxidant activity test by using DPPH method from the white tea using different solvents [Internet]. 2017. Available from: http://dx.doi.org/10.1063/1.5011866

26. Monsen R. A Comparison of Normal Saline Mouthwash and Mouthwash Based on Tea Solution from Salvia Officinalis in Palliative Care: A Randomized Controlled Trial [Internet]. Available from: http://dx.doi.org/10.26226/morressier.5c76c8bbe2ea5a723761258a

27. Daniel PH. Red Tea. [Madras] : Higginbothams; 1969. 334 p.

28. 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 Oct;89(10):1241–8.

29. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19

UNDER PEER REVIEW

and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.

30. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol Macromol. 2018 Dec;120(Pt A):876–85.

31. 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 Nov 26;11:CD011423.

32. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. Hypertens Res. 2020 Aug;43(8):851–3.

33. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol. 2019 Dec;16(12):935–6.

34. 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 Dec 15;148:176–84.

35. 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 Aug 29;42(10):183.

36. 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 Apr;83(4):445–50.

37. 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 Jun 12;23(6). Available from: http://dx.doi.org/10.3390/molecules23061429

UNDER PEER REVIEW

38. 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 Aug 1;8(3). Available from: http://dx.doi.org/10.3390/biom8030068

39. 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 Aug 1;23(4):383–93.

40. 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 Mar 1;135:672–7.

41. 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 Aug;47(8):1198–214.

42. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. Oral Dis [Internet]. 2021 Jun 21; Available from: http://dx.doi.org/10.1111/odi.13937

43. 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 Feb 11; Available from: http://dx.doi.org/10.1111/odi.13798

44. 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 Jun 16;105390.

45. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol. 2021 Jun 14;105375.

46. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS.

UNDER PEER REVIEW

Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humaniz Comput [Internet]. 2018 Dec 14; Available from: https://doi.org/10.1007/s12652-018-1166-8

47. 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 Jul 7;14:2851–61.

48. Masadeh, Masadeh. Antimicrobial Activity of Common Mouthwash Solutions on Multidrug-Resistance Bacterial Biofilms [Internet]. Journal of Clinical Medicine Research. 2013. Available from: http://dx.doi.org/10.4021/jocmr1535w

49. Yaghini J, Naghsh N, Sadeghi SM, Soltani S. Gingival Inflammatory Indices and Dental Stain Index after Using Aloe Vera-Green Tea Mouthwash, Matrica Mouthwash, or 0.2% Chlorhexidine Mouthwash Compared with Placebo in Patients with Gingival Inflammation [Internet]. Vol. 13, The Open Dentistry Journal. 2019. p. 214–20. Available from: http://dx.doi.org/10.2174/1874210601913010214

50. Oguntibeju O. Antioxidant-Antidiabetic Agents and Human Health. BoD – Books on Demand; 2014. 382 p.

51. Hakeem KR, Abdul WM, Hussain MM, Razvi SSI. Oral Health and Herbal Medicine. Springer; 2018. 46 p.

UNDER PEER REVIEW