

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 10 μ l to 50 μ l the antioxidant activity of the standard was greater than the prepared red tea mouthwash.

Conclusion

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

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.

MATERIALS AND METHODS

This study was performed in the Blue Lab of Saveetha Dental College and Hospitals, Chennai. Ethical clearance required for the study was obtained from the institutional committee. The study was performed during January 2021-February 2021.

Preparation of mouthwash : Equal weights of powdered red tea 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 mouthwash was concentrated to 10% and transferred to an Eppendorf tube.

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 $\sim 10^5$. 100 μ l of each bacterial culture was spread on the Luria-Bertani agar plates. Agar wells were made with the help of sterilised cork borers and loaded with different concentrations of extract and plates were incubated for 24 hours at 37°C and diameters of zone of inhibition were recorded in millimetres. Different concentrations of the extract (25 μ l, 50 μ l and 100 μ l) 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 μ l. Each test tube was loaded with DPPH (2,2-diphenyl-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:

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

Absorbance of control

Statistical Analysis : The obtained data was sorted in MS Excel and the results were depicted in graphs and tabulations.

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 25 μ l, 50 μ l and 100 μ l 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 100 μ l (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 10 μ l to 50 μ l the antioxidant activity of the standard was greater than the prepared herbal mouthwash. However at concentrations of 40-50 μ l, the antioxidant activity of the 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 10 μ l concentration and significantly increased at concentrations of 40-50 μ l as observed in this study (Table 2).

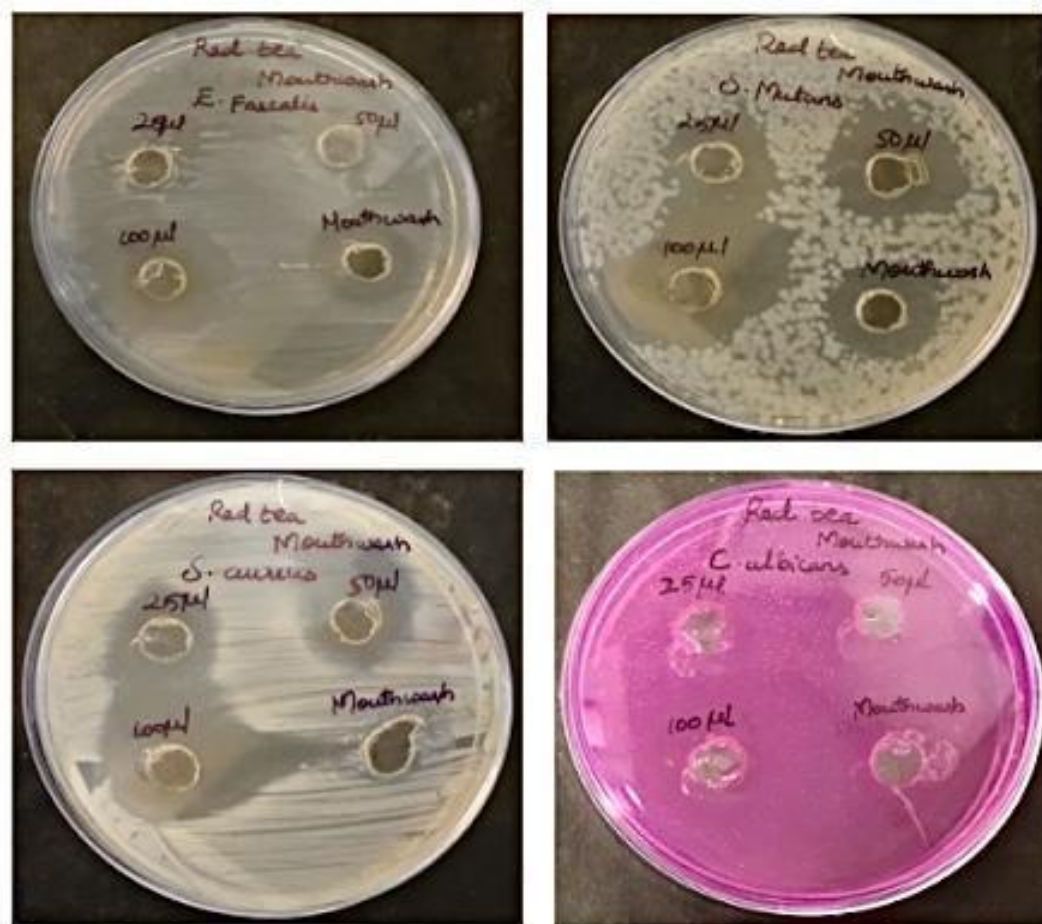


Figure 1 : Zone of inhibition of Red tea mouthwash against *Streptococcus mutans*, *Streptococcus aureus*, *Candida albicans* and *Enterococcus faecalis*

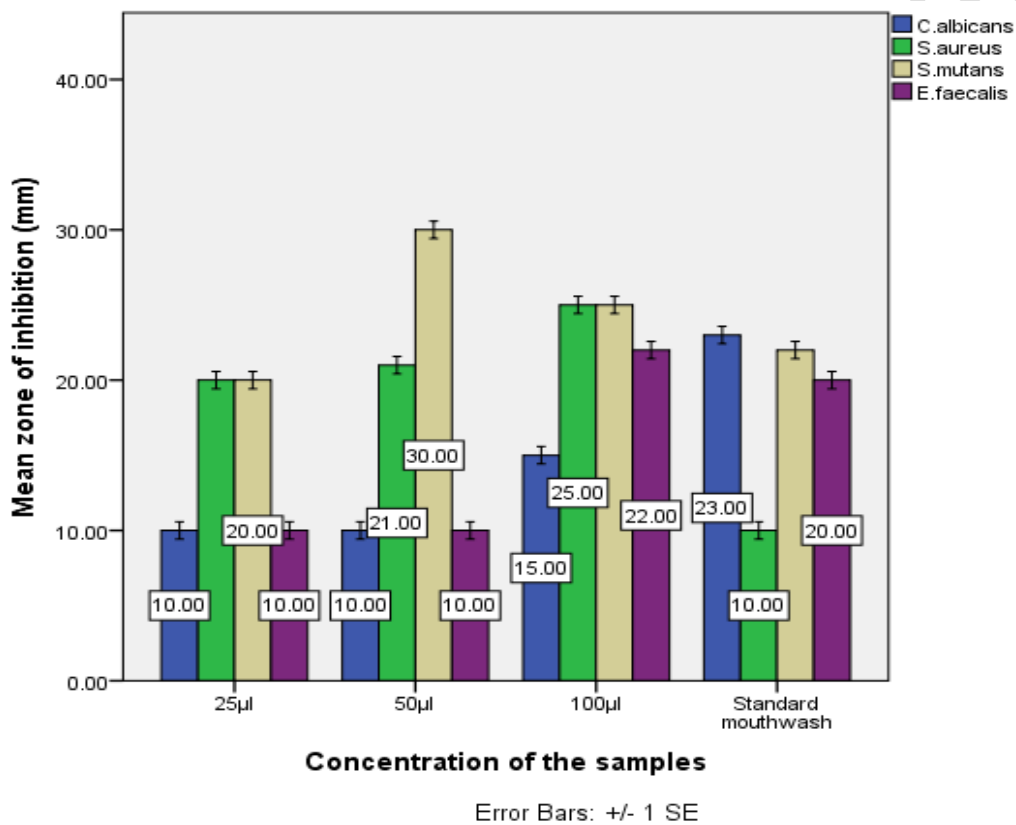


Figure 2 : Bar graph representing the Antimicrobial activity of Red tea mouthwash. X axis shows concentration of the samples and Y axis shows the mean value of zone of inhibition. Blue colour represents the *Candida albicans*, green colour represents *staphylococcus aureus*, yellow represents the *enterococcus 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	25 μ l	50 μ l	1.000
		100 μ l	0.001*
		Standard	0.000*
	50 μ l	100 μ l	0.001*
		Standard	0.000*
		Standard	0.000*
S.aureus	25 μ l	50 μ l	0.630
		100 μ l	0.001*
		Standard	0.000*
	50 μ l	100 μ l	0.005*
		Standard	0.000*
		Standard	0.000*

	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)

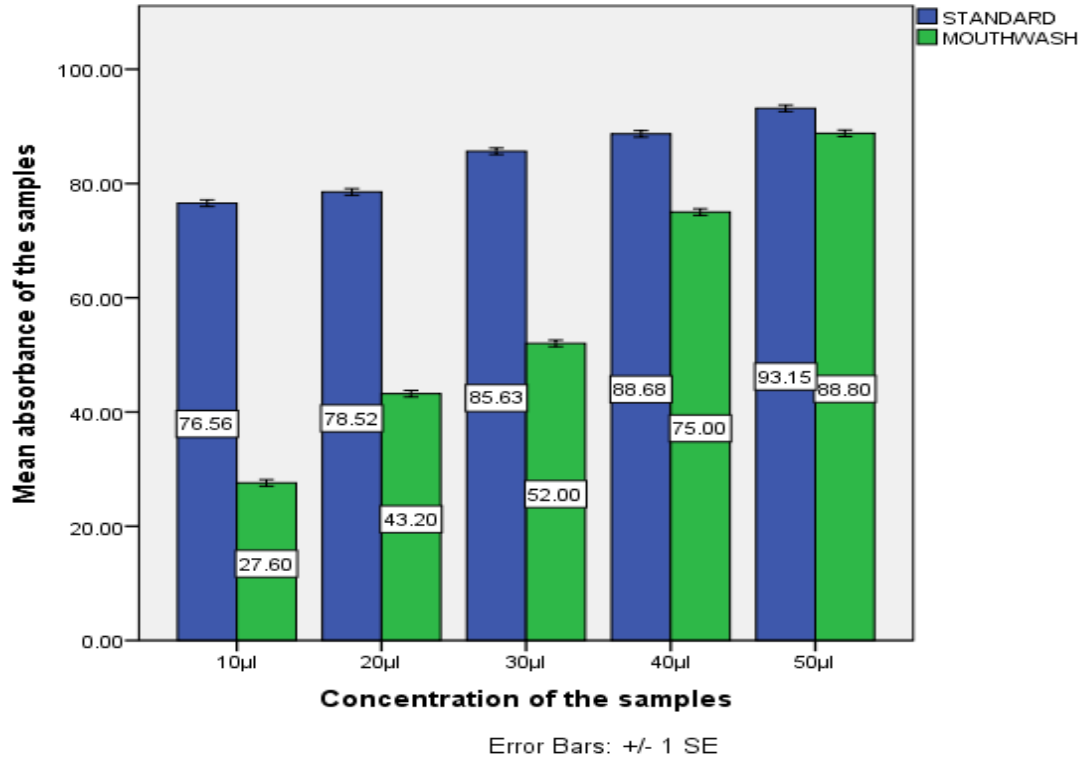


Figure 3: Graph depicts the antioxidant activity of Red tea mouthwash compared to the standard. The Y-axis depicts the mean absorbance values of the samples. The X-axis denotes the various concentrations. The blue bar depicts the standard while the green bar depicts the herbal mouthwash. Herbal formulation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

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)

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 phytochemicals 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 FeCl_3 -EDTA and H_2O_2 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(52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) ((62,63) (64) (65). 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.

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