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Evaluation of Anti-inflammatory Effect of Cinnamaldehyde Mouthwash in Comparison with Chlorhexidine in Patients with Gingivitis

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ABSTRACT

Gingivitis literally means gingival inflammation which is the most common gum disease. Dental plaque is the main cause of gingivitis. Plaque control by dental brush and dental floss is not sufficiently effective. Therefore, the lateral use of chemical plaque control materials is recommended. Chlorhexidine mouthwash is known as the standard of periodontal treatment. But the side effects of chlorhexidine, such as color changes in the teeth and changes in the sense of taste and mucosal burning, led to an increase in efforts to find newer chemicals and herbs as alternatives. The aim of this study was to compare the effect of cinnamaldehyde and chlorhexidine mouthwash on gingival inflammation. 105 participants of both sexes (55 females and 50 males) with age range of: 20 to 45 years old with plaque-dependent chronic gingivitis were randomly divided into three groups (each group was 35): Group 1 (cinnamaldehyde 0.1%), group 2 (chlorhexidine 0.2%), group 3 (placebo). Dental plaque and gingivitis indices were measured at day one, one week later and then on day 21 after oral intake. Scores of periodontal variables were analyzed by SPSS 22 software, Kruskal-Wallis statistical tests, repeated variance analysis, Friedman test and post hoc test. Intra-group comparisons showed a significant difference between the plaque and gingival index in all groups (P <0/001). There was a significant difference between placebo and chlorhexidine groups in both PI and GI indexes. However, there was no significant difference between groups in plaque index and gingival index between cinnamaldehyde group and chlorhexidine mouthwash (P <0/001). Cinnamaldehyde mouthwash was helpful in improving gingival status and its effects were comparable to that of chlorhexidine without its significant side effects.

Keywords: Gingivitis, Cinnamaldehyde Mouthwash, Chlorhexidine, Plaque Index, Gingival Index

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INTRODUCTION

Gingivitis literally means gingival inflammation and includes the most common gingival disease. Various research results indicate that microbial plaque is a major contributor to gingivitis, which can be prevented by the removal of microbial plaques [1]. Today, several ways to remove microbial plaque have been proposed, which involve mechanical and chemical methods. Evidence suggests that mechanical plaque control is not 100 percent effective in many people. As a result, attention has been paid to chemical methods of plaque control [2]. One can mention the chlorhexidine mouthwash which research shows that it has the best effect on oral hygiene [3]. The most important side effect of chlorhexidine is the discoloration of the tooth [4]. The science of identifying and using medicinal herbs is as old as human life. Until the 19th century, the use of natural resources, mostly plants, was the main means of treating diseases. But the rapid progress in chemistry and the lack of natural resources led to the replacement of new chemical compounds with herbal medicines. Antimicrobial, antifungal, and anticancer effects in medicinal plants have been the source of many studies and the outcome of this research can lead to self-sufficiency and independence in the country's pharmaceutical industry. The bacterial resistance to synthetic chemical drugs also requires the investigation and production of a variety of plant antimicrobials [5]. Cinnamon with scientific name of cinnamomum zeylanicum is a shrub of Lauracea and of Laurales species Cinnamon shrub is a small, evergreen tree, 5 to 7 meters tall, a pleasant odor is smelled from all parts of it. The tree leaves are dark green and its yellowish-white flowers will appear in February to early April. Cinnamon is native to Sri Lanka and South India and its dried skin is shaped like tubular pieces that its outer space is reddish-brown and its interior surface is dark brown [6]. Cinnamon's skin contains 0.5 to 2.5 percent essential oil, containing more than 50 different compounds, 80-65 percent of which are cinnamal aldehydes. Other compounds include cinnamic acid, phenolic compounds such as eugenol, fledron and safrole, terpene compounds such as limonene and lanolol, trans-cinnamaldehyde, tannin, coumarin, resin, phenylpropane compounds such as hydroxy cinnamaldehyde and mannitol, whose sweet taste is due to manythol [6]. The most important compounds of cinnamon are cinnamic aldehyd (65-80%) and eugenol (5-10%), which the most antibacterial effect is related to cinnamic aldehyde is [6]. Low dose cinnamaldehyde stimulates the central nervous system and has a palliative effect at high doses. Other effects of cinnamaldehyde include anti-fever, antibacterial, antifungal, accelerated release of catecholamines from the central part of the adrenal gland, poor papaverine effect, increased peripheral blood flow, decreased blood pressure, bradycardia, and increased blood sugar. Angiogenesis and anesthetic effects have been reported for eugenol [7]. Cinnamon tannins have astringent effect. Antimicrobial and antimycotic effect of cinnamon essential oil is probably due to the presence of orthothexine cinnamaldehyde. The volatile oil contained in cinnamon has an anti-inflammatory effect. The fruit extract of cinnamon has anti-mutagenic and antioxidant effects. In vitro extract of cinnamon has a strong anti-tumor effect, and the antispasmodic effect of cinnamon extract has been proven [7]. Our country with its diverse climates of and the cultivation of various medicinal plants has been prominent in the field of traditional medicine and the provision of herbal and natural therapies for many years. Since so far, there has not been a study on the effectiveness of cinnamon mouthwash on gingivitis in Iran and previous studies reported antimicrobial and anti-inflammatory effects of cinnamon, considering the anti-inflammatory and pharmacological effects of this plant, we try to determine the effectiveness of cinnamon on gingivitis, which is an inflammatory process, and to compare it with chlorhexidine mouthwash, when used alone, in a clinic at Sari Dental School.

MATERIALS AND METHODS

The present study is a randomized clinical trial. The study population is composed of patients referring to Sari Dental School in 2017, which their gingivitis was confirmed by periodontologist. These patients are between the ages of 20 and 45 years. To determine the sample size, the results of the Begne study (8) have been used. The mean PI index before the intervention was 91.389 with a standard deviation of 9.168 and on the 14th day, it was equal to 98.69 with a standard deviation of 2.551. Therefore, the sample size including these results and the 95% confidence level, 90% test power, and the two-way test, using the formula for comparison between the two means and using the G-power software, is estimated to be 38 (19

persons in the intervention group and 19 in the control group). Given the fact that there are 3 groups of comparison, so the sample size was changed to 81 (27 in each of the three groups) and also with a 30% drop, it increased to 150 people (35 in each of the three groups).

Preparation of initial formulation of cinnamaldehyde mouthwash 0.1%

To prepare the mouthwash, it was first evaluated with various solvents to achieve a suitable solvent system. For this purpose, the solvents of ethanol, glycerin, propylene glycol, isopropyl alcohol, PEG 200, PEG 300 and PEG 400 were used. For this purpose, the solvents of ethanol, glycerin, propylene glycol, isopropyl alcohol, PEG 200, PEG 300 and PEG 400 were used. After determining the solvent system, the amount of microbial protector needed was calculated. Emulsion has been used in the absence of a solvent system. Investigation of the physical stability of the prepared products in terms of not changing the solubility at three temperatures of environment, refrigerator, and 40 ° C during the two-week period was performed. Determination of the amount of active substance by ultraviolet spectrophotometry was performed. For this purpose, the standard curve of cinnamaldehyde was drawn in three different days and three times each day, and the final standard curve was the basis for determining the value. Finally, the product was evaluated for microbial content (based on the US Pharmacopoeia, USP). The criteria for entering the study include:

Non-smokers, lack of systemic disease and pregnancy, presence of at least 24 teeth except for third molar, no active caries, no antibiotic use during the past 3 months, gingivitis with a probe depth of no more than 4 mm and ultimately insurance of patient collaboration.

Sufficient explanations were given to patients and consent provided by the vice chancellor of the university was seen and signed by the patient. Patients were trained how to use mouthwash in combination with daily oral hygiene and brushing with the bass technique. In order to evaluate plaque and gingival accumulation, PIs (plaque index = Loe and Silness index) and GI (gingival index = Loe and Silness index) were used. The teeth were first dried and examined by a periodontologist using a probe, an explorer and a mirror.An examination was performed on ramjfort teeth. The teeth are teeth number 3, 9, 12, 19, 25, and 28. If one of these teeth was absent, side teeth were used. The situation was entered on the patient checklist. To record the indices, the gingival of each tooth were divided into four levels of Dystofacial papilla, Meso facial papilla, facial margin and the entire lingual margin. According to tables 1 and 2 [9], each level was awarded. By dividing the total points of the four levels by four, the gingival index and the plaque index were obtained. The PI, GI indexes were recorded on the first day of the examination in the patient's case, and then the patient was examined weekly for 3 weeks to evaluate the effects of the treatment. No prophylaxis was performed before the study began. The first group received 10 ml of cinnamaldehyde mouthwash (0.1% concentration), the second group received 10 mg chlorhexidine mouthwash (0.2% concentration) and placebo was given to the control group. We instructed patients to gargle mouthwash after brushing with whitening toothpaste and flossing twice a day (morning and evening) and avoid having to eat and drink for up to 1 hour.

Table 1: Loe & Silness Scale for the gingival index

Score	Status
0	Normal gingiva
1	Slight inflammation, slight discoloration, no bleeding at the touch
2	Medium inflammation, redness, edema and glazing, blemishes on the touch
3	Severe inflammation, the presence of edema and redness, the tendency to self-hemorrhage

Table 2: Loe & Silness scale for the plaque index

Score	Status
0	No plaque at the surface of teeth and gingiva
1	There is a thin shell of sticky plaque in the free gingival margin and the adjoining area and the plaque may only be detected by moving from the root surface
2	Moderate accumulation of soft sediments on the margin of the gingiva adjacent to the tooth surface (visible with the naked eye) with gingival envelope
3	soft materials on the gingiva margin and adjacent dental surfaces along with gingival envelopes

Patients are urged not to use any other mouthwash during treatment. Follow up sessions were performed in the first week and the third week after using mouthwash to record plaque and gum index. Data entry was done in SPSS ver22 software. After refining the data, the normal distribution of variables was performed using Kolmogorov-Smirnov test. Comparison of indices was done before intervention with the last stage of intervention in each group with paired t-test or

its nonparametric equivalents. Comparison of the indices between the intervention and control groups was done with independent T test or its nonparametric equivalents. Also, to compare the mean of the indices in different stages of measurement, we used the repeated variance analysis or its nonparametric eqivalent, Friedman and post hoc test. The criterion of judgment was a significance level of less than 0.05.

RESULTS

Study of the demographic data of the patients under study

This study was performed on 105 patients referring to Sari Dental School in the age range of 20 to 45 years old who suffered from chronic plaque gingivitis after confirmation by a periodontologist. According to all the criteria for entering and leaving the study, 109 patients referring to Sari dental school were enrolled. Of these, 2 patients were excluded due to lack of cooperation, and 2 patients due to acute allergy to Cinnamaldehyde mouthwash.

The cinnamaldehyde mouthwash group consists of 35 patients with an average age of 34.13 years. The chlorhexidine mouthwash group includes 35 people with an average age of 22.22 years.The group receiving placebo mouthwash also includes 35 people with an average age of 33.19 years (Table 3). A total of 55 female and 50 male participated in the study (Table 4).

Table 3: Age of patients participating in the study

Age (year)	Cinnam-aldehyde group	Chlorhexi-dine group	Placebo group	P-value
	34/11±6/7	32/23±6/4	33/91±6/2	0/406

Table 4: Gender of participating patients

Gender	Cinnamaldehyde group	Chlorhexidine group	Placebo group
Male	16	18	16
Female	19	17	19

Investigation of changes in plaque index and gingival index during the study

A) Plaque index (PI)

As shown in Table 5, the plaque index showed the highest decrease at the end of the first week in the chlorhexidine group and then in the Cinnamaldehyde group. The results of the study at the end of the third week showed a significant decrease in plaque index in the chlorhexidine group (p <0.001). The lowest effect was observed in the placebo group, which is statistically significant (p <0.001). These data also show that at the end of the first week there is no significant difference between the three groups (p = 0.61), but with the continuation of treatment, at the end of the third week, these differences have a significant effect (p =0/001).

Table 5: Anova Analysis Results for the Plaque Index for each group

Plaque index	Cinnamaldehyde Group	Chlorhexidine group	Placebo group	intergroup p-value
First visit	1/82±0/4	1/78±0/2	1/74±0/3	0/252
The end of the first week	1/57±0/4	1/43±0/2	1/55±0/2	0/061
The end of the third week	1/26±0/4	1/10±0/2	1/39±0/3	<0/001*
intragroup p-value	<0/001*	<0/001*	<0/001*	

**= p-value<0/05*

Also, a two-to-two comparison between mouthwash consumer groups in the post hoc test showed that there was no significant difference between the placebo and chlorhexidine groups at the end of the first week (p = 0/297), but at the end of the third week there were significant differences(p=0/001). The difference between the Cinnamaldehyde group and the placebo group was not significant in the plaque index during the first week (p = 0/995) and also in the third week (p = 0/99). A comparison between the two groups of chlorhexidine and the Cinnamaldehyde group was not significant at the end of the first week (p = 0/184) and the end of the third week (p = 0/62) and suggests that Cinnamaldehyde herbal mouthwash has a nearly similar effect to chlorhexidine in reducing plaque index (Diagram 1).

B) Gingival index (GI)

Regarding the data obtained from the analysis of variance, the gingival index during the first week of study showed the highest decrease in the chlorhexidine group and then in the Cinnamaldehyde group. The lowest effectiveness at the end of the first week is observed in the placebo group. Results showed no significant

difference between the three groups in the first week (p = 0/33)(Table 6).

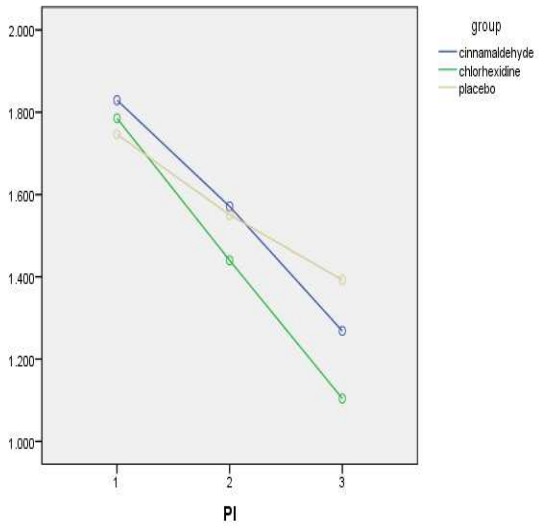

Diagram 1: Evaluation of changes in plaque index at the moment of referral, first week, third week in three groups

Table 6: Anova Analysis Results for gingival index by groups

Gingival index	Cinnamaldehyde group	Chlorhexidine group	Placebo group	Intergroup P-value
First visit	1/57±0/4	1/62±0/2	1/60±0/3	0/807
The end of the first week	1/29±0/3	1/28±0/2	1/44±0/3	0/33
The end of the third week	1/02±0/4	0/96±0/2	1/13±0/3	<0/001*
intragroup p-value	<0/001*	<0/001*	<0/001*	

**=p value<0/05*

In the third week, the highest reduction in gingival index was observed in the chlorhexidine group, and the difference between the three groups at the end of the third week was statistically significant (p <0/001).

By comparing groups two by two, The placebo group did not show a significant difference in gingival index at the end of the first week with both chlorhexidine (p = 0/089) and cinnamaldehyde (p = 0/69) groups, But at the end of the third week, the difference between the placebo group and the other two groups was significant (p 0/001). The difference in gingival index between Cinnamaldehyde and Chlorhexidine at the end of the first week (p <0/993) and at the end of the third week was not statistically significant (p = 0/712) which these results confirm that Cinnamaldehyde Mouthwash can provide a good improvement compared to chlorhexidine in the gingival index.

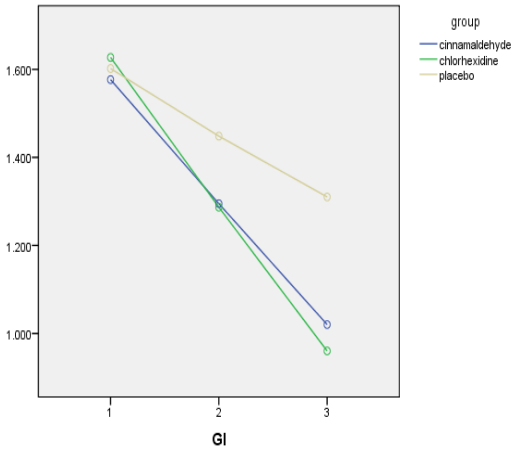

Diagram 2: Evaluation of gingival index changes at first visit, first week, and third week in three groups

DISCUSSION AND CONCLUSION

This study was performed on 105 patients referring to Sari Dental School who suffer from chronic plaque gingivitis. Patients had no systemic disease and did not use cigarettes and alcohol - which have an adverse effect on the prognosis of periodontal treatments (10)- in the months that led to the study. Patients were randomly divided into three groups of 35 (Group 1: Cinnamaldehyde mouthwash, Group 2: Chlorhexidine mouthwash, Group 3: placebo Mouthwash). According to the results, the plaque index and gingival index were significantly decreased during the study period in intra-group comparisons in all three groups. There was no significant difference between the three groups in the comparison between the groups in both plaque and gingival indexes during the first week. But differences were reported statistically significant at the end of the third week. In the evaluation of plaque index and gingival index, the highest reduction was observed in the chlorhexidine group and the difference between the chlorhexidine group and placebo

group was significant. There was no significant difference between the chlorhexidine group and the Cinnamaldehyde group in both indices. This indicates the proper effect of Cinnamaldehyde mouthwash on improving gingival index and dental plaque compared to chlorhexidine. Improvement by herbal mouthwash can be attributed to anti-inflammatory and antibacterial property of Cinnamic aldehyde compound [11]. The placebo group with Cinnamaldehyde had no significant difference in plaque index, but in the gingival index, the difference between the two groups was significant. This was also seen in a study on the effect of toothpaste containing Propolis on dental plaque. The plaque index variable had no significant statistical difference between the control group and the intervention group after the completion of the study. The reason for this is the more precise mechanical plaque control in patients, however, gingival index showed a significant decrease which was due to the antibacterial properties of Propolis [12]. In the present study, considering the oral hygiene education of patients at the time of first visit, as well as the similar composition and formulation of Cinnamaldehyde mouthwash and placebo, and the fact that only the effective ingredient of cinnamaldehyde has been removed from the placebo, it can be argued that the reason for no significant difference in plaque index between two groups of cinnamaldehyde and placebo is proper observance of oral hygiene and improving the level of mechanical plaque control in patients. And a significant reduction in gingival index between the two groups is due to the anti-inflammatory properties of cinnamaldehyde [13, 14]. Cinnamon dates back to 2700 BC and was used in ancient China to improve articular inflammation and respiratory failure. Loghman Hakim prescribed cinnamon for diseases of the stomach and intestines and for severe anger conditions [15]. Cinnamon makes people cheerful, it eliminates infections, and is good for the treatment of anxiety, obsession and strengthening of the nerves, the stomach, the liver, the elimination of oral misconception and smoothing of sound [15]. The new research also considers cinnamon effective in treating bloating, fever and chills, coughing, ear pain, headache, toothache, bad breath, hiccups, bladder infections, colds and flu, rheumatoid arthritis and joint inflammation and wound healing. Cinnamon is a general stimulant of the body, an accelerator of blood flow and anti-inflammation [16]. A lot of research has been done on antibacterial effects and retention ability of herbal essences including essential oils obtained from plants of laurel family [17, 18]. In a study in 2015 it was claimed that cinnamon contains the highest anti-inflammatory properties among 115 examined food substances which is most closely related to E-cinnamaldehyde and O-methoxy cinnamaldehyde [13]. Also, in two studies of Anupama et al. [19] and Dalirestani and colleagues [20], it has been suggested that the use of herbal mouthwashes has the benefits of reducing the side effects of taking chemical and synthetic drugs, and in addition, it is economical which is consistent with the results of the current study and, as observed, herbal mouthwash can show proper therapeutic effect without the presence of synthetic oral mouthwash effects and it can be suggested as an auxiliary treatment or an alternative of chlorhexidine. In another study, the antibacterial effect of cinnamon oil on the growth of Porphyromonus gingivalis bacteria in deep envelopes in patients with chronic periodontitis was examined in laboratory conditions. The results of this study indicate that the essential oil of cinnamon-which its majority is Cinnamaldehyde (6)-at MIC = 750mg / ml concentration has bacteriostatic and at MIC = 1500mg / ml concentration has bactericidal effects, of course, this effect was weaker than conventional antibiotics such as amoxicillin and metronidazole [21]. The results of this study are in line with the results of the present study in terms of the anti-bacterial effect expected. In the study of Subhashini et al., The effect of tea tree oil and essential oil of cinnamon on oral pathogens was investigated, and both extracts showed remarkable effects on S. mutants [22]. Also, a research was done on the effect of cinnamon essential oil and essential oil of cloves on oral microbes in vitro that the cinnamon essential oil showed a stronger effect [14]. The above studies all claim that the extract of cinnamon plant can produce significant anti-inflammatory and antibacterial effects, which is completely consistent with the present study.in the study of Gupta et al. the effect of chlorhexidine mouthwash and cinnamon extract on plaque index and gingival index were compared on 105 medical and dental students. Subjects were divided into three groups of 35, and the first group was given cinnamon extract, the second group was given chlorhexidine mouthwash and the third group was given distilled water. Results were evaluated on days 0, 15 and 30 and showed that the highest

reduction of both indices has occurred in the chlorhexidine group. Results showed no significant difference between the cinnamon extract and the chlorhexidine groups [23]. This study is consistent with the overall results, but there are some differences with the present study. In this study, cinnamon oil extract was used, while in the present study, hydroalcoholic formulation was developed as a mouthwash of Cinnamaldehyde which is the main and effective component of cinnamon extract. Also in the placebo group, a mouthwash was prepared with a combination of herbal mouthwash which lacked only non-active ingredient of Cinnamaldehyde which is different from the mentioned study in which distilled water has been used. Also, the hydroalcoholic formulation is more similar to the type of chlorhexidine alcohol formulation used in the study. In addition, the duration of treatment in this study has been one week shorter. All of the above considerations greatly enhance the accuracy of the results in the present study compared to the aforementioned study, and also in shorter period, we have achieved a similar effect, which can be resulted due to the quality of the drug product and training of the patient's health. In the study of Devand et al., the effect of chlorhexidine mouthwash with a mouthwash containing two herbal extracts of cinnamon and T.chebula on dental plaque was compared. Sixty students were examinedin two groups of 30 subjects in a 3-day period. The results of this study showed no significant difference between the two groups, suggesting that herbal mouthwash can have a good effect compared to chlorhexidine [24]. Other studies have also compared the effects of herbal mouthwashes with chlorhexidine [25]. The results of the above studies are consistent with the results of this study. In another study, the efficacy of a mouthwash containing turmeric plant with chlorhexidine gluconate mouthwash in preventing gingivitis and dental plaque formation, in two groups of 50 and on days 14 and 21 after the start of oral intake was examined. Also, microbial counts were also used during the study. There was no significant difference in gingivitis and microbial counting of mouthwash containing turmeric with chlorhexidine [26], which is consistent with the present study. Only in the prevention of plaque, chlorhexidine mouthwash has been reported to be more effective. Non-consistency of this result can be due to differences in the studied plant as well as differences in the formulation of herbal mouthwash. Overall, the results of this study showed that the effects of cinnamaldehyde mouthwash on reducing plaque and gingival indices are similar and comparable to chlorhexidine mouthwashes and as it was expected from the previous studies on cinnamon and active ingredient, an acceptable effect on reducing gingivitis was observed. The major finding of this study is the lack of significant difference between the effect of chlorhexidine and cinnamaldehyde on the reduction of plaque index and gingival index that due to the numerous complications of chlorhexidine, cinnamaldehyde mouthwash can be suggested as a suitable alternative to it.

According to the results, it can be stated that both chlorhexidine mouthwash and cinnamaldehyde herbal mouthwash are effective in improving gingival and dental plaque index, although conventional and gold standard mouthwash in these cases is chlorhexidine which showed better therapeutic results in the present study, However, due to the small difference between the obtained indices and statistical studies, considering the numerous side effects of chlorhexidine and ultimately the cost-effectiveness of herbal mouthwashes for patients, it can be suggested as a substitute or auxiliary treatment in cases where there is no known allergy to cinnamon. Overall, the present study shows the beneficial effects of cinnamaldehyde mouthwash in reducing gingival inflammation. Given the fact that the oral use of cinnamon has not yet shown a significant side effect, It seems that this plant can be used to treat a variety of oral and dental problems. Also, due to the easy access and satisfaction of patients with the taste and smell of this mouthwash compared to chlorhexidine, which is a chemical medicine, this mouthwash seems to be a good alternative to chlorhexidine.

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