Original Article

A Novel Herbal Formulation versus Chlorhexidine Mouthwash in Efficacy against Oral Microflora

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Received: 06-02-18.
Accepted: 23-03-18.
Published: 24-04-18.

ABSTRACT

Aims and Objectives: The aim of the study was to compare and to analyze the antimicrobial efficacy of 0.12% chlorhexidine and new formulated herbal mouthwash after using for 14 days. The objective was to signify whether the noval herbal combination could be a better alternative mouthwash to Chlorhexidine (CHX).

Materials and Methods: This is a double-blinded, random controlled research study conducted in the Department of Oral Pathology and Microbiology. A total of 200 dental students were selected randomly, comprising of two groups, 100 in each, aged between 18 and 22 years with gingival index of score II. The first group was advised to oral rinse with 0.12% chlorhexidine mouthwash and the second group with new formulated herbal mouthwash for 14 days. Saliva samples were collected on the day 0 (baseline), followed by day 7 and 14 and microbial colony count was performed. The data obtained was statistically analyzed using SPSS version 16. Student's *t*-test was applied for comparison of the mean microbial count between the two groups. Repeated measures analysis of variance followed by Tukey's *post hoc* test was applied to assess the changes from day 0 to day 7 to day 14. The statistical significance level was set at $P < 0.05$.

Results: Microbial colonies were reduced better in chlorhexidine group on the day 7 whereas, on day 14, greater reduction was observed in the herbal group in both gender groups with high statistical significance ($P < 0.001$).

Conclusions: Herbal mouthwash formulation performed effectively well on long-term usage, could be used as an alternative mouthwash to overcome the disadvantages of chlorhexidine.

KEYWORDS: *0.12% chlorhexidine, colony count, herbal, microbial, mouthwash, saliva*

INTRODUCTION

Plaque control is utmost essential for the suppression of gingivitis, dental caries, and halitosis-causing microorganisms. The most commonly used tool in the treatment of supragingival plaque are the tooth brushing either mechanical or electrical, dental floss, or interdental brushing.[1] Other means of plaque control are chemical therapeutic agents such as mouthwashes, sprays, chewing gums and varnishes; aid in an effective home care.[2] Nevertheless, mouthwashes have been accepted as the simplest and easiest mode of oral hygiene aid.[3] This could be the main mode of oral cleansing in medically compromised patients and elderly where adequate oral hygiene maintenance could be a major concern.[4] Chlorhexidine (CHX) has been the most widely used mouthwash and is considered as the gold standard in dental practice for about three decades, but not without certain disadvantages such as taste perturbation, tooth discoloration, oral ulcerations, unilateral, or bilateral parotid swelling.[5]

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_59_18

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How to cite this article: Vinod KS, Sunil KS, Sethi P, Bandla RC, Singh S, Patel D. A novel herbal formulation versus chlorhexidine mouthwash in efficacy against oral microflora. J Int Soc Prevent Communit Dent 2018;8:184-90.

Considering these drawback of CHX mouthwash, alternative antiplaque agents have been developed in the recent years with the use of herbal products. Herbs have been the main source of medications since the ancient times of Charaka and Sushruta and have conquered the confidence of the people of Asia. Naturally available herbs such as tulsi, triphala, neem, honey, ajwain, turmeric, etc., have been commonly used either alone or in combination as safe and effective antibacterial agents.[6]

Hence, this study was planned to use new formulated herbal mouthwash and 0.12% CHX mouthwashes in two different groups of individuals and compare the salivary antimicrobial efficacy of each over the other.

Materials and Methods

This research study is a randomized, controlled, double-blinded study which was carried out in the Department of Oral Pathology and Microbiology, Triveni Institute of Dental Sciences, Hospital and Research Centre, Bilaspur. The study was undertaken after obtaining the ethical clearance from the Institutional ethical clearance board in the month of August 2016. (TIDSHRC/EC 363-A/2016). All the participants were learned about the protocol of the study, and those who complied were only included in the study, and the informed consent was obtained.

Two different mouthwashes were used in the present study. One is the 0.12% chlorhexidine (CHX) containing commercially available mouthwash and the other a newly formulated herbal mouthwash prepared by standardized ayurvedic preparation method using Tulsi (*Ocimum tenuiflorum*), turmeric (*Curcuma longa*), triphala (Amlakki [*Emblica officinalis*], Haritaki [*Terminalia chebula*] bibhitaki [*Terminalia bellirica*]), neem (*Azadirachta indica*), honey and mint (Mentha) leaves.

Preparation of formulated herbal mouthwash

Dry powder of tulsi, neem, triphala, and turmeric of equal quality was suspended in 10 times its quantity of sterile distilled water in a flask and was kept undisturbed for 72 h at 4°C. The aqueous extract thus obtained was decanted and clarified by filtration through double-layered muslin cloth. This solution was then transferred to a porcelain dish and let to evaporate at 40°C. The dried remnant obtained was stored for making the mouthwash solution. 200 g of the powder was suspended in polyethylene glycol, and distilled water of 800 ml and was allowed to evaporate to get the final concentrate. The final concentrate was then diluted with sterile distilled water to make a mouthwash of 20% (w/v) concentration. Two tablespoons of honey and mint extract were added as a natural sweetener and flavoring agent, respectively.[7]

Study design

The participants were excluded from the study if they were diagnosed of nonplaque induced gingivitis or periodontitis, patients who were on antibiotic therapy or have been using mouthwash for the past 3 months, those with any systemic diseases, or having smoke or smokeless tobacco habits.

The sample size was calculated assuming at the most 5% risk, with minimum 80% power and 5% significance level (significant at 95% confidence level. i.e., $Z = 1.96$) and standard deviation of 0.5 and a margin of error (confidence interval) of ± 10, a sample size of 96 in one group would be sufficient. Hence, a sample size of 100 was considered in each group. Sample size was calculated based on formula,

Necessary sample size = $(Z\text{-Score})^2 \times \text{Std Dev} \times (1 - \text{Std Dev})/(\text{Margin of error})^2$

Necessary sample size = $(1.96)^2 \times (0.5 \times 0.5)/(0.10)^2 = 96.04$

The present study comprised 200 dental students 18–22 years of age, with gingival index of Score II (According to Loe and Silness [1963]);[6] were randomly divided into two groups of 100 each ($n = 100$). The first group was advised to rinse with 15 ml 0.12% chlorhexidine (CHX) mouthwash, and the second group with 15 ml of formulated herbal mouthwash twice daily for 30 s for 15 days along with their regular tooth brushing with a standard toothbrush and paste with no other restrictions in par with real-life situation. The participants were motivated on regular intervals by personal and phone contact, to use mouthwash on regular basis. The saliva samples were collected on the day 0 (baseline), followed by day 7 and 14. The assessment of salivary microbes was done by dilution and spread method, where saliva samples were diluted (1:1000) and streaked on blood agar containing gel plates. These plates were then incubated at 35° for 48 h. The growth of microorganisms thus obtained was subjected to microbial colony count using an automated microbial colony counter by a microbiologist who was blinded to the participants allocated. After having the baseline record of colony count for day 0, the counts were further obtained for day 7 and day 14 samples.

Statistical analysis

Statistical analyses were performed using SPSS Version 16.0 (SPSS Inc, Chicago, USA). Student's *t*-test was applied for comparison of the mean microbial count between the herbal group and CHX group. Repeated measures analysis of variance followed by Tukey's *post hoc* test was applied to assess the changes from day 0 to

day 7 to day 14. Statistical significance level was set at $P < 0.05$.

Results

Comparison between herbal and CHX group using "paired t test" revealed that, in relation to the baseline microbial colony count (day 0), to day 7, was found reduced in both the mouthwash groups, but the difference was not statistically significant ($P = 0.35$ and $P = 0.74$ for herbal and CHX respectively), and on day 14, microbial count was reduced in both the groups and was statistically significant ($P < 0.001$) with greater reduction noted in the herbal group [Table 1].

RM-ANOVA test and Tukeys *post hoc* test were performed for comparison in microbial reduction in both genders with respect to herbal and CHX mouthwashes for day 0 (baseline values), day 7, and day 14. The microbial colony was reduced better in CHX group on the day 7 compared to baseline values (day 0) in both males and females and was statistically significant ($P < 0.001$) whereas from day 7 to day 14, microbial count was much better reduced in the herbal group in both the gender groups with high statistical significance ($P < 0.001$), whereas the same in the CHX group though showed reduction in the counts was not statistically significant ($P = 0.097$ and $P = 0.158$ in males and females, respectively) [Table 2 and Graph 1].

The final microbial counts on day 14 from day 0 (baseline) made the noticeable difference where, despite both mouthwashes caused reduction in microbes ($P < 0.001$), the herbal mouthwash group performed far better in both the gender groups with greater reduction in the microbial colony count with high statistical significance ($P < 0.001$) [Table 2 and Graph 1].

Discussion

The primary outcome observed in the study is the reduction in the number of microbial colonies after use of both the mouthwashes. The reduction in microorganisms was marginally better with the chlorhexidine group on day 7 compared to baseline values (day 0), and the difference was not statistically significant, but on the 14th day there was comparatively superior reduction in the microorganism count in the herbal group, and the difference was statistically significant ($P < 0.001$).

The secondary outcome in the study was the subjective an objective observation in the attitude of the subjects in using the mouthwashes. Even though the participants using the mouthwashes were unaware of the nature, those using chlorhexidine found it acceptable as it tasted good and appeared attractive with blue-green color. Those using formulated herbal mouthwash felt it bit uncomfortable to taste and some others found it unattractive in color. However, as all the participants were always kept in contact during the entire study period and with constant motivation, they were suggested to continue using the mouthwash despite with some discomfort.

Strength of the Study

The strength of present study is the noval formulation of herbal mouthwash, with collaboration of various herbal extracts as to enrich the benefits of multiple herbs.

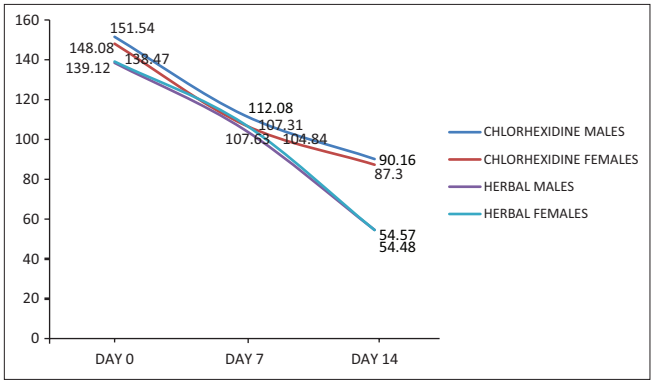
Limitations of the Study

1. The study was performed among the dental students aged between 18 and 22 years which confines to the subjects of relatively younger age group. Nevertheless, the participants were selected on inclusion and exclusion criteria basis to keep bias as minimum as possible
2. The assessment of microbial count was limited to 14 days duration of use of mouthwashes; however,

Table 1: Comparison in microbial colony counts between formulated herbal and 0.12% chlorhexidine mouthwash on 0, 7, and 14 days using "paired *t*-test"

Day of microbial colony count	Herbal mouthwash		CHX mouthwash		*P*
	Mean	SD	Mean	SD	
Males (*n*=100)					
Day 0	138.47	58.34	151.54	58.41	0.26
Day 7	112.08	56.33	104.84	53.72	0.51
Day 14	54.57	22.64	90.16	44.70	<0.001**
Females (*n*=100)					
Day 0	139.12	59.20	148.08	59.97	0.45
Day 7	107.63	51.64	107.31	53.56	0.97
Day 14	54.48	22.98	87.30	46.21	<0.001**
Total (*n*=200)					
Day 0	138.80	58.47	149.50	58.91	0.35
Day 7	109.72	53.77	106.23	53.42	0.74
Day 14	54.53	22.69	88.74	45.24	<0.001**

SD=Standard deviation, n=number. Statistical significance level was set at P<0.05. *P<0.001=Highly significant



Graph 1: Line graph representing the decline in microbial count after using 0.12% chlorhexidine and herbal mouthwash.

Table 2: Changes in microbial count in herbal and 0.12% chlorhexidine mouthwash groups on day 0, 7, and 14 days through multiple comparisons using repeated measures analysis of variance (RM-ANOVA) test and Tukey's *post hoc* test

Gender group	Study group (*n*=100)	Mean (SD)			*P*	Multiple comparison (*P*)
		Colony count day 0	Colony count day 7	Colony count day 14		
Males	Herbal mouthwash (*n*=50)	138.47 (58.34)	112.08 (56.33)	54.57 (22.64)	<0.001**	Day 0 versus day 7 (=0.002*)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (<0.001**)
	CHX mouthwash (*n*=50)	151.54 (58.41)	104.84 (53.72)	90.16 (44.70)	<0.001**	Day 0 versus day 7 (<0.001**)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (0.097)
Females	Herbal mouthwash (*n*=50)	139.12 (59.20)	107.63 (51.64)	54.48 (22.98)	<0.001**	Day 0 versus day 7 (0.003*)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (<0.001**)
	CHX mouthwash (*n*=50)	148.08 (59.97)	107.31 (53.56)	87.30 (46.21)	<0.001**	Day 0 versus day 7 (<0.001**)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (0.158)
Total (males + females)	Herbal mouthwash (*n*=100)	138.80 (58.47)	109.72 (53.77)	54.53 (22.69)	<0.001**	Day 0 versus day 7 (<0.001**)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (<0.001**)
	CHX mouthwash (*n*=100)	149.83 (58.91)	106.23 (53.42)	88.74 (45.24)	<0.001**	Day 0 versus day 7 (<0.001**)
						Day 0 versus day 14 (<0.001**)
						Day 7 versus day 14 (<0.001**)

SD=Standard deviation, n=number. Statistical significance level was set at P<0.05. Statistical significance level was set at P<0.05, **P<0.001=Highly significant

a longer term study would provide better analysis of the herbal mouthwashes.

Several studies have been published using herbal extracts either individually or in combinations as mouthwashes and compared with CHX for their efficiency. The herbal mix used in the present study is unique and new and to the best of our knowledge is the first of its kind in combination.

Baratakke *et al*. studied triphala combination mouthwash and compared with CHX and found no significant difference between both in reducing the plaque and gingival scores and concluded that both were equally effective and triphala mouthwashes could in future replace CHX as they have no side effects and is cost-efficient.[8]

A similar observation was noted by Pradeep *et al*., where triphala mothwash was tested against CHX, and it was noted that the plaque, gingival, and oral hygiene index-simplified values were reduced similarly with both the mouthwashes in comparison with placebo group. They concluded that triphala was compatible with CHX and can be used as a potential therapeutic agent in treatment of gingivitis.[9] Sushma *et al*. subjected the dentures from patients to microbial analysis after cleaning the dentures with triphala churna and chlorhexidine gluconate and

found triphala to be more efficient than conventional chlorhexidine in reducing the *Candida albicans* count.[10]

Mahajan *et al*. in their study reported that tulsi and neem showed antimicrobial property but was less than that of chlorhexidine. Tulsi is found to be active against Candida species and hence is advantage to add one as a component in herbal mouthwash compared to chlorhexidne which acts against normal oral microflora increasing the chances of candidiasis on long-term use.[11] Mallikarjun *et al*. studied the ethanolic exctracts of tulsi (*Ocimum sanctum*) on periodontal pathogens in comparison with doxycycline. They found tulsi to exhibit an inhibition zone on the agar gel similar to doxycycline particularly against *Aggregatibacter actinomycetemcomitans*, but less inhibition against *Porphyromonas gingivalis* and *Prevotella intermedia*. They concluded that tulsi could possibly used as an effective and affordable adjunct along with standard care in management of periodontal diseases.[12]

Anand *et al*. found 3% neem to be an effective antimicrobial solution in reducing (87% reduction) the streptococcal mutans on the toothbrush bristles.[13] Datta *et al*. used neem extract as an endodontic irrigating agent and showed that 0.94% and 1.88% of the extract was found effective against Enterococcus faecalis and *C. albicans,* respectively, when compared to 3% sodium hypochlorite and 2% chlorhexidine.[14]

Heyman *et al*., in an effort to understand the antioxidant properties of neem leaf by ethanol extraction, found that neem leaf extract showed prominent dose-dependent antibacterial action against *P. gingivalis* but had no effect on *F. nucleatum*.[15]

Chatterjee *et al*. in their study used 1% turmeric mouthwash in comparison with 2% chlorhexidine and found that the turmeric mouthwash as effective as CHX in reducing the gingival and plaque index and concluded that turmeric mouthwash could be used as adjunct similar to CHX after scaling and root planning.[16]

The components of the formulated herbal mouthwash in the present study were selected after detailed understanding of their pharmacological actions so that the combination is at its best. The antibacterial effect of the herbal blend could be attributed to the cumulative effect of each individual component. Sushrutha Samhita emphasize triphala to have anti-inflammatory, analgesic, hemostatic, and wound-healing properties.[8] Alongside, triphala has been attributed with other additive properties such as antioxidant, antibacterial, antimutagenic, antinoeplastic, chemo and radioprotective, and antidental caries as well with broad clinical application.[17] The phenolic and nonhenolic components, the tannic acid, chebulic acid, and flavonoids in triphala provide the antibacterial action and therapeutic potential, especially against streptococci and *C. albicans*.[10]

Neem (*Azadirachta Indica*) contains azadarachitin, the chief active component, which is an effective antimicrobial agent. Trimethylamine, nimbidin, nimbin, nimbolide, chlorides, lectin, and fluorides are other major components and silica, sulfur, Vitamin C, tannins, saponins, flavonoids, and sterols as minor components. These elements provide neem with its antiseptic and anti-inflammatory effect.[18] The polyphenolic tannins present in the neem could effectively bind to the bacterial surface proteins causing bacterial aggregation and loss of glycosyltransferase activity.[13] The antioxidant activity of neem extract gets further amplified following adherence to bacteria. Neem leaf extracts contain polyphenols that help adhere to the oral mucosa and are effective as synergetic antioxidants in periodontal diseases.[15]

The antimicrobial activity of *Ocimum sanctum* (Tulsi) is attributed to the essential oils contained in them, namely, eugenol, caryophyllene, germacrene-A, clemene, and caryophylline oxide. These oils render tulsi active against wide array of organism such as streptococci, staphylococci, Shigella, Salmonella and recently been tested against *A. actinomycetemcomitans*, a prominanat periodontal pathogen. The phenolic nature of the essential oils in tulsi exert membrane destabilizing effect in the microbial strains and stimulate leakage of cellular potassium which is lethal for the bacteria.[19]

Turmeric is known for its anti-inflammatory, antimicrobial, antioxidant, immunostimulant, and antiseptic properties. The anti-inflammatory action is due to its selective inhibitory action of prostaglandin E2 synthesis and thromboxane and inflammatory mediators of arachidonic acid metabolism.[20] As the molecules are lipophilic in nature, cause rapid permeability of the cell membrane as in the process of apoptosis and induces change in the structure and integrity of the bacterial cells.[16]

As per the literature, there are a few controversies regarding the duration of the studies conducted using herbal mouthwashes. Parwani *et al*. in a 4 days "*de novo*" plaque formation study concluded that 0.2% CHX mouthwash performed better than the herbal mouthwash. However, they were of opinion that the 4 days clinical study would be very short period to evaluate the effectiveness of the herbal mouthwash.[21] Similar was the conclusion by Moran *et al*. in their study comparing triclosan and CHX mouthwash in a 4 days plaque regrowth model.[22] In 2011, Chatterjee *et al*., in their study, found that the herbal oral rinse was equally effective in reducing the periodontal index as that of

chlorhexidine.[23] The same can be said about the results of our study on 7th day values where both mouthwashes were almost similar in activity with CHX faring marginally better, but on an extended use for 14 days, greater effect was observed in the herbal group.

CHX though has stood the test of times also come with a price tag of certain disadvantages on long-term usage, such as the teeth staining and the taste alteration.[6,24] It is also believed that continued and increased use of chlorhexidine may cause the emergence of new strains of micobacteria with reduced susceptibility.[11]

Combining several such herbal extracts as in the present study would certainly formulate mouthwash with a wide array of benefits in reducing the pathogenic oral microorganisms. As the benefits of the mouthwashes are better delivered on long-term usage, it is desirable to switch to herbal mix mouthwashes as they are traditionally accepted, culturally amicable, economically feasible, safe, and efficient too.

Further researches regarding new herbal combinations as mouthwashes on oral pathogenic organisms at different age groups in various clinical conditions are required to implicate the benefits of herbal extracts over chlorhexidine.

Conclusions

With few limitations in the study, it can be concluded that 0.12% CHX mouthwash was better at 7th day in reducing the microbial colony count, while on a prolonged usage for 14 days the herbal preparation was found to perform much better than 0.12% CHX proving the new formulated herbal mouthwash an affordable alternative in reducing the microbial count. However, further long-term clinical studies are essential to provide much-required standardization and qualification of the various combinations of herbal mouthwashes to counter the disadvantages of the gold standard chlorhexidine.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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