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# Comparison of antimicrobial efficacy of chemical mouthwash and herbal mouthwash: An ex vivo study on orthodontic arch wires

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***Abstract***---Background: The use of mouth rinse for oral hygiene maintenance has been advocated as an adjuvant to mechanical aids of plaque removal. The aim of this study was to compare the antimicrobial effect of herbal and chemical mouth rinse on bacterial accumulation on orthodontic arch wires. Materials and Methods: The study sample consisted of 15 numbers of 0.016 Nickel Titanium (NiTi) arch wires removed from patients' mouths, which were split into three segments of 1 cm each. Wires were dipped in solutions of Chlorhexidine (Group A), Befresh Herbal (Group B) and Distilled water (Group C) individually for 30 seconds and then in normal saline for one minute. 0.5microlitre of this saline solution was inoculated in a Brain heart infusion medium (BHI) for 24 hours and then examined for bacterial count. The obtained results were subjected to One-way ANOVA, Bonferroni and post hoc tests for statistical analysis. Results: Group A showed the maximum reduction in the bacterial colony forming units (CFU) after 24 hours with a mean value of 65.67cfu. Group B showed a mean value of 336.87cfu. There was a statistically significant difference between the Chlorhexidine group and the Control group. However, the difference between the results of Group A (Chlorhexidine) and Group B (Befresh Mint Mouth Wash) was not statistically significant. Conclusion: Chlorhexidine mouth rinse had more reduction in the bacterial cfu/ml than the herbal mouth rinse group, but it was not statistically significant. Hence, herbal mouth


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rinse can be used as an adjuvant to chemical mouth rinse in orthodontic subjects.

***Keywords***---chlorhexidine gluconate, cinnamomum zeylanicum, herbal, oral hygiene.

## Introduction

Oral hygiene mainly involves the use of mechanical agents like toothbrush, that facilitate the removal of plaque accumulated around the teeth. However, this alone is not sufficient to prevent plaque accumulation, as it is a constant process [1]. Adjuvant chemical agents such as mouth rinses are necessary to exhibit an antiplaque activity in the oral cavity. Even though it is difficult to prevent the accumulation of plaque, the bacterial accumulation in the plaque can be reduced by the help of these agents [2]. Orthodontic therapy in general, warrants the need for good oral hygiene maintenance. The use of several metallic and non-metallic components during orthodontic therapy, facilitates plaque accumulation [3]. This leads to the formation of white spot lesions and further progresses to caries formation, gingivitis, periodontitis, halitosis [4-5].

Chlorhexidine is a chemical mouth rinse of the bisbiguanide group that possesses the substantivity property, making it the gold standard of chemical antimicrobial agents [6]. It is most commonly used in a concentration of 0.2%, which provides adequate antimicrobial activity However, Long term use of chlorhexidine mouth rinse is contraindicated as it can cause staining of teeth [7]. Other side effects such as alteration of taste perception also has been noted with long term chlorhexidine usage [8]. Chlorhexidine mouth rinse and other fluoride containing mouthwashes also hinder the mechanical properties of orthodontic components by causing leaching of metallic ions and also altering their surface tomography [9-10]. Such shortcomings have necessitated the search for a viable alternative that can overcome these adverse effects and can be a valuable alternative to chemical mouth rinse.

Herbal mouth rinses are non-alcoholic and they do not possess any known side effects [11]. Befresh mint mouth rinse used in this study is an herbal, non-alcoholic mouth rinse, that is composed of 0.05% cinnamon oil, 0.05% clove oil, 0.05% eucalyptus oil and 0.30% spearmint oil [12]. Cinnamomum zeylanicum (Cinnamon) is a member of the lauraceae family. Historically, cinnamon has been used in the treatment of various conditions such as cold, flatulence, nausea, diarrhoea, etc. It is also known to possess antibacterial and antifungal action. Cinnamon is also seen to inhibit the growth of Salmonella enterica and Escherichia coli, which are food borne bacteria [13]. It has been a natural remedy in the treatment of halitosis as well, due to its aromatic properties of the essential oil, cinnamaldehyde. The menthol content in the spearmint oil gives it properties of a local anaesthetic and makes it a powerful anodite and an antiseptic agent [11]. Clove oil is also effective in its antibacterial, antiviral, antifungal and spasmolytic properties. This herbal concoction of mouth rinse is completely devoid of alcohol, added sugar or any preservatives [14]. The cinnamon and clove oils are especially seen to be effective against eurotium, aspergillus and

penicillium groups of microorganisms [15]. Cinnamon based mouthwash has also been found to possess antiplaque, antimicrobial and anti-inflammatory properties [16]. Herbal mouth rinses do not cause any deleterious changes to the orthodontic components, nor does it affect the surface coating of any orthodontic arch wires and brackets [10]. This makes it ideal for usage on a prolonged duration without any adverse side effects. The mouth rinse used in the study is a commercially available cinnamon-based herbal mouth rinse (Befresh Mint Mouthwash; BPRL Private Limited). The aim of this study was to compare and evaluate the antimicrobial effect of chlorhexidine with herbal mouth rinse in subjects undergoing orthodontic treatment.

**Methodology**

This in vitro study involved the collection of 15 samples of 0.016 NiTi arch wire from patients undergoing fixed orthodontic appliance therapy at the department of Orthodontics, Saveetha dental college. Informed consent was obtained for the participants in the study and ethical clearance was obtained from the scientific review board of Saveetha dental college (SDC/SIHEC/2020/DIASDATA/0619-0320). The collected arch wires were cut into 3 segments of 1cm length each. Each segment of the cut arch wire was used for analysis of the following groups.

- Group A- Chlorhexidine group.
- Group B - Test Group containing herbal mouth rinse (Befresh Mint Mouthwash; BPRL Private Limited).
- Group C - Control group containing distilled water.

The wire segments were immersed in these solutions for 30 seconds. Following exposure, the wire segments were taken out of their interventions and were placed in 3 different 2ml cuvettes filled with 0.5ml of normal saline and was gently agitated to unload the bacterial colonies from the arch wire, into the normal saline (Fig. 1).


Fig. 1. Three 1cm segment wires placed in 2ml cuvettes of Group A, Group B, and Group C, filled with 0.5ml of normal saline

After 1 minute, 0.5 microlitre of the sample from each group was inoculated in BHI (Brain heart infusion) medium for 24 hours and the readings were taken using a colony counter application (Fig. 2).

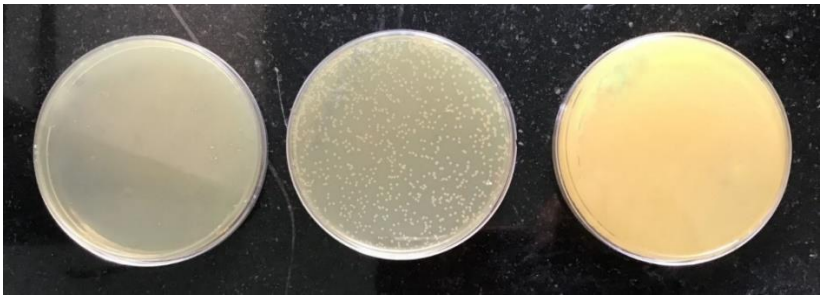


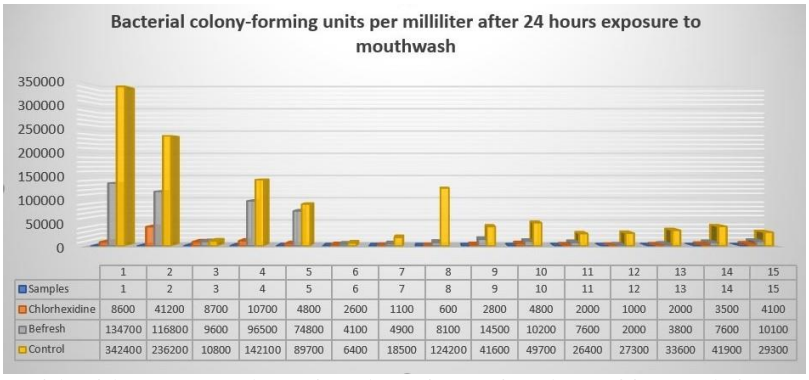
Fig. 2. Bacterial colony forming units (CFU) seen Brain heart infusion media (BHI) 24 hours after inoculation of sample A, B, and C

**Statistical Analysis**

The obtained results were statistically analysed using IBM SPSS software version 23 for windows. One-Way ANOVA test was performed to test the significance of the obtained quantitative variables. Bonferroni and Post hoc tests were performed to test between and within the groups for statistical significance of the obtained values.

**Results**

The results of the study are depicted in (Table 1) which shows the results of One way ANOVA, mean and the standard deviation of the bacterial colony forming units per millilitre in the three groups. One-way ANOVA test results for significance of the obtained variables show that the p value is 0.007, which is statistically significant. The graphical representation of the descriptive statistics show that there is more reduction of bacterial colony-forming units per millilitre (cfu/ml) in Group A as compared to Group B (Fig. 3).




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Chlorhexidine | 8600 | 41200 | 8700 | 10700 | 4800 | 2600 | 1100 | 600 | 2800 | 4800 | 2000 | 1000 | 2000 | 3500 | 4100 |
| Befresh | 134700 | 116800 | 9600 | 96500 | 74800 | 4100 | 4900 | 8100 | 14500 | 10200 | 7600 | 2000 | 3800 | 7600 | 10100 |
| Control | 342400 | 236200 | 10800 | 142100 | 89700 | 6400 | 18500 | 124200 | 41600 | 49700 | 26400 | 27300 | 33600 | 41900 | 29300 |

Fig. 3. Graphical interpretation showing the reduction of bacterial CFU between three groups. x axis represents the samples and y axis represents the colony-forming units (cfu) per millilitre (ml). The chlorhexidine group shows the maximum reduction in cfu/ml when compared with the Befresh group and the control group

Bonferroni and Post hoc tests show a statistically significant difference in the cfu/ml values between intervention group A (Chlorhexidine) and the control group

C. However, there is no statistically significant difference in the obtained values of cfu/ml between the two test groups A (Chlorhexidine) and B (Herbal Mouth rinse), and also the intervention group B (Herbal Mouth rinse) and test group C, in spite of there being a reduction in the CFU in Group B. (Table 2).

**Discussion**

The arsenal of herbal mouth rinses that are being discovered and manufactured are ever increasing. The challenge lies in estimating which among these herbal mouth rinses is closest to being called comparable, if not better than the "gold standard" in chemical aid of oral hygiene maintenance, which is chlorhexidine. Due to the recent increase in awareness of the side effects associated with chemical mouth rinse, the usage of herbal mouth rinse has increased manifolds [17]. The results of this study show that there is a statistically significant difference between the obtained variables in all three groups. The descriptive statistics show that the chlorhexidine group has the maximum reduction in the colony forming units per millilitre, when compared to the Befresh and control group. This is in accordance with the findings of other studies comparing chlorhexidine with cinnamon based as well as other herbal mouth rinse concoctions [18–20]. Bonferroni and Post hoc tests done to compare the three groups between each other and evaluate the reduction in bacterial colony forming unit, showed a statistically non-significant result between the Chlorhexidine and Befresh Herbal mouth rinse group as well as the herbal mouth rinse and control group. The chlorhexidine and the control group however, showed statistically significant results as p value was <0.05. Similar results were reported by Gupta et al., when they compared chlorhexidine with a herbal mouth rinse made out of Cinnamon and *Terminalia Chebula* [21]. Bay et al., in his study has also concluded that there is no significant difference in the reduction of bacterial colony forming units between chlorhexidine and cinnamon based mouth rinse [22]. Kripal et al., had done a similar study in an in vivo setting where they have evaluated the Plaque and gingival index and baseline, 1st, 2nd and 3rd revisits [11]. He concluded that the Befresh herbal and chlorhexidine mouth rinse were equally effective in reducing plaque and gingivitis in patients.

On the contrary, Nagappan N et al., in his study stated that chlorhexidine has a better efficacy against *S.mutans* when compared with herbal mouth rinse [23]. Parwani SR et al., also concluded that 0.2% chlorhexidine gluconate remained the best antiplaque agent [24]. Systematic review and meta analysis done by Manipal S et al., states that chlorhexidine can still be regarded as the "gold standard" of mouth rinses as sufficient evidence is not present in favor of herbal mouth rinses [25]. Although highly effective, chlorhexidine mouth rinses have certain drawbacks, that make them unsuitable for orthodontic purposes. Orthodontic treatments go on for longer duration and this necessitates the use of mouth rinse for a prolonged period of time. Chlorhexidine mouth rinse have been shown to cause staining of teeth and alteration of taste perception on prolonged usage [26]. Desquamation of oral mucosa and soreness of the oral cavity have also been reported as a result of idiosyncratic reactions to the long-term use of chlorhexidine mouth rinse [8] Furthermore, chemical mouth rinses containing fluoride cause corrosion of orthodontic brackets and wires [27].

Scanning electron microscopic evaluation of orthodontic arch wires exposed to chemical mouth rinse show changes in the surface tomography and characteristics [28]. Study conducted by Singh et al., shows that Shear bond strength is affected more in the presence of alcohol containing chemical mouth rinse as compared to herbal mouth rinse [29]. Leaching of metal ions from stainless steel brackets have also been observed to be maximum in the presence of chlorhexidine as opposed to colgate plax and listerine, as reported by Danaei et al (9).Usage of chlorhexidine mouth rinse is also not warranted for long term purposes as it can be cytotoxic and deleterious to the oral tissues [30]. Cinnamon based mouth rinse also has been observed to have antifungal, antiplaque and anti-inflammatory activity [31]. Cinnamon in particular, is effective against gram positive as well as gram negative bacteria [32]. The mechanism of action of cinnamon against bacterial pathogens are still unclear, yet it is believed to be the property of hydrophobicity, which enables these essential oils in breaking down the lipid molecules in the bacterial cell, causing cell death [33]. On the other hand, some allergic responses to cinnamon oil in the form of contact dermatitis and stomatitis have also been reported [34]. The main limitation of this study was the evaluation of CFUs instead of a real time PCR which is a more quantitative method. A short-term evaluation is also a limitation since orthodontic treatment is done for longer durations.

## Conclusion

Within the limitations of this study it can be concluded that even though chlorhexidine group has more reduction in the bacterial cfu/ml than Befresh mouth rinse group the difference in antimicrobial activity was not significant statistically, hence herbal mouthwashes can be considered as an alternative to chlorhexidine in orthodontic subjects.

## Conflict of Interest

None.

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**Tables**

Table 1
One-way ANOVA test results showing Mean and Standard deviation of the bacterial colony-forming units per millilitre (cfu/ml) in the three groups and the P value which is 0.007, suggesting that it is statistically significant

| | N | Mean (cfu/ml)[1] | Std. Deviation | Std. Error | P value |
|---|---|---|---|---|---|
| Chlorhexidine (Group A) | 15 | 6566.67 | 10053.689 | 2595.85 | 0.007* |
| Befresh (Group B) | 15 | 33686.67 | 46614.373 | 12035.77 | |
| Control (Group C) | 15 | 81340.00 | 95369.663 | 24624.34 | |
| Total | 45 | 40531.11 | 67782.710 | 10104.45 | |

[1]cfu/ml - colony-forming units per milliliter.
*P value is less than 0.05, indicating that it is statistically significant**.**

Table 2
Bonferroni and Post hoc tests show that the results comparing the herbal mouthwash and chlorhexidine mouthwash are insignificant as p value is 0.456

| Groups | Mean Difference | P value |
|---|---|---|
| Chlorhexidine (A)[1] – Befresh (B) | -27120.00 | .456 |
| Befresh (B)[2] – Control (C) | -47653.333 | .098 |
| Control (C)[3] – Chlorhexidine (A) | 74773.333 | .005 |

[1](A) - Group A;
[2](B) - Group B;
[3](C) – Group C.

**Legends for illustrations**

- **Fig. 1:** Three 1cm segment wires placed in 2ml cuvettes of Group A, Group B, and Group C, filled with 0.5ml of normal saline.
- **Fig. 2:** Bacterial colony forming units (CFU) seen Brain heart infusion media (BHI) 24 hours after inoculation of sample A, B, and C.

- **Fig. 3:** Graphical interpretation showing the reduction of bacterial CFU between three groups. x axis represents the samples and y axis represents the colony-forming units (cfu) per millilitre (ml). The chlorhexidine group shows the maximum reduction in cfu/ml when compared with the Befresh group and the control group.