Original article

Comparison of *Andrographis paniculata* and Chlorhexidine Mouthwash on Anti-plaque, Anti-gingivitis and Side Effects

Jananya Plianrungsi[1] and Promphakkon Kulthanaamondhita[1]

[1]Department of Oral Diagnostic Science, College of Dental Medicine, Rangsit University, Pathum Thani, Thailand

Abstract

The purpose of this study was to evaluate the effects of *Andrographis paniculata (Ap)* mouthwash compared with chlorhexidine (chx) mouthwash on anti-plaque, anti-gingivitis, tooth staining, and burning sensation. This study was a double-blind, randomized two group experiments. Thirty-four healthy volunteers were enrolled in this study. Participants were assigned into two groups and mouthwash was given according to the group. Gingival index, plaque index, stain index and visual analog scale for burning sensation were recorded at baseline and three weeks after the experiment. Then, participants were switched between groups and the data were recorded. The results had shown that the baseline data were not significantly different between the groups. Both mouthwashes can reduce the gingival index but were not significantly different from baseline and between the groups. Both mouthwashes can significantly reduce plaque index but were not statistically significant between the groups. Chx significantly caused more staining on the teeth compared to *Ap* and the burning sensation reflected as VAS score was significantly lower in *Ap.* It can be concluded that *Ap* mouthwash can effectively reduce plaque accumulation, produce less staining and discomfort. This herbal mouthwash can be used as an adjunctive to mechanical oral hygiene procedures and as an alternative to chlorhexidine mouthwash.

Keywords: Andrographis paniculata, Chlorhexidine, Dental plaque, Gingivitis, Staining

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Correspondence to:
Promphakkon Kulthanaamondhita, Department of Oral Diagnostic Science, College of Dental Medicine, Rangsit University, Pathum Thani, 12000 Thailand Tel: 0863747157 Email: promphakkon.k@rsu.ac.th

Introduction

Dental plaque removal is an important issue in oral health promotion. Dental plaque is the mass of bacteria that starts accumulating on the surface of a tooth as a sticky biofilm. Plaque deposition brings about the inflammatory changes on the periodontium that can lead to the destruction of periodontal tissues and loss of periodontal attachment. If adequate control measures are not undertaken, the gradual build-up of plaque over time will lead to tooth decay and gingival diseases.[1]

Gingivitis is one of the most common oral diseases which can occur with every individual.[2] Normally, the mechanical and chemical plaque controls are used to remove the plaque. Chemical plaque control like mouthwash is widely used for adjunctive therapy. The most widely

prescribed anti-plaque and anti-gingivitis chemical agent is chlorhexidine gluconate (chx) containing mouthwashes.[3] Chx has been reported to be the gold standard antiplaque and antigingivitis agent and its effects in combination with or without mechanical plaque control measures havebeen widely discussed in the literature.[4-9] However, the long-term use of chlorhexidine gluconate mouthwash has been found to be associated with several side effects including teeth discoloration, staining, and a burning sensation, mucous membrane irritation, and taste disturbance.[3,4,10-12]

Nowadays, people pay more attention to natural products, including herbal mouthwash due to its low toxicity, ease of availability and lack of microbial resistance of herbal agents. Bamboo, Triphala, pomegranate mouthwash has been studied for a long time and advantages of these herbs are verified by much evidence.[13-17]

Andrographis paniculata (Ap) is an alternative choice due to its properties. *Ap* has been used in the treatment of various diseases, such as cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia and malaria for centuries in Asia, America and Africa.[18] It also has been used in the treatment of oral diseases such as oral cancer in which dehydroandrographolide of *Ap* inhibits cell migration and the invasion of cancer cells.[19] Inhibitory activity against *P. gingivalis* has also been found in *Ap* combined with a 95% ethanol extract which can aid in the treatment of periodontal diseases.[20] Furthermore, subgingivally delivered *Ap* gel had been investigated and found to aid in adjunctive chronic periodontitis treatment and to improve the periodontal conditions in periodontitis patients during the maintenance phase.[21-24]

Myseptic Mybacin® is a commercially available mouthwash that contains *Ap* extracts, water, alcohol and other essential oils. The manufacturer claims it can be used as an adjunctive mouthwash to help prevent dental plaque build-up, the development of gingivitis and to improve halitosis.

A previous study by our group[25] found that both chx and Ap mouthwash were able to reduce the gingival index but are not significantly different from baseline and between the groups. Therefore, to overcome the bias and limitations in the previous study, this study was developed to further evaluate the effect of these mouthwashes in reducing the severity of gingivitis and also evaluate the side effects caused by both mouthwashes.

In addition, existing evidence in support of mouthwash formulation containing *Ap* as a single active ingredient against plaque and gingivitis is still limited, and studies that so far investigated the effectiveness of *Ap* had many methodological limitations.

Therefore, the aim of this study was to compare the commercially available *Ap* mouthwash and the commercially available chx mouthwash on anti-plaque, anti-gingivitis, tooth staining, and burning sensation.

Materials and methods

1. The recruitment of participants

The study recruited 40 healthy participants from dental students at the College of Dental Medicine, Rangsit University among the age group of 18-30 years old. The study was approved by the Ethics Committee of Rangsit University (RSUERB2020-015) and the participants were included in the study after signing informed consent and voluntarily agreeing to participate in this project.

Inclusion criteria

- Healthy participants aged between 18-30 years old
- Dental students of College of Dental Medicine, Rangsit University
- BOP ≥ 10%
- Probing depth ≤ 3 mm
- No clinical attachment loss
- No radiographic bone loss
- Periodontal diagnosis as localized or generalized dental plaque induced gingivitis (AAP/EFP 2018)

Exclusion criteria

- Pregnancy and lactation
- Gingival enlargement
- Requiring daily medication
- Allergy to any component used in this study

- Any fixed or removable orthodontic appliances or prosthesis
- Physical and mental retardation that can interfere with oral hygiene
- Alcoholism
- Severe malalignment teeth
- Smoking
- Participants had received periodontal therapy or antibiotics within 6 months prior to the study
- Tooth loss due to periodontal disease

The participants were randomly divided into the following two groups of 20 patients each: Group A were given commercially available 0.12% Chx mouthwash (Mybacin®) and Group B were given commercially available *Ap* mouthwash (Myseptic Mybacin®).

2. Data collection

Two calibrated examiners held all of the examinations and were trained and well calibrated. The intra and inter-examiner reliability was calculated. Cohen's kappa coefficient (k)[26] for intra and inter-examiner reliability was 0.81 and 0.79 respectively. The data collection processes were in total of five visits as shown by the flow chart in Figure 1.

First visit

All participants received oral prophylaxis using Gracey curettes and sickle scaler by the operators and were approved by an instructor at the Oral Diagnosis Clinic, College of Dental Medicine, Rangsit University. Oral hygiene instructions using the modified Bass brushing technique were given. The participants received the same dental hygiene set comprised of a toothbrush (soft-bristled brushes) and tube of toothpaste (Colgate®) to use until the end of study. Normal saline solution (NSS) was given to the participants and they were instructed to rinse 15 ml twice daily immediately after tooth brushing during this period.

Second visit

One week after the first visit, the participants were requested to return all the bottles at this visit to evaluate the compliance. Clinical examination using mouth mirror, explorer and William probes comprising of Silness & Löe plaque index (PI)[27], Löe & Silness Gingival index (GI)[28], modified Lobene stain index (SI)[29] and visual analog scale (VAS)[30] for burning sensation were performed by the same examiners. The participants were randomly divided into two groups, A for Chx mouthwash and B for *Ap* mouthwash. All of the mouthwashes were packed in similar colored bottles but labeled differently and were administered to the participants by the examiners. The participants were also unaware of which mouthwashe they had been given and were asked to rinse with 15 ml of mouthwash for 30 seconds immediately after toothbrushing in the morning and before bedtime.

Third visit

Three weeks after the second visit, participants were recalled for a second measurement by the same examiners. Again, the participants were requested to return all the bottles at this visit to evaluate the compliance. Clinical examinations comprising of PI, GI, SI and VAS were performed. Oral prophylaxis using Gracey curettes and sickle scaler was done to set the clinical parameter to zero. Normal saline solution (NSS) was given to the participants and they were instructed to rinse 15 ml twice daily immediately after tooth brushing during this period.

Fourth visit

One week after the third visit, once again the compliance of the participants were measured by the return of all the bottles and participants were recalled for a third measurement by the same examiners. Clinical examinations comprising of PI, GI, SI and VAS were performed. Mouthwashes were administered by switching types of mouthwash between groups. Participants were asked to rinse with 15 ml of mouthwash for 30 seconds immediately after toothbrushing in the morning and before bedtime.

Fifth visit

Three weeks after a fourth visit, participants were recalled for the final measurement by the same examiners. Once again, the participants were requested to return all of the bottles at this visit to evaluate for compliance. Clinical examinations comprising PI, GI, SI and VAS were performed. Oral prophylaxis using Gracey curettes and sickle scaler was done.

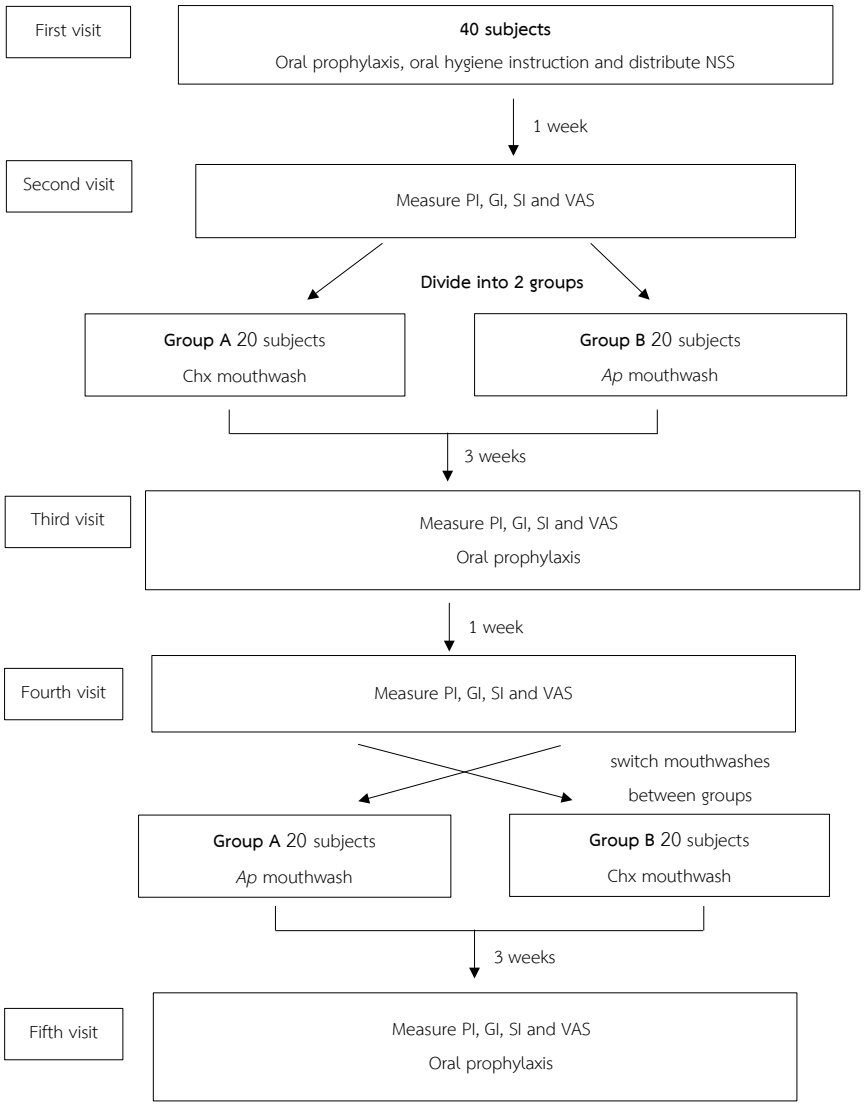

Figure 1 *Study design flowchart*

3. Data analysis

The statistical software SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The normal distributions of the clinical measurements were assessed using the Shapiro-Wilk test. For the clinical measurements, the descriptive analysis of PI, GI, SI and VAS is presented as mean ± SD and median (min, max). After investigating the normal distribution of the data, the PI and GI scores were not normally distributed so the mean PI and GI scores between groups were examined using the Mann-Whitney U test and the differences of mean within a group were assessed by the Wilcoxon signed rank test. Whereas, the SI and VAS scores were normally distributed so the mean SI and VAS scores between groups were examined using the independent t test and the differences within the groups were assessed by a paired *t* test. Significant differences were defined as $p<0.05$.

Results

This study was approved by the Research Ethics Office of Rangsit University (RSUERB2020-015) and all the participants signed the informed consent before starting the study. Of the 40 participants, six were excluded from the study due to loss of follow up. Thus 34 participants completed the study, 17 participants in the Chx group and 17 participants in *Ap* group. The mean age of the participants was 22.09 ± 2.24 years old. There were more females than males (Table 1).

Table 1 *Demographic data at the participant level. Data reported as mean ± SD*

Characteristics	
Number of participants	34
Age: mean ± S.D.	22.09±2.24
Sex	
Female	23 (67%)
Male	11 (33%)

1. Gingival index

The baseline gingival index (GI1) was not significantly different between the Chx and the *Ap* mouthwash groups. After finishing the experiment, the gingival index (GI2) was not significantly different between the Chx and the *Ap* mouthwash groups. Both mouthwashes slightly decreased the gingival index but not significantly from baseline (Table 2).

Table 2 *Descriptive data and comparison of gingival index (GI) between Chx and Ap mouthwash. Data reported as mean ± SD and median*

Clinical parameter	Chlorhexidine		*Andrographis paniculata*		*P*-value
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
GI1	1.15 ± 0.36	1.25 (0.38, 1.75)	1.15 ± 0.28	1.19(0.64, 1.71)	0.787
GI2	1.12 ± 0.39	1.19 (0.25, 1.83)	1.09 ± 0.25	1.08 (0.33, 1.58)	0.418

2. Plaque index

There was no significant difference in the baseline plaque index (PI1) between the Chx and the *Ap* mouthwash groups. The plaque index at the end of the experimental period (PI2) was significantly lower in both groups compared with baseline. Whereas, the plaque index between both groups after the experiment were not significantly different from each other (Table 3).

Table 3 *Descriptive data and comparison of plaque index (PI) Chx and Ap mouthwash. Data reported as mean ± SD and median (p<0.05).*

Clinical parameter	Chlorhexidine		*Andrographis paniculata*		*P*-value
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
PI1	1.31 ± 0.35	1.27(0.67, 2.13)	1.30 ± 0.35	1.36(0.42, 1.88)	0.811
PI2	1.06 ± 0.39	1.04(0.42, 1.96) *	1.15 ± 0.36	1.15(0.54, 1.83) *	0.320

3. Stain index

No analysis was performed on the baseline stain data because no participants presented with staining after the first visit. The overall scores revealed that Chx mouthwash significantly demonstrated more stain deposited for both extent, intensity, and total of both. When analyzing each area, only the gingival area significantly demonstrated more extent and intensity of staining in the Chx mouthwash compared to the *Ap* mouthwash. There was a trend toward less proportions of the heavy stain intensity and the total area but not statistically different from each other (Table 4, Fig. 2 and Fig. 3).

Table 4 *Descriptive data and comparison of stain index (SI) between Chx and Ap mouthwash. Data reported as mean ± SD and median (p<0.05)*

Clinical parameter Stain index		Chlorhexidine		*Andrographis paniculata*		*P*-value
		Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
Extent	Gingival	0.20 ± 0.28	0 (0, 1)	0.04 ± 0.18	0 (0, 1)	0.008*
	Approximal	0.08 ± 0.21	0 (0, 1)	0.01 ± 0.03	0 (0, 0.17)	0.056
	Body	0.04 ± 0.15	0 (0, 0.83)	0.05 ± 0.03	0 (0, 0.17)	0.142
	Total	0.32 ± 0.52	0.17 (0, 2.67)	0.05 ± 0.22	0 (0, 1.25)	0.009*
Intensity	Gingival	0.19 ± 0.25	0 (0, 0.92)	0.04 ± 0.16	0 (0, 0.92)	0.005*
	Approximal	0.09 ± 0.25	0 (0, 1.33)	0.03 ± 0.171	0 (0, 1)	0.058
	Body	0.04 ± 0.13	0 (0, 0.67)	0.00 ± 0.01	0 (0, 0.08)	0.170
	Total	0.32 ± 0.52	0.13 (0, 2.67)	0.05 ± 0.21	0 (0, 1.17)	0.008*
Extent + intensity: Total		0.64 ± 1.04	0.29 (0, 5.33)	0.11 ± 0.44	0 (0, 2.42)	0.008*

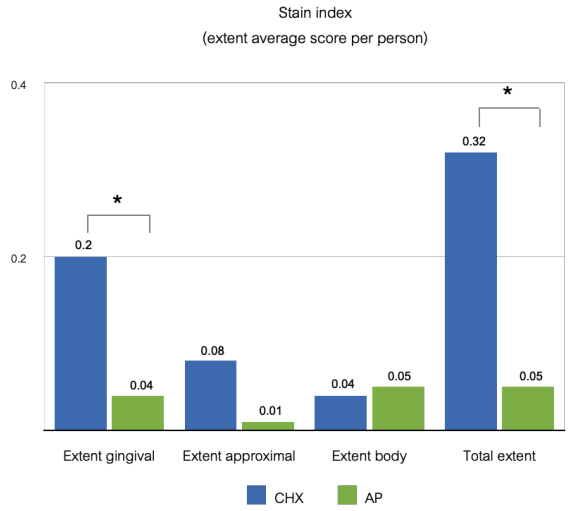

Figure 2 *Comparison of stain index (extent) between Chx and Ap mouthwash. Data reported as mean ± SD (p<0.05)*

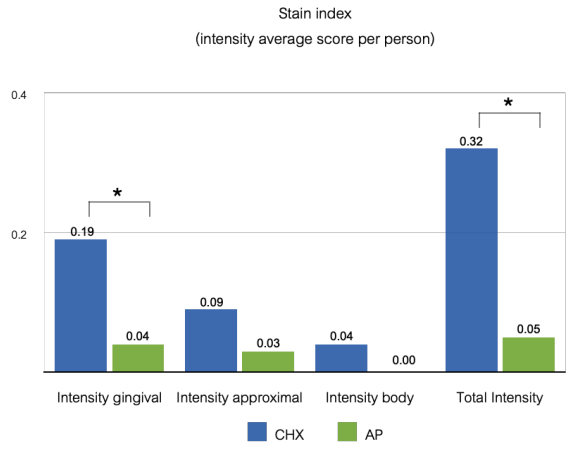

Figure 3 *Comparison of stain index (intensity) between Chx and Ap mouthwash. Data reported as mean ± SD (p<0.05)*

4. Burning sensation

The VAS was used to represent the burning sensation experienced by the participants. There was more burning sensation observed in the Chx group. Furthermore, the means of VAS between the two groups were also significantly different (Fig. 4).

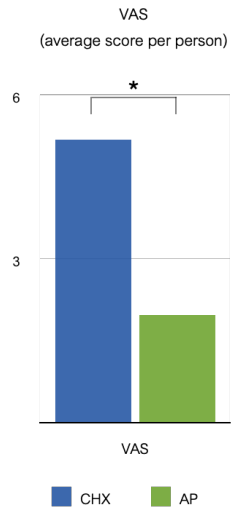

Figure 4 *Comparison of visual analog scale (VAS) of burning sensation between Chx and Ap mouthwash. Data reported as mean ± SD (p<0.05)*

Discussion

This study was further developed from the previous study[25] to overcome the bias and limitations with additional investigation that aims to compare both

types of mouthwashes in reducing the severity of gingivitis and its side effects. A previous study had shown that there was no difference in the reduction of GI between both mouthwashes, so the investigations on PI, SI and burning sensation were performed in order to evaluate other aspects of gingivitis reduction, and in addition, evaluate the side effects after using both mouthwashes.

This study was a double-blind, randomized two group experiments evaluating the effect of *Ap* compared with chx on the development of plaque and tooth staining, decreasing the severity of gingivitis and burning sensation. The results revealed that after 21 days, *Ap* significantly reduced the plaque accumulation comparable to chlorhexidine. Moreover, *Ap* significantly generated lower staining on teeth and less burning sensation was experienced by the participants when compared with chx.

In this study, the gingival index after the experiments which indicates the status of the gingival health revealed no significant differences from the baseline in both groups. Previous studies have shown that chx can improve the gingival index towards better gingival health when used concurrently with mechanical plaque control.[3,4,8,9,31,32]

Thawonrungroj *et al*. found that subgingivally delivered *Ap* gel was able to significantly reduce the gingival index in chronic periodontitis patients with mostly moderate gingival inflammation when compared to scaling and root planing alone.[22] A recent study by Kuphasuk and Prommas[24] which studied the effect of subgingivally delivered *Ap* gel during a supportive periodontal therapy also support the ability of *Ap* in improving the gingival health in a chronic periodontitis patient as shown by the reduction of the gingival index.

In comparison to our study, *Ap* mouthwash did not significantly reduce the gingival index when compared to baseline. This was due to the different types of the gingival disease, different preparation of the agent, delivery method and the initial status of the gingival health of the participant which, in our study, were quite mild to moderate gingival inflammation. Most of the participants presented very mild gingival inflammation that may not show further improvement after the mouthwash was used. Furthermore, oral prophylaxis procedures given to the participants before the experiment could reduce the gingival inflammation that resulted in no difference in gingival inflammation between the two groups.

Moreover, concentration and preparations of *Ap* may also have an effect on the outcome of this study. *Ap* gel most widely used in the previous study contains 0.5625 mg/mL of *Ap* extracts.[22,23] The concentration of commercially available *Ap* mouthwash was not provided by the manufacturer. However, the preparation in gel form may provide better retention in the target area and also provide the direct effect of the substance into the gingiva, which in turn could provide better results in improving gingival health. The use of mouthwash also required patient compliances while the gel preparation can only be used by professional application and applied during the recall intervals. These differences may account for the different results obtained from this study.

Previous studies had investigated the possible anti-inflammatory mechanism of *Ap* and found that andrographolide, an active ingredient form *Ap* extracts, was responsible for the anti-inflammatory property. Overproduction of nitric oxide (NO) and prostaglandin-E2 (PGE2), inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), plays a significant role in the inflammatory processes. The methanol extract of *Ap* and andrographolide incubated with macrophages have been reported to inhibit LPS-stimulated NO production in a concentration-dependent manner. Andrographolide has also been reported to suppress IL-2 production and T-cell proliferation in a mixed lymphocyte reaction and to inhibit dendritic cell maturation and antigen presentation[33,34].

Bacterial plaques have been proven to have a role in the etiology of dental caries and periodontal diseases. The use of mouthwashes as disinfectants can aids mechanical methods to reduce plaques.[8] Chx as a gold standard appears to be the most effective antimicrobial agent for the reduction of plaque.[9,35,36] Our study has shown that *Ap* mouthwash can significantly reduce plaque accumulation and comparable

with chx mouthwash. The results are also in agreement with previous studies that showed significant effects of *Ap* on plaque reduction when compared to baseline.[22-24]

Several studies in the past have shown that herbal and essential oil-containing mouthwash had similar properties in plaque reduction when compared to chlorhexidine. Charles *et al.*[37] found that at six months, the essential oil and chlorhexidine mouthrinse produced statistically significant PI reductions compared with the control and were not statistically significantly different from each other with respect to plaque and gingivitis reduction. Priya *et al.*[38] investigated the green tea mouthwash compared to chlorhexidine mouthwash and observed a significant decrease in PI in both of the groups. These results may support the use of *Ap*, an herbal medicine, to be used as an adjunctive to chlorhexidine for chemical plaque control.

Teeth staining and burning sensation are one of the side effects when using chx mouthwash. The brown deposition on the teeth can be esthetically unpleasing, and coupled with an unpleasant sensation, may discourage the patient to use the mouthwash and decrease the compliance of the patient.[6,10,11] Our study found that *Ap* significantly caused less staining on the teeth when compared to chx. Moreover, the patient reported outcome reflected as a VAS score on the sensation perceived after the use of both mouthwashes also indicates significantly better results than chlorhexidine.

Yaghini *et al.*[39], studied the effect of aloe vera-green tea and matrica mouthwash compared to 0.2% chlorhexidine mouthwash. The results showed that all three mouthwashes can significantly reduce gingival index, plaque index and bleeding on probing with matrica mouthwash being the most potent in the gingival index and plaque index reduction. In addition, both aloe vera-green tea and matrica mouthwash produced significantly lower stains on the teeth when compared to chlorhexidine.[39] A recent study by Kamolnarumeth *et al.* which studied the effect of chx mixed with hydrogen peroxide and compared to chlorhexidine alone showed that 0.2% chlorhexidine significantly produced more staining on the teeth and the side effects of burning sensation were more pronounced in the chx only group.[40] Both studies support the use of a different preparation or alternatives to reduce the teeth staining and help improve patient compliance.

Also, there were some limitations to the present study. Most of the recruited participants were mild gingivitis patients which may not reflect the general population that may have more severe forms of gingival diseases. Also, due to the time limits of the study, the washout period between two mouthwashes was reduced to seven days. Newcombe *et al.*[41] studied the efficacy of oral hygiene agents with concerns about the residual effect especially in chx mouthwash. The results showed that the residual effect of chx was different from other mouthwashes and suggested that a longer washout period, such as 10 days, is preferable. Previous studies that comparable mouthwashes, especially with chx, also used a washout period of 14 days or more. From this aspect, the shorter washout period in our study could affect the results and a longer washout period is mandatory in order to differentiate the effects of each mouthwash.

Furthermore, the compliance of the participants, one of the most critical parts of the study, were hard to control. The amount of the dispensed mouthwash and the duration that the participants used could also have an impact. In this study, the participants were instructed to use 15ml of mouthwashes for 30 seconds. Lang and Brecx[9] suggested that the use of 10ml of chx mouthrinse for 30-45 seconds were adequate for an optimal dose of 30 % of the applied chlorhexidine to be bound in the oral cavity. A recent review by James *et al.*[32] investigated 51 studies to evaluate the chx mouthrinse as an adjunctive treatment for gingival health and found that most studies used 10-15ml of the solution for 30 seconds. This suggests that the amount and duration of the mouthwash used in our study were adequate and in compliance with most of the previous studies.

To determine the compliance of the participants, they were instructed to bring their own bottle of mouthwash to determine whether they had been using the mouthwash regularly as instructed. Although the procedure may seem promising, complete compliance cannot be ascertained by this method. Moreover, the order of the mouthwash that participants received could affect their compliance. The group who received chx mouthwash as their first mouthwash may experience the burning sensation and unpleasant taste that might lead them to refrain from continuing to use the mouthwash as instructed. In contrast, the group that received *Ap* mouthwash first may have better compliance. From this aspect, the results obtained may be compromised

Conclusion

Within the limitations of the study, it can be concluded that *Ap* mouthwash can effectively reduce plaque accumulation with less staining deposition and burning sensation. The results support the use of *Ap* as an adjunctive to mechanical oral hygiene procedures and as an alternative to chlorhexidine for the antiplaque properties. Since the number of studies about *Ap* mouthwash are still limited, further research with a larger sample size is required in order to support the use of this valuable medicinal plant.

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References

1. Listgarten MA. The role of dental plaque in gingivitis and periodontitis. ***J Clin Periodontol*** 1988;15(8):485-7.

2. Trombelli L, Farina R, Silva CO, Tatakis DN. Plaque-induced gingivitis: Case definition and diagnostic considerations. ***J Clin Periodontol*** 2018;45(S20):S44-S67.

3. Charles CH, Mostler KM, Bartels LL, Mankodi SM. Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. ***J Clin Periodontol*** 2004;31(10):878-84.

4. Van Strydonck DA, Slot DE, Van der Velden U, Van der Weijden FJJocp. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. ***J Clin Periodontol*** 2012;39(11):1042-55.

5. Sreenivasan PK, Prasad KVV. Effects of a chlorhexidine mouthwash on clinical parameters of gingivitis, dental plaque and oral polymorphonuclear leukocytes [PMN]. ***Contemp Clin Trials Commun*** 2020;19:100473.

6. Richards D. Chlorhexidine mouthwash plaque levels and gingival health. ***Evid Based Dent*** 2017;18(2):37-8.

7. Osso D, Kanani N. Antiseptic mouth rinses: an update on comparative effectiveness, risks and recommendations. ***J Dent Hyg*** 2013;87(1):10-8.

8. Löe H, Schiott CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. ***J Periodontal Res*** 1970;5(2):79-83.

9. Lang N, Brecx MC. Chlorhexidine digluconate–an agent for chemical plaque control and prevention of gingival inflammation. ***J Periodontal Res*** 1986;21:74-89.

10. Bas K, Yilmaz F. A Rare Complication of Chlorhexidine: Buccal Mucosal Burn. ***Indian J Surg*** 2020;82(6):1250-1.

11. Flotra L, Gjermo P, Rolla G, Waerhaug J. Side effects of chlorhexidine mouth washes. ***Scand J Dent Res*** 1971;79(2):119-25.

12. Gurgan CA, Zaim E, Bakirsoy I, Soykan E. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: a double-blind clinical study. ***J Periodontol*** 2006;77(3):370-84.

13. Saima S, Ahmad R. Efficacy of chlorhexidine vs herbal mouthwash in college students: A comparative study. ***Int J Appl Dent Sci*** 2019;5:403-6.

14. Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of Streptococcus mutans. ***J Ethnopharmacol*** 2004;92(2-3):281-9.

15. Hekmatpou D, Mehrabi F, Rahzani K, Aminiyan A. The Effect of Aloe Vera Clinical Trials on Prevention and Healing of Skin Wound: A Systematic Review. ***Iran J Med Sci*** 2019;44(1):1-9.

16. Gupta P, kaur Bagga S. Efficacy of Chlorhexidine v/s Herbal mouthwash mouth wash in college students: A comparative study. ***J Adv Med Dent Scie Res*** 2018;6(7):78-81.

17. Aspalli S, Shetty VS, Devarathnamma MV, Nagappa G, Archana D, Parab P. Evaluation of antiplaque and antigingivitis effect of herbal mouthwash in treatment of plaque induced gingivitis: A randomized, clinical trial. ***J Indian Soc Periodontol*** 2014;18(1):48-52.

18. Kumar S, Patel S, Tadakamadla J, Tibdewal H, Duraiswamy P, Kulkarni S. Effectiveness of a mouthrinse containing active ingredients in addition to chlorhexidine and triclosan compared

with chlorhexidine and triclosan rinses on plaque, gingivitis, supragingival calculus and extrinsic staining. ***Int J Dent Hyg*** 2013;11(1):35-40.
19. Hsieh MJ, Chen JC, Yang WE, Chien SY, Chen MK, Lo YS, *et al.* Dehydroandrographolide inhibits oral cancer cell migration and invasion through NF-κB-, AP-1-, and SP-1-modulated matrix metalloproteinase-2 inhibition. ***Biochem Pharmacol*** 2017;130:10-20.
20. Amornchat C KP, Kraivaphan V, Triratana T. The antibacterial activity of Andrographis paniculata crude extracts on oral bacteria. ***J Dent Assoc Thai*** 1991;414:177-84.
21. Boonchaipanichwatana P. The comparative clinical and microbiological effects of Andrographis paniculata gel and minocycline ointment as an adjunct in the treatment of early onset periodontitis: Thesis, Mahidol University; 2001.
22. Thawornrungroj S, Kuphasuk Y, Petmitr S, Srisatjaluk R, Kitkumthorn N. The application of Andrographis paniculata gel as an adjunct in the treatment of chronic periodontitis: clinical and microbiological effects. ***NUJST*** 2014;19(2):38-49.
23. Teparat-Burana T, Samarnsotthiwong C, Kasetsuwan J, Sirirat M, Rojanapanthu P. The efficacy of subgingivally delivered Andrographis paniculata gel to treat patients with periodontitis. ***DENT*** 2015;2015:1-14.
24. Kuphasuk Y, Prommas C. The comparative clinical evaluation of subgingivally delivered andrographis paniculata gel and 10% doxycycline gel as an adjunct to treatment of chronic periodontitis in patients involved on supportive periodontal therapy. ***M Dent J*** 2020;40(2):69-76.
25. Thaosridee S, Temrungsee T, Saksricharoen K, Sintuchai N, Plianrungsi J, and Kulthanaamondhita P, editors. Comparing the effect of Andrographis paniculata and chlorhexidine mouthwash on anti-gingivitis properties. RSU International Research Conference 2021 on Science and Technology; 2021: Rangsit University.
26. Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. ***Biometrics*** 1977;33(1):159-74.
27. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. ***Acta Odontol Scand*** 1964;22(1):121-35.
28. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. ***Acta Odontol Scand*** 1963;21(6):533-51.
29. Macpherson LMD, Stephen KW, Joiner A, Schäfer F, Huntington E. Comparison of a conventional and modified tooth stain index. ***J Clin Periodontol*** 2000;27(11):854-9.
30. Sirintawat N, Sawang K, Chaiyasamut T, Wongsirichat N. Pain measurement in oral and maxillofacial surgery. ***J Dent Anesth Pain Med*** 2017;17(4):253-63.
31. Herrera D. Chlorhexidine mouthwash reduces plaque and gingivitis. ***Evid Based Dent*** 2013;14(1):17-8.
32. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, *et al.* Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. ***Cochrane Database Syst Rev*** 2017;3(3):1-194.
33. Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and Clinical Pharmacology of Andrographis paniculata and Its Major Bioactive Phytoconstituent Andrographolide. ***Evid Based Complement Alternat Med*** 2013;2013:846740-.
34. Dai Y, Chen S-R, Chai L, Zhao J, Wang Y, Wang Y. Overview of pharmacological activities of Andrographis paniculata and its major compound andrographolide. ***Crit Rev Food Sci Nutr*** 2019;59(sup1):S17-S29.
35. Hase JC, Attstrom R, Edwardsson S, Kelty E, Kisch J. 6-month use of 0.2% delmopinol hydrochloride in comparison with 0.2% chlorhexidine digluconate and placebo. (I). Effect on plaque formation and gingivitis. ***J Clin Periodontol*** 1998;25(9):746-53.
36. Lorenz K, Bruhn G, Heumann C, Netuschil L, Brecx M, Hoffmann T. Effect of two new chlorhexidine mouthrinses on the development of dental plaque, gingivitis, and discolouration. A randomized, investigator-blind, placebo-controlled, 3-week experimental gingivitis study. ***J Clin Periodontol*** 2006;33(8):561-7.
37. Charles C, Mostler K, Bartels L, Mankodi SJJocp. Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6—month clinical trial. ***J Clin Periodontol*** 2004;31(10):878-84.
38. Priya BM, Anitha V, Shanmugam M, Ashwath B, Sylva SD, Vigneshwari SK. Efficacy of chlorhexidine and green tea mouthwashes in the management of dental plaque-induced gingivitis: A comparative clinical study. ***Contemp Clin Dent*** 2015;6(4):505-9.
39. 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. ***Open Dent J*** 2019;13(1):214-20.
40. Kamolnarumeth K, Thussananutiyakul J, Lertchwalitanon P, Rungtanakiat P, Mathurasai W, Sooampon S, *et al.* Effect of mixed chlorhexidine and hydrogen peroxide mouthrinses on developing plaque and stain in gingivitis patients: a randomized clinical trial. ***Clin Oral Invest*** 2021;25(4):1697-704.
41. Newcombe RG, Addy M, McKeown S. Residual effect of chlorhexidine gluconate in 4-day plaque regrowth crossover trials, and its implications for study design. ***J Periodontal Res*** 1995; 30(5):319-24.