Comparing the effect of *Andrographis paniculata* and chlorhexidine mouthwash on anti-gingivitis properties

Siriem Thaosridee, Kornpawat Saksricharoen, Nichapa Sintuchai, Tharin Temrungsee, Jananya Plianrungsi and Promphakkon Kulthanaamondhita*

College of Dental Medicine, Rangsit University, Pathum Thani, Thailand
*Corresponding author, E-mail: promphakkon@gmail.com

Abstract

Gingivitis is one of the most common oral diseases that can occur in every individual. Normally, mechanical and chemical plaque controls are used to remove the plaque. Chemical plaque control like mouthwash is widely used for adjunctive therapy. Chlorhexidine mouthwash is the gold standard mouthwash used in aiding the treatment of periodontitis but it still has undesirable side effects. *Andrographis paniculata* mouthwash becomes an alternative due to its properties. The objective of this study is to compare the gingivitis control effects of *Andrographis paniculata* with a commercially available chlorhexidine gluconate mouthwash. Forty healthy subjects aged 18-30 years old were randomly divided into two groups of 20 patients each; Group A Chlorhexidine mouthwash and Group B Andrographis paniculata mouthwash. All subjects received oral hygiene instructions, scaling, and prophylaxis. The collected data comprised gingival index (GI) at baseline and after using the designated mouthwash for 3 weeks. The results had shown that the baseline data are not significantly different from each other. Both types of mouthwash can reduce the GI but not significantly different from the baseline and each other. In conclusion, *Andrographis paniculata* can be used as an adjunctive to mechanical oral hygiene procedures and as an alternative to chlorhexidine, especially in patients whose self-performed oral hygiene may be limited.

Keywords**:** *gingivitis, gingival index, Andrographis paniculata, chlorhexidine gluconate*

1. Introduction

Dental plaque removal is an important issue in oral health promotion. Dental plaque is a mass of bacteria that starts accumulating on the surface of a tooth as a sticky biofilm. Plaque deposition brings about inflammatory changes on the periodontium that can lead to the destruction of tissues and loss of attachment. If adequate control measures are undertaken, gradual build-ups of plaque over time will lead to tooth decay and gingival diseases (Sharma, 2010).

Gingivitis is one of the most common oral diseases that can occur in every individual. Normally, mechanical and chemical plaque controls are used to remove the plaque. Chemical plaque control like mouthwash is widely used for adjunctive therapy. Chlorhexidine mouthwash is the gold standard mouthwash used in aiding treatment of periodontitis but it still has side effects such as taste alteration, excess formation of supragingival calculus, soft-tissue lesions in young patients, allergic responses, and staining of teeth and soft tissues (Bas & Yilmaz, 2020; Flötra et al., 1971; Gurgan et al., 2006; Sreenivasan & Prasad, 2020).

Today people pay more attention to natural products, including herbal mouthwash, due to their low toxicity, ease of availability, and lack of microbial resistance of herbal agents. Bamboo, Triphala, and pomegranate mouthwash have been studied for a long time, and the advantages of these herbs are verified by many pieces of evidence (Aspalli et al., 2014; Limsong et al., 2004; Saima & Ahmad, 2019). *Andrographis paniculata* is an alternative due to its properties such as treatment of various diseases; cancer, diabetes, high blood pressure, ulcer, leprosy, bronchitis, skin diseases, flatulence, colic, influenza, dysentery, dyspepsia, and malaria for centuries in Asia, America and Africa continents (Kumar et al., 2013). It has also been used for the treatment of oral diseases such as oral cancer which dehydroandrographolide of *Andrographis paniculata* inhibits cell migration and invasion of cancer cells and enhances the healing of recurrent aphthous stomatitis lesion (Hsieh et al., 2017). The antimicrobial activity of this herb against oral bacteria; *Streptococcus mutans* and *Porphyromonas gingivalis*, has been reported by using four sequential extraction fractions, namely, hexane, methylene chloride, ethanol 95%, and water, through the agar diffusion method. The hexane and

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methylene chloride fraction showed inhibitory activity against *Streptococcus mutans*, while the ethanol fraction exhibited inhibitory activity against both bacteria. The mechanism responsible for this antimicrobial activity is still unclear but the active ingredient, ehydroandrographolide, may play an important role (Amornchat et al., 1991).

However, existing evidence in support of mouthwash formulation containing *Andrographis paniculata* as a single active ingredient against plaque and gingivitis is still limited, and so far studies that investigated the effectiveness of *Andrographis paniculata* had many methodological limitations. Therefore, this study aimed to compare the gingivitis control effect of *Andrographis paniculata* with a commercially available chlorhexidine gluconate mouthwash.

2. Objectives

To compare the anti-gingivitis effect between *Andrographis paniculata* and chlorhexidine mouthwash.

3. Materials and Methods

The study recruited 40 subjects from dental students of the College of Dental Medicine, Rangsit University, aged between 18-30 years old. The study was approved by the Ethics Committee of Rangsit University, and the subjects were included in the study after signing the informed consent form and voluntarily agreeing to participate in this project. Two calibrated examiners held all of the examinations and were trained and well-calibrated. Intra-examiner and inter-examiner reliability were tested by Kappa analysis.

In the 1st visit, all subjects received oral prophylaxis by Gracey curettes and sickle scaler 1 week before the start of the study by the operators who were approved by the instructor at the Oral Diagnosis Clinic, College of Dental Medicine, Rangsit University. Oral hygiene instructions for the modified Bass brushing technique were given. The subjects received the same dental hygiene set comprising a toothbrush (soft-bristled brushes) and a tube of toothpaste (Colgate®) to use until the end of the study. The subjects were told to rinse with normal saline solution during this period.

In the 2nd visit, 1 week after the 1st visit, the subjects were recalled for the first measurement by the same examiners. Clinical examination comprising of the gingival index (GI) was performed. The subjects were randomly divided into two groups; the control group (chlorhexidine mouthwash) and the test group (*Andrographis paniculata)* mouthwash. All of the mouthwash was packed in a similar color of bottles but labeled differently, after that they were randomly administered to the subjects by the operator. The subjects were also unaware of which mouthwash they have been administered. They were asked to rinse with 15 ml of mouthwash for 30 seconds after toothbrushing in the morning and before bedtime.

In the 3rd visit, 3 weeks after the 2nd visit, the subjects were recalled for the second measurement by the same examiners. Clinical examination comprising of the gingival index (GI) was performed. Oral prophylaxis by Gracey curettes and sickle scaler was done.

The statistical software SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The normal distribution of the clinical measurements was assessed using the Shapiro-Wilk test. For the clinical measurements, the descriptive analysis of the gingival index is presented as mean ± SD and median (min, max) to evaluate the efficacy of two types of mouthwash. The differences within each group of GI scores were assessed by the Wilcoxon signed-rank test. The mean GI scores between the groups were compared using the Mann-Whitney U test. Significant differences were defined as $p<0.05$.

4. Results and Discussion

This study was approved by the Research Ethics Office of Rangsit University (RSUERB2020-015), and all the participants signed the informed consent form before starting the experiment. Of the 40 participants, 6 were excluded from the study due to a loss of follow-up. Thus, 34 participants completed the study; 17 participants in the chlorhexidine group and 17 in the Andrographis paniculate group.

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The subject's demographics in the two groups were not significantly different (Table 1). The mean age of the subject was 22.09 ± 2.24 years old. There were more females than males in both groups (Female: 67.65% and male 22.35%).

Table 1 Subject's demographic data

Characteristics	**Control group N (%)**	**Test group N (%)**	**p value**
Number of participants	17 (100%)	17 (100%)	
Age: mean ± S.D.	21.71±1.51	22.47±2.76	0.162
Sex			
Female	12 (70.5%)	11 (64.7%)	0.604
Male	5 (29.5%)	6 (35.3%)	

The baseline (GI1) was not significantly different between the Chlorhexidine and *Andrographis paniculata* mouthwash groups (GI1= 1.15 vs. 1.15). After finishing the experiment, the gingival index (GI2) was not significantly different between the Chlorhexidine and *Andrographis paniculata* mouthwash groups (GI2 = 1.12 vs. 1.09). Both types of mouthwash slightly decreased the gingival index but not significantly from the baseline ($p<0.05$) (Table 2 and Figure 1).

Table 2 Descriptive data and comparison of the gingival index (GI) between the Chlorhexidine and *Andrographis paniculata* mouthwash

Clinical parameter	**Chlorhexidine (control)**		**Andrographis paniculata (test)**		**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

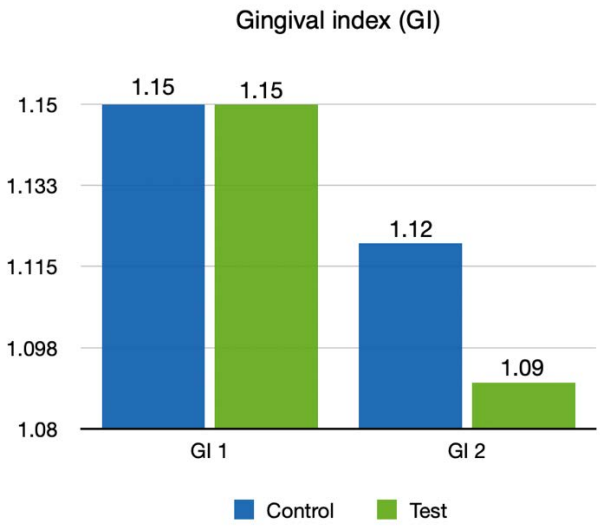

Figure 1 Comparison of the gingival index (GI) between the Chlorhexidine (test) and *Andrographis paniculata* (control) mouthwash

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This study was a double-blind, randomized two-group experiment that evaluated the effect of *Andrographis paniculata* compared with chlorhexidine on the development of gingivitis. After the experiments, the gingival index that indicates the status of the gingival health revealed no significant differences from the baseline in both groups. Previous studies have shown that chlorhexidine can improve the gingival index towards better gingival health when using concurrently with mechanical plaque control (Charles et al., 2004; Herrera, 2013; James et al., 2017; Lang & Brecx, 1986; Löe & Schiott, 1970; Van Strydonck et al., 2012).

Thawonrungroj et al. found that subgingivally delivered *Andrographis paniculata* gel was able to significantly reduce the gingival index in a chronic periodontitis patient with mostly moderate gingival inflammation when compared with scaling and root planning alone (Thawornrungroj et al., 2014). A recent study by Kuphasuk and Prommas who studied the effects of subgingivally delivered *Andrographis paniculata* gel during a supportive periodontal therapy also supports the ability of *Andrographis paniculata* in improving gingival health in a chronic periodontitis patient as shown by a reduction of the gingival index (Kuphasuk & Prommas, 2020).

In comparison to this study, *Andrographis paniculata* mouthwash did not significantly reduce the gingival index when compared with the baseline, which might be due to different types of gingival diseases, different preparations, delivery methods used in the study, and the initial status of the subject's gingival health that, in this study, were quite assorted to mild with slight moderate gingival inflammation. Most of the subjects were presented with very mild gingival inflammation that may not show further improvement after the mouthwash was used. Further research with a larger sample size with more severe gingival inflammation may be required to differentiate the effects of chemical plaque control from normal oral hygiene practice procedures.

Moreover, concentration and preparations of *Andrographis paniculata* may also affect the outcome of the study. *Andrographis paniculata* gel most widely used in the previous study contains 0.5625 mg/mL of *Andrographis paniculata* extract (Hamasakwattanakul, 2004; Kuphasuk & Prommas, 2020). *Andrographis paniculata* mouthwash commercially available also contains 0.6 mg/mL, which is similar to the gel preparation. However, the preparation of the gel form may provide better retention of the substance in the target area compared with mouthwash. The use of mouthwash also required the patient's compliances while the gel preparation can only be used by the professional applications and apply during the recall intervals. These differences may account for the different results obtained from this study.

The present study also has some limitations. Most of the recruited subjects were mild gingivitis patients, which may not reflect the general population that may have more severe forms of gingival diseases. Furthermore, the subject's compliances, one of the most critical parts of the study, are hard to control. The subjects were instructed to bring their bottle of mouthwash to determine whether the subject has been using the mouthwash regularly as instructed. Although the procedure may seem promising, yet complete compliance cannot be ascertained by this method.

5. Conclusion

This study has shown that *Andrographis paniculata* mouthwash can reduce the gingival index, the gingival parameter that indicates gingival health. The results support the use of *Andrographis paniculata* as an adjunctive to mechanical oral hygiene procedures and as an alternative to chlorhexidine for the antiplaque properties, especially in patients whose self-performed oral hygiene is limited. Since the number of the study about *Andrographis paniculata* mouthwash are still limited, further research with larger sample size is required to support the use of this valuable medicinal plant.

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