Original Article

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Evaluation of mouthwash containing anthocyanin extract for the control of dental plaque formation and gingivitis in orthodontic patients

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Objective: To evaluate the effect of mouthwash containing anthocyanin extract for controlling dental plaque formation and gingivitis in orthodontic patients.

Materials and Methods: Patients treated with orthodontic fixed appliance were assigned to use anthocyanin mouthwash (n=7) and sterile water (n=7). Initially, all of the participants received a prophylaxis and instructions on how to brush and floss. Measurements were recorded for Plaque indices (PI) and Gingival indices (GI) before and after intervention. Mean PI and GI scores were compared statistically between the groups and within groups using independent t-test and paired t-test. The significance level was set at $p<0.05$.

Results: The PI and GI scores after receiving the intervention in the anthocyanin mouthwash group were significantly lower compared to the control group ($p<0.05$). Within the group, PI and GI scores for the anthocyanin mouthwash group significantly reduced ($p<0.05$) after using mouthwash.

Conclusion: This study shows that the use of anthocyanin mouthwash can reduce the amount of plaque and gingivitis in patients undergoing orthodontic treatment.

Keywords: anthocyanins, gingivitis, mouthwash, orthodontics, plaque

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Introduction

Orthodontic patients treated with a fixed orthodontics appliance will have a change in oral condition by a bracket attached to the tooth surface. After the patient was treated with fixed appliances, it was discovered that the plaque increased, causing gingival inflammation and leading to gingivitis [1]. Furthermore, orthodontic appliances greatly reduce the effectiveness of natural oral cleansing forces as well as mechanical biofilm removal by toothbrushing [2].

In addition to brushing and flossing, using additional mouthwash can help reduce the build-up of microbial plaque and early stage of dental caries in orthodontic patients [3]. There are several types of mouthwashes used in dentistry containing various active agents including chlorhexidine, essential oils, Triclosan, Cetylpyridinium chloride, and herbs that have been reported to be effective in reducing dental biofilm formation and gingivitis [4]. Among these agents, Chlorhexidine is the most studied and effective antiseptic for plaque inhibition and prevention of gingivitis [5]. However, because of

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its adverse effects after continuous use such as staining of teeth and tongue, altered taste sensation, and desquamation of oral mucosa, it should not be indicated for long-term periods [6]. Hence, finding newer antiplaque and antimicrobial agents with a natural origin such as plant extracts is important to reduce adverse effects upon long-term application.

Black rice is a local plant widely found in Thailand. The essential oil extracted from the black rice is anthocyanins. Anthocyanins are flavonoids found in many fruits, vegetables, and flowers that give them a vibrant red to blue colour. It has the benefits of anti-inflammatory, antimicrobial, anticarcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties [7]. In dentistry, it was found that anthocyanin extract was able to resist apoptosis of the gingival epithelium and reduce the incidence of dental caries in rat experiments [8, 9]. In the previous study, it was shown that anthocyanins can exhibit effective anti-inflammatory properties against 5-FU-induced oral mucositis by inhibiting NF-ϰB activation [10]. Moreover, anthocyanins can inhibit HSC-2 cell proliferation, and this may involve TGF-β1 downregulation. This could be useful for the treatment of patients with squamous carcinoma [11]. Anthocyanins are promising candidates for the engineering of new pharmaceutical drugs and can be used as an alternative or adjuvant therapy capable of preventing the occurrence of many disorders [12]. Many studies have examined the safety of flavonoids and failed to indicate any adverse effects or toxicity issues of anthocyanins [13]. Due to the many benefits and safety of anthocyanins, the anthocyanin mouthwash was developed.

The objective of this study is to evaluate the effect of mouthwash containing anthocyanin extract for controlling dental plaque formation and gingivitis in orthodontic patients.

Materials and Methods

Certificate of Approval for this study was obtained from the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COA.No.MU-DT/PY-IRB 2022/016.2903).

Subject

This prospective study included 14 patients who were undergoing treatment of fixed appliances in the Orthodontic Clinic at the Faculty of Dentistry, Mahidol University.

The inclusion criteria were 18-40 years of age, treatment with the fixed appliance (stainless steel bracket), healthy, and no systemic disease.

The exclusion criteria were patients with craniofacial deformity including cleft lip and palate, smoking, pregnancy, breastfeeding, allergy to mouthwash, xerostomia, taking NSAIDs, steroid, antibiotic, or anticancer agent, and patients with periodontitis.

Subjects who met the criteria were informed about the design and objective of the study and asked to sign the informed consent before entering the study. The participants were divided into an experimental group (Anthocyanin mouthwash) and a control group (Sterile water).

Procedure

At the beginning of the study, demographic information like age and sex of participants was collected. All the participants were given instructions on how to brush and floss and will be asked to stop using their regular mouthwash. Each participant will receive an initial prophylaxis with full-mouth scaling and polishing by the same dentist 2 weeks before they begin using mouthwash.

The anthocyanins were purified from Black rice (*Oryza sativa* L.) using a Hypersil Gold C18 column with a chromatogram at 530 nm. The chromatogram indicated the presence of Cyanidin-3-glucoside (C3G) and Pelargonidin-3-glucoside (P3G). The anthocyanins was extracted in ethanol and contained approximately 0.85 mg/g of C3G and 0.076 mg/g of P3G in the dry extract, as used in a previous study [10].

The participants were allocated into Group A and Group B with 7 participants in each group. The examiner and the participants were blinded with regard to the mouthwash allocated to them.

Two weeks later, all gingival examinations of the participants were carried out by the dentist, according to the study by Marsh PD and Bradshaw DJ [14]. The examined gingival indices were the plaque index (Modified Silness and Loe index)[15] and the gingival index (Silness and Loe index) [16]. Subjects in Group A and Group B will receive 1 liter of mouthwash in identical bottles. The participants were requested to rinse the mouthwash every morning and evening after tooth brushing about 15-20 ml, for 1 minute.

After a month, the examined gingival indices were collected. The participants were recalled along with the mouthwash bottles assigned to check for the volume used. The process of this study is shown in the workflow (Figure 1).

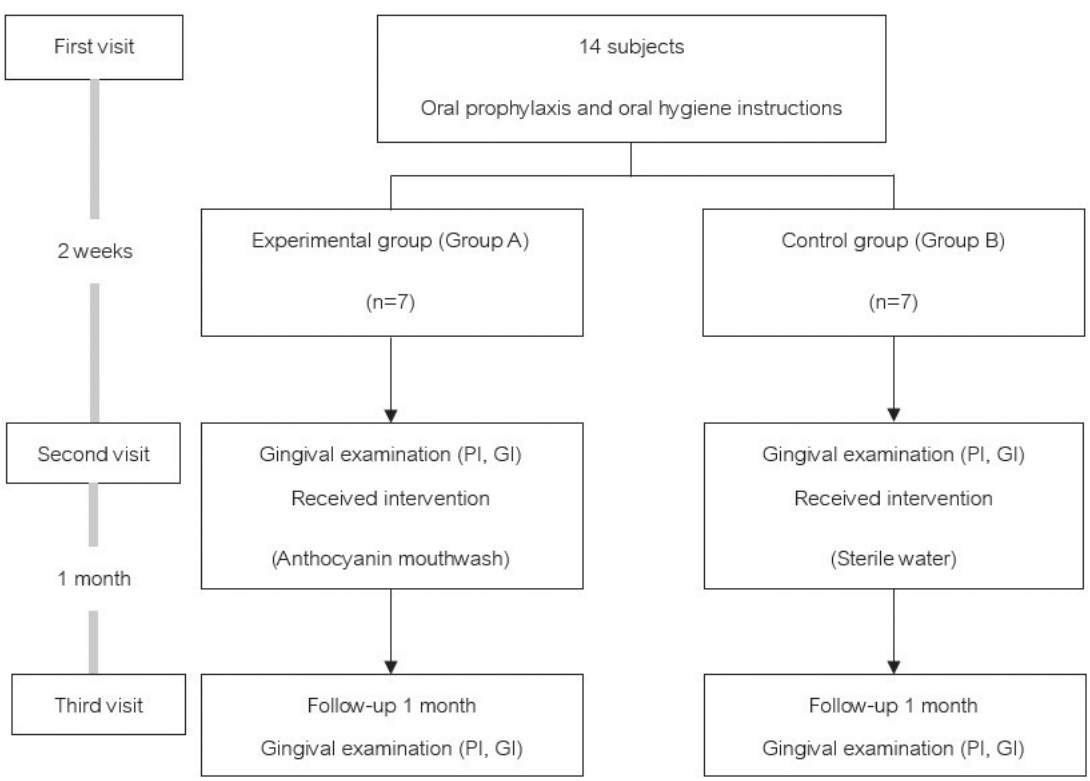

Figure 1 Workflow of the study process

Methods for gingival examinations

The Plaque index (Modified Silness and Loe index)

The location as related to the four areas around the bracket. (gingival, mesial, distal, incisor) Each of the four areas of the tooth is given a score from 0 to 3. Selected teeth have been used to represent the entire dentition. Plaque indices were obtained from six teeth (tooth 16, tooth 12, tooth 25, tooth 36, tooth 32 and tooth 44).

0 = No plaque in the gingival area.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface.

2 = Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin or adjacent tooth surface, which can be seen by the naked eye.

3 = Abundance of soft matter within the gingival pocket or on the gingival margin and adjacent tooth surface.

The scores from the four areas of the tooth may be added and divided by four to give the PI for the tooth. Finally, by adding the indices for the teeth and dividing by the total number of teeth examined.

Score 0.1-1.0 = Mild inflammation

Score 1.1-2.0 = Moderate inflammation

Score 2.1-3.0 = Severe inflammation

The Gingival Index (Silness and Loe index)

The location as related to the four areas. (buccal, mesial, distal, lingual) Each of the four gingival areas of the tooth is given a score from 0 to 3. Gingival indices were obtained from six teeth (tooth 16, tooth 12, tooth 25, tooth 36, tooth 32 and tooth 44).

0 = Normal gingiva

1 = Mild inflammation — slight change in color, slight edema. No bleeding on probing

2 = Moderate inflammation—redness, edema and glazing. Bleeding on probing

3 = Severe inflammation — marked redness and edema. Ulceration. Tendency to spontaneous bleeding.

The scores from the four areas of the tooth may be added and divided by four to give the GI for the tooth. Finally, by adding the indices for the teeth and dividing by the total number of teeth examined.

Score 0.1-1.0 = Mild inflammation

Score 1.1-2.0 = Moderate inflammation

Score 2.1-3.0 = Severe inflammation

Data analysis and outcome measurements

The effectiveness of mouthwash in reducing plaque and gingival inflammation was measured from plaque index and gingival index. The statistical software SPSS version 29.0.1 (IBM Corp., Armonk, NY, USA) was used for data analysis. The normal distributions of measurements were assessed using the Shapiro-Wilk test. The PI and GI scores were normally distributed, so the mean of PI and GI scores between groups were examined using the independent t-test, and the differences of the mean within group were assessed by the paired t-test. The significance level was set at $p<0.05$.

Results

Of the 14 participants, the anthocyanin mouthwash group included 7 subjects (mean age 25.42 years; age range 22 to 34 years). The control group included 7 subjects (mean age 24.71 years; age range 21 to 37 years). No significant differences were found among the groups (Table1).

Table 1 General characteristics

Characteristics	Experimental Group (n=7)	Control Group (n=7)	*p*-value
Age, years (mean ± SD)	25.42 ± 5.22	24.71 ± 5.85	0.436
Sex, n (%)			
Female	6 (85.71)	6 (85.71)	0.769
Male	1 (14.29)	1 (14.29)	
Number of remaining teeth, n (mean ± SD)	25.71 ± 1.80	26.29 ± 2.93	0.668

Table 2 shows the mean of PI and GI scores before and after receiving anthocyanin mouthwash and sterile water. The baseline measurements (Pre) of PI and GI scores were not significantly different between the two groups ($p>0.05$). After receiving the intervention (Post), the PI and GI scores were significantly different between the two groups ($p<0.05$). Within the group, PI and GI scores for the experimental group reduced significantly ($p<0.05$) after using anthocyanin mouthwash, while the PI and GI scores in the control group were not significantly different.

Discussion

Orthodontic patients often experience plaque buildup and resulting gingivitis [1, 2]. During orthodontic therapy, the development of retentive surfaces around fixed appliances that are attached to teeth appears to be the cause of an increase in dental plaque and inflammatory reaction [17]. The main components of fixed appliances are able to reduce the physiological mechanism of self-cleaning by the tongue and cheeks [18]. The excess composite around the bracket base is the critical site for plaque accumulation due to its rough surface and the presence of a distinct gap at the composite-enamel interface [19]. Several findings revealed that the plaque index and gingival index values in the treatment groups were higher than the non-treatment group [20, 21] .

Anthocyanins have a wide range of health-promoting properties. They are members of the flavonoid group of phytochemicals that have been shown to have antimicrobial activity, antioxidative properties, and anti-inflammatory effects.

Table 2 Mean (SD), Plaque index (PI), and Gingival index (GI) scores for each group

	Experimental Group (n=7)	Control Group (n=7)	Significance between group (*p*-value)
PI scores	Pre 1.54 (0.37)	Pre 1.30 (0.24)	0.181
	Post 0.91 (0.29)†	Post 1.33 (0.34)	0.034*
GI scores	Pre 1.49 (0.17)	Pre 1.49 (0.15)	0.935
	Post 0.99 (0.22)†	Post 1.42 (0.16)	0.001**

Statistically significant difference within group, † $p<0.05$

Statistically significant difference between groups, *$p<0.05$, **$p<0.01$

Anthocyanins exhibit antimicrobial effects attributed to their ability to interact with DNA, proteins, and sulfhydryl groups, disrupting vital microbial activities. By interfering with AKT, ATPase, and superoxide dismutase activities, anthocyanins repress the citric acid cycle, microbial metabolism, and enzyme function, resulting in impeding microbial development, replication, and biofilm formation [12]. Anthocyanins exhibit anti-inflammatory properties by suppressing NF-ΚB activation in monocytes and reducing the plasma concentration of pro-inflammatory mediators [10]. Previous studies have indicated that anthocyanins can promote the migration of rat dermal fibroblasts and possess antioxidant properties [22]. Additionally, anthocyanins enhance the mRNA expression of collagen type I alpha 2 and upregulate type I collagen protein levels in stimulated rat dermal fibroblasts without inducing cytotoxicity [22]. Extensive research and human studies have failed to report any adverse effects associated with anthocyanins [13]. This safety record of anthocyanins makes it a valuable natural compound with many health benefits.

This study evaluated the effect of anthocyanin mouthwash in orthodontic patients. The results revealed that after 1 month, anthocyanin mouthwash significantly reduced the plaque accumulation comparable to sterile water. In the anthocyanins group, the plaque and gingival indices were reduced from moderate to mild levels of inflammation. The results were in agreement with previous studies demonstrating the effectiveness of the grape seeds mouthwash containing phenolic compounds like anthocyanins in controlling plaque and gingivitis [23].

Previous studies had investigated the possible antimicrobial activity of anthocyanins. It has been shown in several studies to limit the growth of biofilms in the oral cavity. The anthocyanins has the potential to prevent the growth of S. mutans biofilm on the occlusal surface of human teeth [24]. Compared to our study, previous research focused on the antimicrobial activity of anthocyanins, particularly in the context of Streptococcus mutans biofilm formation on extracted natural teeth. While our study concentrated on the overall impact of anthocyanins-containing mouthwash on plaque and gingivitis in orthodontic patients, both studies found that anthocyanins can reduce biofilm formation, which has been proven to play a role in the etiology of dental caries and periodontal diseases.

Also, there are some limitations to the present study. Due to the small sample size, the results may not reflect the general population, and there are confounding factors between subjects. Additional studies should be conducted with a larger sample size and using a cross-over technique to eliminate the inter-subject factors. The use of negative-control mouthwash in comparison confirms that anthocyanin mouthwash can decrease plaque and gingivitis in orthodontic patients.

Conclusion

This study shows that the use of anthocyanin mouthwash can reduce the amount of plaque and gingivitis in patients undergoing orthodontic treatment. Since the number of subjects in this study is still limited, further study with a larger sample size is required in order to support the use of this mouthwash.

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