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ORIGINAL RESEARCH

Effectiveness of Duration in Rinsing 4% Basil Leaf Extract (*Ocimum africanum Lour.*) on Saliva pH of Fixed Orthodontic Users

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KEYWORDS

Salivary pH;
Basil Leaf Extract;
Ocimum;
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ABSTRACT

Fixed orthodontics may compromise the patient's oral health and reduce salivary pH, thereby increasing the risk of dental caries. One way of increasing pH of saliva is by using natural ingredients such as basil leaf as a mouthwash. This study aimed to determine the effectiveness of duration in rinsing 4% basil leaf extract (*Ocimum africanum* Lour.) on saliva pH of fixed orthodontic users. This study used a quasi-experimental pre test-post test control group design with 30 respondents from Diponegoro University's Faculty of Medicine. Saliva samples were obtained for pH measurements before and after rinsing. The samples were divided into 6 groups, namely the treatment group rinsing with 4% basil leaf extract solution and the control group rinsing with distilled water for a duration of 1 minute, 2 minutes, and 3 minutes. The results were analysed using One-Way ANOVA and Tukey HSD post hoc tests. One-Way ANOVA results showed a significant difference with a value of $p=0.000$ ($p<0.05$) in salivary pH between the treatment group and control group. LSD post hoc tests showed that the treatment group rinsing 4% basil leaf extract with a duration of 1 minutes did not significantly increase pH, but rinsing durations of 2 minutes and 3 minutes had effectively increased pH. The conclusion of this study is that rinsing with 4% basil leaf extract for 2 minutes or longer effectively increases salivary pH in users of fixed orthodontics to reach neutral to alkaline levels.

1. INTRODUCTION

Malocclusion is defined as a developmental condition presenting with deviation of the normal teeth alignment to other teeth within the same arch and/or opposing arch.[1] According to the World Health Organization (WHO), malocclusion is considered one of the most important oral health issues just behind caries and periodontal diseases.[2] A previous systematic review and meta-analysis evaluated the prevalence of malocclusion and revealed a worldwide prevalence of 56% with the highest occurrence in Africa (81%), followed by Europe (72%), America (53%), and Asia (48%).[3] Correction of malocclusion is achieved by orthodontic treatment through the use of dental appliances. Although severe cases of malocclusion require the use of fixed orthodontic appliances, these appliances may compromise the patient's oral health by increasing areas of plaque retention and hampering efforts to maintain oral hygiene. Accumulation of dental plaque can lead to alterations in the oral microbiota and reduction of pH, resulting in increased risk of developing carious lesions or periodontal disease.[4–6]

Saliva plays a significant role in maintaining oral health owing to its unique composition of electrolytes, immunoglobulins, proteins, enzymes, mucins, and nitrogenous substances. These components serve a multifunctional purpose such as providing lubrication in the aid of mastication, speech, and swallowing functions; acting as a protective barrier against irritants and microorganisms; buffering action through the neutralization of acids; maintaining tooth integrity by preserving the balance of remineralization and demineralization processes; as well as providing immune and non-immune antibacterial defences. Modulation of intraoral pH in particular is contingent on the buffering and cleansing action of saliva along with salivary flow rate.[7] However, these saliva parameters have been observed to undergo changes during the course of orthodontic treatment such as a decrease in salivary pH alongside increased concentrations of bacteria namely *Streptococcus mutans* and *Lactobacillus*.[8] A decrease in salivary pH can be alleviated through systemic stimulation via the sense of taste, such as mouth rinsing, as well as chemical stimulation through the taste produced.[9]

Chlorhexidine is one of the most commonly used antibacterial mouthwash for inhibiting plaque formation, yet it has been linked to numerous adverse effects that include xerostomia, burning pain, changes in the perception of taste, discoloration of the teeth, and detachment of the oral mucosa which limits it's long-term use in patients.[2] As a result, the use of herbal-derived

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mouthwashes are increasing in demand as an alternative. Commonly known species within the *Ocimum* genus are rich in essential oils and secondary metabolites that include terpenoids, phenolic compounds, coumarins, and flavonoids which have garnered much interest due to its broad applications in traditional medicine. One species in particular known as *Ocimum africanum* Lour. or lemon basil, exhibits antimicrobial, gastroprotective, antioxidant, anticarcinogenic and anticholinesterase activity. Despite these numerous pharmacological activities, *Ocimum africanum* Lour. is still underrepresented in research compared to its sibling species such as *O. sanctum* and *O. basilicum*.[10] The flavonoids contained in basil leaf give it an astringent and bitter taste when consumed which has potential to stimulate the secretion of salivary glands and affect the salivary pH. It's eugenol contents can also help decrease the degree of acidity within the mouth.[11] When compared with the antimicrobial action of synthetic mouthwashes, herbal based ones can have additional anti-inflammatory and antioxidant effects that benefit not just plaque control but also promote gingival health.[12]

A previous study by Agnes (2014) demonstrated the efficacy of ethanol extract of basil leaf at 4% concentration in increasing the pH of saliva.[13] A concentration of 4% basil leaf from ethanol extraction was also determined to have the highest inhibitory effect against *Streptococcus mutans*.[14] Good efficacy must also be balanced with good compliance and hence mouthwashes are generally recommended to rinse with for 30 seconds to 1 minute. Borrowing from the method of Hervina[15] (2017) in determining the optimal rinsing duration for increasing salivary pH using treatment groups of rinsing for 1 minute, 2 minutes, and 3 minutes, this study was conducted to determine the effectiveness of rinsing with 4% basil leaf extract (*Ocimum africanum* Lour.) on saliva pH at different durations so that it can be used in preventive efforts against dental and oral diseases, especially caries in fixed orthodontics users.

2. METHODOLOGY

This study was a quasi-experimental study with pretest-posttest non-equivalent control group design and multiple intervention duration to determine the effectiveness of the duration of rinsing with 4% basil leaf extract (*Ocimum africanum* Lour.) on saliva pH. The sample size for this study was calculated using Federer's formula, with the minimum number of samples in each group being 4 subjects and total sample size at least 24 subjects. In this study, 5 subjects were assigned for each group, therefore the total sample size needed was 30 subjects. The sampling method used was a purposive sampling technique according to the inclusion and exclusion criteria. The inclusion criteria for samples in this study were fixed orthodontic users at Diponegoro University's Faculty of Medicine, willing and cooperative, healthy, patients treated by orthodontic specialists, and duration of fixed orthodontic treatment at >12 months. The exclusion criteria were alcohol consumption, smoking, uncooperativeness, consumption of drugs (such as antihypertensives, antidiabetics, and antihistamines), a history of chronic diseases (such as diabetes mellitus, Sjögren's syndrome, Bell's palsy, and HIV), and oral diseases (such as dental caries, gingivitis, and periodontitis). The samples were non-randomly divided into 6 groups at the researcher's discretion, namely the treatment group rinsing with 4% basil leaf extract (*Ocimum africanum* Lour.) and the control group rinsing with distilled water for different rinsing durations of 1 minute, 2 minutes, and 3 minutes.

The basil leaf extraction process began with sun drying the basil leaves for 5 days and grinding them using a blender to obtain basil leaf in powdered form. The amount of powdered simplicia required for the study was 500 grams. The preparation of extract was carried out using the maceration method. The maceration method was done by soaking the powdered simplicia in 4000 mL of 96% ethanol solution within a maceration vessel for 3 days with one stirring. The resulting liquid ethanol extract was filtered using a paper filter at room temperature which was then evaporated using a vacuum rotary evaporator with a pressure setting of 140 mmHg and rotary speed of 30 rpm. Afterwards, the extract is concentrated using a water bath at 55°C and a chiller temperature at 10°C. The 4% basil leaf extract solution was obtained from a dilution process with a ratio of 6 mL of basil leaf extract and 144 mL of distilled water.

Collection of saliva sample was done using the spitting method by instructing respondents to let their saliva pool on the base of the mouth before spitting it out every 1 minute, then repeat for 10 minutes or until 10 mL of saliva was obtained. Respondents did not eat or drink for 60 minutes prior to the study. At the time of the study, respondents were instructed to consume half a slice of bread (Roti Sari Roti®) within 2 minutes and rest for 5 minutes. Saliva was collected from each treatment and control group before rinsing, then transferred to a saliva pot. pH measurement was done using a digital pH meter (Hanna®H198107). Calibration of the pH meter was done prior to the sampling by immersing the electrode in buffer solutions of 7,01 pH and subsequently 4,01 pH, followed by neutralisation with distilled water. For the treatment group, respondents were instructed to rinse with 4% basil leaf extract for a duration of 1 minute (P1), 2 minutes (P2), and 3 minutes (P3). For the control group, respondents were instructed to rinse with distilled water for 1 minute (P4), 2 minutes (P5), and 3 minutes (P6). Afterwards, the steps for collection and measurement of saliva pH were repeated.

The results were analysed using Statistical Package for the Social Sciences (SPSS) 27 software. Prior to parametric testing, the data is analysed to ensure it meets the assumptions of normality and homogeneity. Normality test was performed using Shapiro-Wilk test due to the sample size of less than 50 ($n < 50$) and homogeneity test was performed using Levene's test. Once these assumptions were met, One-Way ANOVA analysis was performed to determine whether or not there is a statistically significant difference between the group means. Furthermore, a Tukey HSD test was performed to determine which specific group means differ significantly. Ethical clearance of this research was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University with the registered letter number 504/EC/KEPK/FK-UNDIP/IX/2024.

3. RESULTS

Table 1 shows the mean values and standard deviation of pH measurement of saliva for fixed orthodontic users before and after rinsing with 4% basil leaf extract and distilled water. The mean delta (Δ) value was obtained from the difference in salivary pH before rinsing and after rinsing for each group. The highest increase of salivary pH was found in group P3 with a mean value of 0.72, followed by group P2 with a mean value of 0.26 and group P1 with a mean value of 0.24. The lowest increase of salivary pH was found in group P6 with a mean value of 0.06, followed by group P4 and P5 with a mean value of 0.08.

Group	N	Mean ± Standard Deviation		
		Pre-test	Post-test	Δ
P1 (4% Basil Leaf Extract 1 minute)	5	6.56±0.114	6.80±0.070	0.24
P2 (4% Basil Leaf Extract 2 minutes)	5	6.78±0.130	7.04±0.151	0.26
P3 (4% Basil Leaf Extract 3 minutes)	5	6.70±0.158	7.42±0.083	0.72
P4 (Aquades 1 minute)	5	6,62±0.130	6.70±0.122	0.08
P5 (Aquades 2 minutes)	5	6.68±0.130	6.76±0.114	0.08
P6 (Aquades 3 minutes)	5	6.60±0.158	6.66±0.167	0.06

Table 1. Mean and Standard Deviations of Pre-Test and Post-Test Salivary pH Measurements

Figure 1. Comparison of Pre-Test and Post-Test Salivary pH Measurements

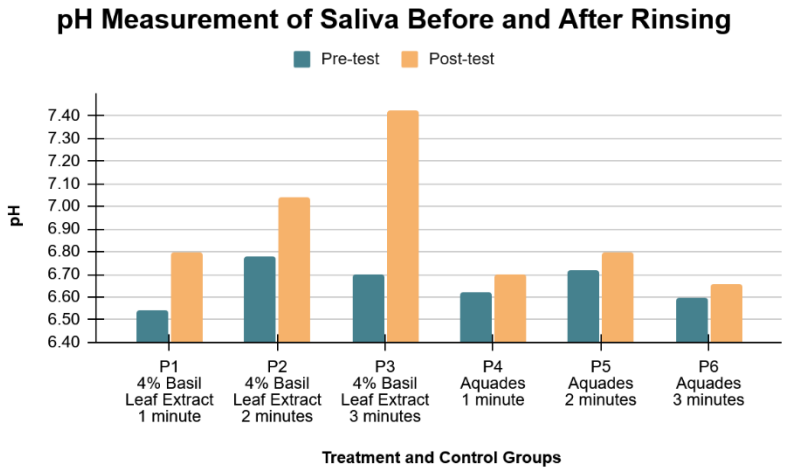

Table 2 shows the results of the Shapiro-Wilk normality test. The measured pre-test and post-test data for each group showed that the *p*-values are greater than 0.05, which indicates that the pH measurements are normally distributed ($p > 0.05$). Therefore, a test of homogeneity can be performed with Levene's test

Group		Shapiro-Wilk		
		Statistic	df	Sig.
P1 (4% Basil Leaf Extract 1 minute)	*Pre-test*	.961	5	.814
	Post-test	.883	5	.325
P2 (4% Basil Leaf Extract 2 minutes)	*Pre-test*	.902	5	.421
	Post-test	.914	5	.492

Group		Shapiro-Wilk		
		Statistic	df	Sig.
P3 (4% Basil Leaf Extract 3 minutes)	*Pre-test*	.987	5	.967
	Post-test	.881	5	.314
P4 (Aquades 1 minute)	*Pre-test*	.902	5	.421
	Post-test	.833	5	.146
P5 (Aquades 2 minutes)	*Pre-test*	.902	5	.421
	Post-test	.961	5	.814
P6 (Aquades 3 minutes)	*Pre-test*	.987	5	.967
	Post-test	.881	5	.314

Table 2. Results of Shapiro-Wilk Test for Normality of Pre-Test and Post-Test Salivary pH Measurements

Table 3 shows the results of Levene's test of homogeneity. Based on the results of the test, the *p*-values are greater than 0.05 in both the pre-test and post-test measurements, therefore the variances in pH of saliva for the pre-test and post-test measurements are equal ($p > 0.05$). Based on the results of the normality and homogeneity tests, all assumptions for calculating a One-Way ANOVA are met.

pH Saliva	Levene's Test			
	Statistic	df1	df2	Sig.
Pre-test	.157	5	24	.976
Post-test	.014	5	24	.314

Table 3. Results of Levene's Test for Homogeneity of Pre-Test and Post-Test Salivary pH Measurements

Table 4 shows the results of One-Way ANOVA analysis at a confidence interval of 95% ($\alpha = 0.05$). Based on the results of the analysis, there is a significant difference in the increase of salivary pH across the treatment and control groups, $F(5,24) = 27.503$, $p < .05$, $\eta^2 = .851$, indicating a large effect. Since the ANOVA result was significant, a post-hoc test was conducted using Tukey HSD test to examine pairwise differences among the groups and identify which groups differed significantly.

	pH Saliva	Sum of Squares	df	Mean Square	F.	Sig.
Pre-test	Between Groups	.158	5	.032	.1660	.183
	Within Groups	.456	24	.019		
	Total	.614	29			
Post-test	Between Groups	2.086	5	.417	27.503	<.001
	Within Groups	.364	24	.015		
	Total	2.450	29			

Table 4. Results of One-Way ANOVA for Pre-Test and Post-Test Salivary pH Measurements

Group	P1 (4% Basil Leaf Extract 1 minute)	P2 (4% Basil Leaf Extract 2 minutes)	P3 (4% Basil Leaf Extract 3 minutes)	P4 (Aquades 1 minute)	P5 (Aquades 2 minutes)	P6 (Aquades 3 minutes)
P1 (4% Basil Leaf Extract 1 minute)	-	.051	.000*	.791	.995	.486
P2 (4% Basil Leaf Extract 2 minutes)	.051	-	.000*	.003*	.016*	.000*
P3 (4% Basil Leaf Extract 3 minutes)	.000*	.000*	-	.000*	.000*	.000*
P4 (Aquades 1 minute)	.791	.003*	.000*	-	.970	.995
P5 (Aquades 2 minutes)	.995	.016*	.000*	.970	-	.791
P6 (Aquades 3 minutes)	.486	.000*	.000*	.995	.791	-

Note: *The mean difference is significant at the 0.05 level

Table 5. Results of Tukey's Post Hoc Test for Post-Test Salivary pH Measurements

Table 5 shows the results of Tukey's HSD test at a confidence interval of 95% ($\alpha = 0.05$). Post hoc comparisons demonstrated that salivary pH increase was significantly higher in participants that rinsed with 4% basil leaf extract for 2 minutes and 3 minutes when compared to the control groups that rinsed with distilled water for 1 minute, 2 minutes, and 3 minutes. However, there was a marginally significant difference between 4% basil leaf extract 1 minute group and the 4% basil leaf extract 2 minutes group with a p-value of .051 ($p > 0.05$). There was also a significant difference between the 4% basil leaf extract 1 minute group and the 4% basil leaf extract 3 minutes group. There were no significant differences between the 4% basil leaf extract 1 minute group and the control that rinsed with distilled water for 1 minute, 2 minutes, and 3 minutes. When taken into account, these results suggest that 4% basil leaf extract does have an effect on the salivary pH and that longer durations of rinsing would increase pH levels even more. However, it should be noted that rinsing with 4% basil leaf extract for 1 minute does not appear to significantly increase the pH of saliva.

4. DISCUSSION

The use of fixed orthodontic appliances can often cause a decrease in saliva pH because fixed orthodontic appliances have a complex design that bacteria adhere to and grow in the oral cavity as well as reduce the oral cavity's self-cleaning function, thereby decrease in saliva pH can increase the risk of demineralization of teeth and inflammation of the gums.[16] Previous studies have also documented changes in the properties of saliva and oral microbiota that occur following fixed orthodontic treatment. [17–19] Observed changes in the saliva include an increase in buffering capacity and salivary flow rate alongside a decrease in saliva pH.[17] Pathogenic microbiota such as *Streptococcus mutans* and *Lactobacillus acidophilus* are shown to increase in count following weeks after orthodontic treatment, whereas commensal microbes such as anaerobic bacteria (*Centipeda*, *Anaeroglobus*, *Actinomyces*, *Veillonella*) and *Candida albicans* show a notable rise and may become pathogenic due to an imbalance in the oral microbiota (dysbiosis).[18,19] Based on the pre-test measurements of saliva pH, it was found that the saliva pH of fixed orthodontic users in this study tended to fall into the acidic to neutral category. These findings are in line with those of prior investigations by Rusip et al. (2024) and Melati et al. (2024) that examined salivary pH of fixed orthodontic wires and fixed orthodontic appliance users with an average of 6.16 $\pm$ 0.735 pH and 5.85 $\pm$ 0.398 respectively.[20,21]

Maintaining pH balance of the saliva can be done by mechanical and/or chemical stimulation to increase the salivary flow rate. Mechanical stimulation via chewing motions induce activation of the parasympathetic nervous system, which in turn releases acetylcholine causing vasodilatation of the salivary gland ducts and stimulation of the salivary glands, mainly of the parotid glands.[22] Chemical stimulation via the sense of taste stimulates secretion of saliva from the parotid and submandibular glands in considerable amounts.[23] Salivary flow rate can be increased by rinsing with natural ingredients, one of which is basil leaf extract. Based on the results of the post-test measurements, it can be seen that rinsing with basil leaf extract can significantly increase saliva pH compared to rinsing with distilled water, resulting in neutral to alkaline conditions that prevent tooth decay. This is because distilled water does not contain any active chemicals, whereas basil leaves contain key compounds that have the ability to neutralize saliva pH by increasing the production of saliva. Phytochemical analysis of basil leaves

revealed compounds such as alkaloids, anthocyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolics, saponins, quinone, steroids, terpenoids, and tannins that are present in various parts of the plant.[24] The flavonoid content in basil leaf gives it a bitter, astringent, and unique taste that chemically stimulates the salivary glands to secrete more saliva. Good saliva production helps neutralize acidity in saliva. Tannin compounds can reduce bacterial adhesion to the tooth surface by suppressing the activity of the enzyme glucosyltransferase (GTF) produced by *Streptococcus mutans*.[13,25] This is reinforced by the antibacterial properties of basil leaves, which contain apigenin, a cariostatic substance that can prevent acid-producing bacteria by inhibiting enzyme activity.[25]

Based on the results of the 4% basil leaf extract group, it showed that rinsing with 4% basil leaf extract for 1 minute did not have a significant effect on saliva pH, but rinsing with 4% basil leaf extract for 2 minutes and 3 minutes produced a significant increase of pH. These results indicate the need for higher concentration of basil leaf extract to produce more significant results at shorter durations of rinsing. A longer rinsing duration will have an impact on increasing saliva pH, as evidenced by the most significant increase in pH after rinsing with 4% basil leaf extract for 3 minutes. According to Indriana (2011), the type and intensity of the stimulus will determine each component within it as well as the quantity of saliva produced. Saliva pH is more easily increased by chemical stimuli than mechanical stimuli, and the longer the stimulus is given, the greater the increase in saliva pH.[26]

Although this study has established the effective durations of rinsing with 4% basil leaf extract to increase salivary pH in fixed orthodontics users, the evidence of this study should be viewed considering the limitations of the study. All the samples selected in this study were fixed orthodontic users from Diponegoro University's Faculty of Medicine and thus the results cannot be generalized to other populations. Sampling of the saliva did not include measurements of the baseline unstimulated salivary due to limitations of the pH meter used requiring larger amounts of saliva to properly measure the salivary pH. All of the samples were taken in the afternoon which may affect the composition of saliva due to changes following the circadian rhythm, therefore usage of a repeated-measures design should reduce the individual variability of measured saliva pH after each treatment. Despite showing a large effect size, the statistical power of this study may be affected by its small sample size and requires larger sample sizes to ensure a more representative distribution of the population.

5. CONCLUSION

Mouth rinsing with 4% basil leaf extract (Ocimum africanum Lour.) for durations of 2 minutes or longer is effective in increasing saliva pH in fixed orthodontic users compared to rinsing with distilled water for durations of 1 minute, 2 minutes, and minutes. Rinsing with 4% basil leaf extract for 1 minute did not yield a significant increase of saliva pH, therefore more research is needed on testing of higher concentrations of extract with larger sample sizes to obtain more significant results of that are comparable to the optimal rinsing duration of 1 minute with conventional mouthwashes.

Conflict of Interest

The authors declare no conflict of interests in this study.

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Authors Contribution

Conceptualization: LAP, NH, DFU; Methodology: LAP, NH, DFU; Formal Analysis: LAP, NH, DFU; Funding: LAP; Data Curation: LAP; Administration: LAP; Original Draft Writing: LAP, NH, DFU; Supervision and Editing: NH, DFU.

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