ORIGINAL ARTICLE

The Effectiveness of Red Pomegranate (*Punica granatum Linn*) Extract Mouthwash Against the Number of Oral Bacteria Colony

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ABSTRACT

Introduction: The percentage of Indonesian population experiencing oral and dental diseases based on the 2007 and 2013 RISKESDAS data, rise from 23.2% to 25.9%. People need to maintain healthy teeth and mouth by brushing their teeth and using mouthwash. Chlorhexidine is mouthwash that has been proven to be effective in reducing the number of plaque microorganisms attached to teeth and oral mucosa, but its long-term use can cause tooth stain, allergies, tissue desquamation and resistance. Red pomegranate can be used as an alternative because it contains flavonoids and tannins which are thought to have antibacterial ability. Therefore, this study aims to prove that red pomegranate extract mouthwash can reduce the number of oral bacteria colonies. **Methods:** 25 subjects were divided into 5 groups, namely K1: gargle aquades; K2: Chlorhexidine mouth rinse; K3, K4, K5 each rinse with 0.02%, 0.2% and 2% red pomegranate extract mouthwash. Saliva is collected before and after gargling, then planted in Petri dish using BHIA media with a spread plate method. Calculation of the amount of bacterial colony done 24 hours after the media was incubated. Counting the number of bacterial colonies using a colony counter. Data were analyzed by Anova and LSD tests. **Result:** There were significant difference between all group on decreased of number of oral bacteria colony (p= 0.00). **Conclusion:** Red pomegranate extract mouthwash effective decreased of number of oral bacteria colony and the most effective was 2% concentration.

Keywords: Red pomegranate extract, Mouth rinse, Chlorhexidine, Antibacterial

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INTRODUCTION

Dental and oral diseases in Indonesia are still widely found, from the Ministry of Health data in 2010 showing that caries prevalence in Indonesia reaches 60-80% of the population, and ranks 6th as the most suffered disease. The proportion of population with dental health problems in the last 12 months in 2013 according to Indonesian characteristics in the <1 year age group is 1.1%, aged 1-4 years 10.4%, aged 5-9 years 28.9%, and aged 10-14 years 25.2%. The percentage is said to be far from the target of the World Organization (WHO) which wants 50% of children aged 5-6 years to be free of dental caries (1).

This dental and oral disease is caused by the presence of bacteria that colonize in the oral cavity to form a biofilms/plaque layer (2). Colony of opportunistic bacteria will result in various pathological conditions such as caries, halitosis, gingivitis, angular cheilitis, and others. Several factors can cause microbes survive in the oral cavity, such as low body defenses, saliva that always wet the oral cavity, and also the unique form of oral cavity. Examples of bacteria that are always found in the oral cavity are the group Streptococcus (*Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius*), *Lactobacillus sp*, and so on (3).

The methods to maintain the cleanliness of the oral cavity from bacterial colonies is divided into 2, namely mechanically (using a toothbrush and toothpaste) and chemically (using mouth rinse). Mouth rinse is a formulation that has several functions, such as freeing the oral cavity from plaque and bacteria that cause disruption, providing a fresh oral cavity, minimizing the severity of periodontal disease, and preventing unpleasant breath odor. Mouth rinse has an advantage compared to a toothbrush because it can reach areas that are difficult to reach by a toothbrush (4).

Chlorhexidine is one of the mouthwashes on the market that has a broad-spectrum antibacterial ability. Chlorhexidine has a bactericidal and bacteriostatic effect

on gram-positive and negative bacteria (5). Chlorhexidine has been proven effective against oral bacteria because it can reduce the number of plaque microorganisms by as much as 80% by ionically attached to the surface of the teeth and mucosa of the oral cavity for several hours (6). Long-term use of chlorhexidine can give adverse effects on the oral cavity such as discoloration/staining of teeth and restoration, triggering calculus formation, allergic reactions, discomfort shortly after rinsing. In addition, the use of chlorhexidine also causes desquamation of the oral mucosa (5,6).

The side effects of chlorhexidine mouthwash can be prevented by developing natural mouthwash that has the same function as chlorhexidine but has minimal side effects. One of the plants that can be used as an alternative mouthwash is red pomegranate with the scientific name *Punica granatum Linn* (7). Red pomegranate has been widely used by the people of Indonesia because it is useful as an anti-inflammatory, antioxidant, and antibacterial. The active ingredients contained in red pomegranates that function as antibacterial are polyphenols, flavonoids, tannins and alkaloids (8). Therefore, researchers want to prove the effectiveness of red pomegranates in reducing the number of bacterial colonies in the oral cavity.

MATERIALS AND METHODS

Ethical Clearance

This study had been approved by ethical clearance from Ethical Committee of Medical Research, Faculty of Dentistry, Jember University, Indonesia (No: 531/UN25.8/KEPK/DL/2019).

Design

This research has been carried out in July 2019. This pure experimental study used a pre-post control group design. The manufacture of red pomegranate extract was carried out in the Bioscience Laboratory of the Dental Hospital of Jember University, making mouthwash in the Pharmacy Laboratory of the Faculty of Pharmacy, Jember University and measurement of bacterial colonies in the Microbiology Laboratory of the Faculty of Dentistry, University of Jember.

Samples

The study subjects consisted of 25 people, male sex,18-22 years old, did not suffer from systemic diseases, did not consume antibacterial drugs, did not use prostheses or ortho devices, did not smoke, had moderate OHI-S. Subjects were divided into 5 groups, namely: K1 (negative control), K2 (positive control), K3 (treatment1), K4 (treatment 2), K5 (treatment 3).

Research procedures

Research subjects were asked to collect saliva in pots. Next the subjects were asked to rinse, at K1 (negative control), the subjects rinsed with sterile aquades; K2 (positive control), subjects rinse with chlorhexidine; K3 (treatment 1), subjects gargled with red pomegranate extract mouthwash 0.02%; K4 (treatment 2), subjects gargled with 0.2% red pomegranate extract mouthwash; K5 (treatment 3), subjects gargled with 2% red pomegranate extract mouthwash. Furthermore, subjects were again asked to collect saliva in pots. Then the saliva was diluted to 10^{-5} using the serial dilution method and implanted in the medium of Brain Heart Infusion Agar (BHIA) on Petri dish by the spread plate method. Observations were did 24 hours after the media were incubated, counting the number of bacterial colonies using a colony counter.

Data analysis

The data obtained were analyzed using SPSS 20, with different ANOVA test followed by LSD. To calculate the effectiveness index of mouthwash using the deGarmo formula.

RESULTS

The results of research on the effectiveness of red pomegranate extract mouthwash in reducing the number of oral bacteria colonies can be seen in Figure 1. The results of Anova test and LSD test can be seen in Table I and II.

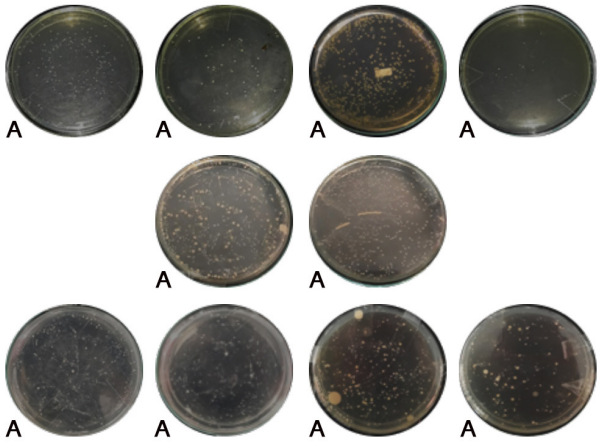

Figure 1: Bacterial colony : (a) Pretest aquadet, (b) Posstest aquadest, (c) Pretest 0.2% Chlorhexidine, (d) Posttest 0.2% Chlorhexidine, (e) Pretest 0.02% Red Pomegranate Extract, (f) Posstest 0.02% Red Pomegranate Extract, (g) Pretest 0.2% Red Pomegranate Extract, (h) Posttest 0.2% Red Pomegranate Extract, (i) Pretest 2% Red Pomegranate Extract, (j) Posttest 2% Red Pomegranate Extract

Table I shows that red pomegranate mouthwash extract can decrease the number of bacteria-colony in the oral cavity. Red pomegranate extract mouthwash 0.02% can reduce bacteria by 29.4 x 10^5 CFu/ml, red pomegranate extract mouthwash 0.2% can reduce bacteria by 48.6 x 10^5 CF/ml, while red pomegranate extract mouthwash can reduce bacteria by 2% a total of 87.4 x 10^5 CF/ml. The negative control group that rinsed using distilled water showed less effective results in reducing oral bacteria by only 6.6 x 10^5 CFu/ml. In the positive control

Table I: The different of oral bacteria colony between group by Anova test

Group	Mean ± SD			Anova (p)
	Pre	Post	Delta	
K1 (aquadest)	155.2 ± 107.1	149.0 ± 10.9	6.6 ± 30.6	
K2 (0.2% Chlorhexidine)	232.6 ± 43.2	33.2 ± 17.9	194.2 ± 29.9	
K3 (0,02% RPE)	168.0 ± 110.4	141.8 ± 115.5	29.4 ±12.9	0.000*
K4 (0.2% RPE)	149.6 ± 83.7	100.8 ± 71.4	48.6 ±14.1	
K5 (2% RPE)	227.6 ± 94.1	140.0 ± 62.4	87.4 ± 24.4	

Table II: The different of oral bacteria colony between group by Anova test

Group	K1	K2	K3	K4	K5
K1	-	0.000*	0.135	0.010*	0.001*
K2	0.000*	-	0.000*	0.000*	0.000*
K3	0.135	0.205	-	0.205	0.019*
K4	0.010*	0.000*	0.205	-	0.000*
K5	0.001*	0.000*	0.019*	0.000*	-

group that rinsed with Chlorhexidine 0.2%, it was seen that the decrease of bacterial colonies was 194.2 x 10^5 CFu/ml.

Data from the research results were then carried out data analysis. ANOVA test results show a significance value of p = 0.000, it means that there were differences in the entire study group, so it can be interpreted that the treatment group has the ability to reduce the number of oral colonies of bacteria. Then proceed with a LSD, there were significant difference between group K1 and K2 (p= 0.000), K1 and K4 (p= 0.010), K1 and K5 (p= 0.001), K2 and K3 (p= 0.000), K2 and K4 (p= 0.000), K2 and K5 (p= 0.000), K3 and K5 (p= 0.019), K4 and K5 (p= 0.000). Based on the calculation of the effectiveness index formula, it was found that the red pomegranate extract 2% mouthwash was most effective.

DISCUSSION

The results of the study and the data analysis showed that pomegranate extract mouthwash with 0.02%, 0.2%, and 2% concentrations can reduce the number oral bacterial colonies. Decreasing the number of bacterial colonies after gargling with red pomegranate extract mouthwash with 0.02%, 0.2%, and 2% concentrations can reduce the number of bacterial colonies This proves that the higher of the concentration used, made the higher of active ingredient contained in the red pomegranate extract so that the ability to inhibit oral cavity bacteria was also increasing (8).

The chemical content of red pomegranate extracts such as: flavonoids, polyphenols, alkaloids, antosianin and tannins acts as an antibacterial agent that can inhibit the growth of oral bacteria (8). The content of flavonoids in red pomegranates has 3 ways as an antibacterial role, namely by inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism (9). One of the flavonoid contents in red pomegranate, quercetin, works by increasing the permeability of bacterial cell membranes and damaging membrane potential, thereby causing disruption of bacterial ATP production, membrane transport, movement of bacteria, and the final bacterial was death (10).

The control group which rinsed using mouthwash Chlorhexidine showed the most optimal results which can reduce the number of oral bacterial colonies by 194.2 x 10^5 CFU/ml. The mechanism of action of Chlorhexidine as an antibacterial agent by inhibiting bacterial cell wall synthesis by ionic binding strong with bacteria. Chlorhexidine which is cationic and bacterial in nature is anion causing bacterial adhesion to the tooth surface will be inhibited, because Chlorhexidine which has been in contact with bacteria that colonize on the tooth surface will loose and mix with saliva, so there is no invasion into the host body (6). Chlorhexidine also reduces the formation of acid in dental plaque and can anticipated the process of dental caries by preventing the formation of dental plaque and suppressing cariogenic bacterial populations in the oral cavity. Chlorhexidine remains effective in killing bacteria within 2-3 days (11).

The effectiveness of pomegranate extract mouthwash with 0.02%, 0.2%, and 2% concentrations was still not equivalent to the effectiveness of Chlorhexidine mouthwash. Factors that influence the ability of antibacterial effectiveness in mouthwash are the concentration of active ingredients in solution, the time of contact between active ingredients and bacteria, the temperature of the solution, the ability of microorganisms to survive, and the presence of other organic materials that can inhibit the contact of mouthwash with bacteria (12). The use of the whole extract method used in maceration of red pomegranate extract can cause the active substances contained in the red pomegranate extract to weaken each other's antibacterial power, thus causing the effectiveness of red pomegranate extract mouthwash to be unequal to the Chlorhexidine mouthwash (13).

Chlorhexidine mouthwash, although more effective, but has shortcomings when used for the long term, such as discoloration/tooth stain and restoration, triggering calculus formation, allergic reactions, discomfort shortly after rinsing, and desquamation of the oral mucosa (5,6). Therefore, red pomegranate extract mouthwash was safer to use compared to Chlorhexidine. This statement was supported by Jayanti's research that discusses about toxicity test of red pomegranate extract against BHK-21 fibroblast cells and the results show that fibroblast cells were more proliferating and not toxic (14). Red pomegranate extract mouthwash also has anti-inflammatory and antioxidant properties that work synergistically, thus helping the antibacterial ability to reduce the number of bacterial colonies (15).

CONCLUSION

In this study it was proven that red pomegranate extract

mouthwash 0.02%, 0.2%, and 2% can reduce the number of oral bacterial colonies. In addition, among the three concentrations of red pomegranate extract mouthwash used, red pomegranate extract mouthwash with 2% concentration was the most effective in reducing the number of oral bacterial colonies.

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REFERENCES

1. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim). 2017;11(2):72–80.
2. Kriebel K, Hieke C, Мьller-Hilke B, Nakata M, Kreikemeyer B. Oral Biofilms from Symbiotic to Pathogenic Interactions and Associated Disease -Connection of Periodontitis and Rheumatic Arthritis by Peptidylarginine Deiminase. Front Microbiol. 2018;9:53. Published 2018 Jan 30. doi:10.3389/fmicb.2018.00053
3. Allaker RP, Stephen AS. Use of Probiotics and Oral Health. Curr Oral Health Rep. 2017;4(4):309–318. doi:10.1007/s40496-017-0159-6
4. Mervrayano, J., Rahmatini, dan Elizabeth Bahar. Perbandingan Efektivitas Obat Kumur yang Mengandung Chlorhexidine dengan Povidone Iodine terhadap Streptococcus mutans. Jurnal Kesehatan Andalas; 2015.
5. Bhate D, Jain S, Kale R, Muglikar S. The comparative effects of 0.12% chlorhexidine and herbal oral rinse on dental plaque-induced gingivitis: A randomized clinical trial. J Indian Soc Periodontol. 2015;19(4):393–395. doi:10.4103/0972-124X.153478
6 Saleem HG, Seers CA, Sabri AN, Reynolds EC. Dental plaque bacteria with reduced susceptibility to chlorhexidine are multidrug resistant. BMC Microbiol. 2016;16:214. Published 2016 Sep 15. doi:10.1186/s12866-016-0833-1
7. Dabholkar CS, Shah M, Kathariya R, Bajaj M, Doshi Y. Comparative Evaluation of Antimicrobial Activity of Pomegranate-Containing Mouthwash Against Oral-Biofilm Forming Organisms: An Invitro Microbial Study. J Clin Diagn Res. 2016;10(3):ZC65–ZC69. doi:10.7860/JCDR/2016/16478.7475
8. Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. J Food Sci Technol. 2014;51(12):4132–4137. doi:10.1007/s13197-013-0956-4
9. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Scientific World Journal. 2013;2013:162750. Published 2013 Dec 29. doi:10.1155/2013/162750
10. Ireneusz Górniak, Rafał Bartoszewski, Jarosław Króliczewski. Comprehensive review of antimicrobial activities of plant flavonoids Phytochemistry Reviews volume 18, pages 241–272.2019
11. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett. 2014;162(2 Pt A):22–38. doi:10.1016/j.imlet.2014.08.017
12. Umar D, Dilshad B, Farhan M, Ali A, Baroudi K. The effect of pomegranate mouthrinse on Streptococcus mutans count and salivary pH: An in vivo study. J Adv Pharm Technol Res. 2016;7(1):13–16. doi:10.4103/2231-4040.173266
13. Kadam NS, Kunte SS, Patel AR, Shah PP, Lodaya RR, Lakade LS. Comparative evaluation of the effect of pomegranate peel extract and chlorhexidine 0.2% mouthwash on salivary pH in children between 6 and 8 years of age: An in vivo study. J Int Oral Health 2019;11:40-4
14. Zakaria M, Mostafa B. Comparing pomegranate extract and chlorhexidine mouthwashes in treatment of recurrent intraoral herpes. J Arab Soc Med Res 2018;13:53-9
15. Hernawati, Sri. Mekanisme Kerja Ekstrak Buah Delima Terstandart Terhadap Degradasi Sel Mukosa Rongga Mulut Mencit Yang Mengalami Transformasi Melalui Ekspresi BCL-2, VEGF, Wild p53, dan Apoptosis. Disertasi. Surabaya: Fakultas Kedokteran Universitas Airlangga; 2012.