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EFFECTIVENESS TEST OF 0.2% CHLORHEXIDINE AND HIBISCUS (*HIBISCUS ROSA SINENSIS* L.) EXTRACT AGAINST *STREPTOCOCCUS SP* BACTERIA ON DENTAL PLAQUE

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ABSTRACT

Background: *Streptococcus sp* colonies are Gram-positive coccus bacteria that play a role in the formation of dental plaque and cause dental caries. However, dental caries can be prevented with the gold standard mouthwash, namely chlorhexidine. In other studies, many mouthwashes have been developed using traditional ingredients that have antibacterial potential, one of which is hibiscus flower (*Hibiscus rosa sinensis* L.). This is due to the presence of chemical compounds that can inhibit the growth of *Streptococcus sp* in dental plaque. **Objectives:** Testing the effectiveness of 0.2% chlorhexidine and *H. rosa sinensis* L. extract against *Streptococcus sp* in dental plaque. **Method:** Laboratory experimental research with *pre and post test only control group design* on 6 groups. Group K- (aquades), K+ (chlorhexidine), *H. rosa sinensis* L. extract 6.25% (P1), 12.5% (P2), 25% (P3), 40% (P4). Each of these groups consisted of 4 subjects. Data were obtained by counting the number of *Streptococcus sp* colonies before and after gargling which had been streak-cultured on *blood agar* media. The Shapiro Wilk test was used to check the normality of the data, if the significant results $P \geq 0.05$. Followed by the *One Way* ANOVA parametric test. While the not significant results with the *Kruskall Wallis* non-parametric test. **Results:** There was a significant difference between the 4 concentrations of *H. rosa sinensis* L. extract 6.25% (P1), 12.5% (P2), 25% (P3), and 40% (P4) with the control (+) ($P = 0.00$) in inhibiting the growth of *Streptococcus sp* bacteria, where a concentration of 25% (P3) was the most effective in inhibiting the growth of *Streptococcus sp* bacteria with a difference between *pretest* and *post test* of 12.5%. **Conclusion:** All *H. rosa sinensis* L. extract at various concentrations (6.25%, 12.5%, 25%, 40%) was effective compared to 0.2% chlorhexidine in inhibiting the growth of *Streptococcus sp*. Administration of various concentrations of *H. rosa sinensis* L. mouthwash extract inhibited the growth of *Streptococcus sp*, and at a concentration of 25% proved to be the most effective in inhibiting the growth of *Streptococcus sp*, which was 12.5% when compared to K+ (0.2% chlorhexidine).

Keywords: *H. rosa sinensis L., Streptococcus sp, chlorhexidine*

INTRODUCTION

Based on Regional Health Research (RISKESDAS) in 2018 the prevalence of caries in Indonesia was 88.8%.[1] Dental caries is the demineralization of tooth hard tissue by the activity of microorganisms in plaque. The process of plaque formation consists of three stages, namely pellicle formation, bacterial colonization and plaque maturation.[2] Gram-positive *Streptococcus sp* coccus such as *S. mutans*, *S. Mitis*, and *S. Sanguis* are the dominant bacteria in dental plaque.[3–7] Antiseptic mouthwashes are used to reduce plaque buildup and minimize dental caries, one of which is chlorhexidine mouthwash .[8,9]

Chlorhexidine 0.2% mouthwash is the gold standard mouthwash as an antiplaque and broad spectrum antibacterial which effectively reduces *Streptococcus sp* in dental plaque.[10] The mechanism of chlorhexidine against plaque bacteria (*S. Mutans*) is by precipitating cytoplasmic acid proteins, changes in permeability and leakage of cell membranes.[11] The side effects of using 0.2% chlorhexidine are in the form of tooth discoloration and a decrease in tongue sensitivity.[11] Chlorhexidine also has reversible and non-reversible side effects. The dangers include discoloration and changes in taste. Discoloration in the form of a brownish yellow color on the interproximal teeth and tongue and one-third gingiva. This is due to the interaction between chlorhexidine and several food materials. Changes in taste occur, namely bitterness and numbness on taste differentiation for minutes to hours after gargling depending on the degree of sensitivity of the oral mucosa. This effect occurs when routine use and long term of more than 2 years or if the user does not follow the rules correctly. when the usage of Chlorhexidine is stopped the effect will

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gradually disappear.[11] Because of the side effects it causes, the use of another alternative mouthwash, one with traditional antibacterial ingredients, is necessary.

H. rosa sinensis L. is a plant originating from East Asia.[12,13] *H. rosa sinensis* L. has been widely developed as a traditional medicine as an antioxidant, antibacterial, antipyretic and anti-inflammatory.[14] *H. rosa sinensis* L. has flavonoids, saponins, tannins, alkaloids, polyphenols, riboflavin, thiamine, water, hibicetin, vitamins.[15] Flavonoid phenol groups with their antibacterial mechanism, namely protein denaturation and damage to cell membranes.[15] Saponins have an antibacterial mechanism by reducing surface tension, resulting in increased permeability and release of intracellular compounds.[16] Tannins can inhibit bacteria by binding to bacterial proteins and disrupting membranes.[17] Alkaloid compounds heterocyclic nitrogen and has groups of carbon, hydrogen and oxygen compounds and has an antibacterial mechanism by inhibiting nucleic acid synthesis.[18]

Based on the description above, it is necessary to conduct research on how much antibacterial effect 0.2% chlorhexidine has compared to hibiscus flower extract (*H. rosa sinensis* L.) on the growth of *Streptococcus sp* bacteria in dental plaque, so that it can reduce the occurrence of dental caries.

MATERIALS AND METHODS

This experiment was laboratory experimental with *pre and post test only control group design*. This study used *H. rosa sinensis* L. extract with a total of 24 samples in this experiment divided into 6 treatment groups. The statistical test in this study was the *Shapiro Wilk* test and the *One Way* ANOVA test.

This study was conducted by 24 subjects by taking oral swab samples before and after rinsing for 7 days. Inclusion criteria in this study were willing to participate, women, 25-35 years old age, fasting 1 hour before sampling, healthy, and measure the plaque index using the Oral Hygiene Index Simplified (OHI-S). Exclusion criteria included consuming drugs, systemic diseases, use of braces and dentures.

The first stage was making *H. rosa sinensis* L. extract by maceration for 3×24 hours. When the extraction process has been completed and produces a thick extract, the next step is the process of making *H. rosa sinensis* L. extract mouthwash in various concentrations. *H. rosa sinensis* L. extract was prepared in concentrations of 6.25%, 12.5%, 25%, and 40%, and each concentration was weighed and diluted with distilled water.

The next step was to do a swab in the oral cavity of the subjects which complied with the inclusion and exclusion criteria, using transport media and giving mouthwash to the probands, as well as instructing the use of mouthwash. The pretest sample was stored in an ice box and immediately brought to the microbiology laboratory for culture, incubated for 1x24 hours and observation and colony counting of *Streptococcus sp*. After 7 days, post-test samples were taken by taking a swab of the dental plaque using transport media, and immediately taken to the microbiology laboratory for culture, incubation for 1 x 24 hours and observation and counting of *Streptococcus sp* colonies.

Streptococcus sp cultures on MHA media were incubated for 24 hours at 37˚C and dilution was carried out by means of 1-2 ose colonies of *Streptococcus sp* mixed with 1 ml of 0.9% NaCl in a tube until turbidity was obtained according to the standard Mc Farland turbidity of 0.5. (1.5 x 10^2 CFU/ml). Then do the colony counting using a colony counter. Each colony of microorganisms that have been counted is marked with a marker.

Table 1. Descriptive and Normality test result

Strepto-coccus sp	Group	Mean ± SD	Median (min – max)	p[£]
Pre test	P1	76,50±5,32	76,5 (70-83)	0,869*
	P2	83,25±16,60	82,5 (68-100)	0,161*
	P3	82,50±10,79	84 (68-94)	0,712*
	P4	91,00±11,46	91 (77-105)	0,858*
	K+	85,75±14,61	86,5 (68-102)	0,962*
	K–	77,75±2,63	78,5 (74-80)	0,369*
Post test	P1	68,00±4,08	68 (63-73)	0,683*
	P2	73,00±16,02	71 (59-91)	0,241*
	P3	70,00±12,83	71,5 (53-84)	0,794*
	P4	77,00±14,35	79,5 (59-90)	0,518*
	K+	75,00±150,0	0 (0-300)	0,001
	K–	73,50±4,36	72,5 (70-79)	0,274*
Delta	P1	-8,50±1,29	-8,5 (-10-(-7))	0,972*
	P2	-10,25±1,89	-9,5 (-13-(-9))	0,086*
	P3	-12,50±2,08	-12,5 (-15-(-10))	0,995*
	P4	-14,00±9,38	-18 (-20-0)	0,017
	K+	-10,75±162,1	-86,5 (-102-232)	0,007
	K–	-4,25±2,87	-4 (-8-(-1))	0,625*

* Normal ($P > 0,05$); [£] Shapiro-wilk

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RESULTS

The *Shapiro Wilk* test analysis as shown in Table 1. above shows the 6 sample groups in the *pretest* with significant normality test results. In the *post test* treatment, four concentrations of *H. rosa sinensis* L. extract, and control (-) had significant normality test results. However, in the *post test* control (+) treatment, the results were not significant. The results of the difference between the *pretest* and *post test* at the three extract concentrations of *H. rosa sinensis* L., and the control (-), in the normality test showed normal data of $P \geq 0.05$. Meanwhile, there was one concentration of hibiscus flower extract (*H. rosa-sinensis* L.), and the control (+) results were not significant. In the *Shapiro Wilk* test which has been carried out with normal results, it is continued with the *One Way* ANOVA parametric test, while in some results it is continued with the *Kruskall Wallis* non-parametric test. Then for the difference test between the *pretest* and *post test*, the data is normally distributed using the *paired t* test, while the data is not normally distributed using the *Wilcoxon* test.

Table 2 shows the differences results between groups. The results obtained were $P < 0.05$ in the difference between the *pretest* and *post test* in the *H. rosa sinensis* L. extract sample group at concentrations of 6.25%, 12.5%, 25%, and 40%. It can be said that these results have a significant difference in inhibiting the growth of *Streptococcus sp*. colonies. Meanwhile, the difference between the *pretest* and *post test* in the control (+) and control (-) treatment groups was $P > 0.05$ or not significant.

Diagram in Figure 1 shows that the mean difference between *pretest* and *post test* in the K- (aquades) group is 4.25, K+ (chlorhexidine) is 10.75, *H. rosa sinensis* L. extract concentration is 6.25% of 8.5, 12.5% of 10.25, 25% of 12.5, and 40% of 14. The diagram shows the difference in the average *H. rosa sinensis* L. extract at a concentration of 25% showing effective results in inhibiting the growth of *Streptococcus sp.* colonies when compared to K+ (chlorhexidine).

Table 2. Differences test result

Group	*Streptococcus sp*		*P*	Delta
	Pre Test	***Post Test***		
P1	76,50 ± 5,32	68,00 ± 4,08	0,001¶*	-8,50 ± 1,29
P2	83,25 ± 16,60	73,00 ± 16,02	0,002¶*	-10,25 ± 1,89
P3	82,50 ± 10,79	70,00 ± 12,83	0,001¶*	-12,50 ± 2,08
P4	91,00 ± 11,46	77,00 ± 14,35	0,058¶	-14,00 ± 9,38
K+	85,75 ± 14,61	75,00 ± 150,0	0,715†	-10,75 ± 162,1
K–	77,75 ± 2,63	73,50 ± 4,36	0,060¶	-4,25 ± 2,87
P	0,515§	0,609‡		0,105‡

* Significant ($P < 0,05$); § One Way ANOVA; ‡ Kruskal Wallis; ¶ Paired t; † Wilcoxon

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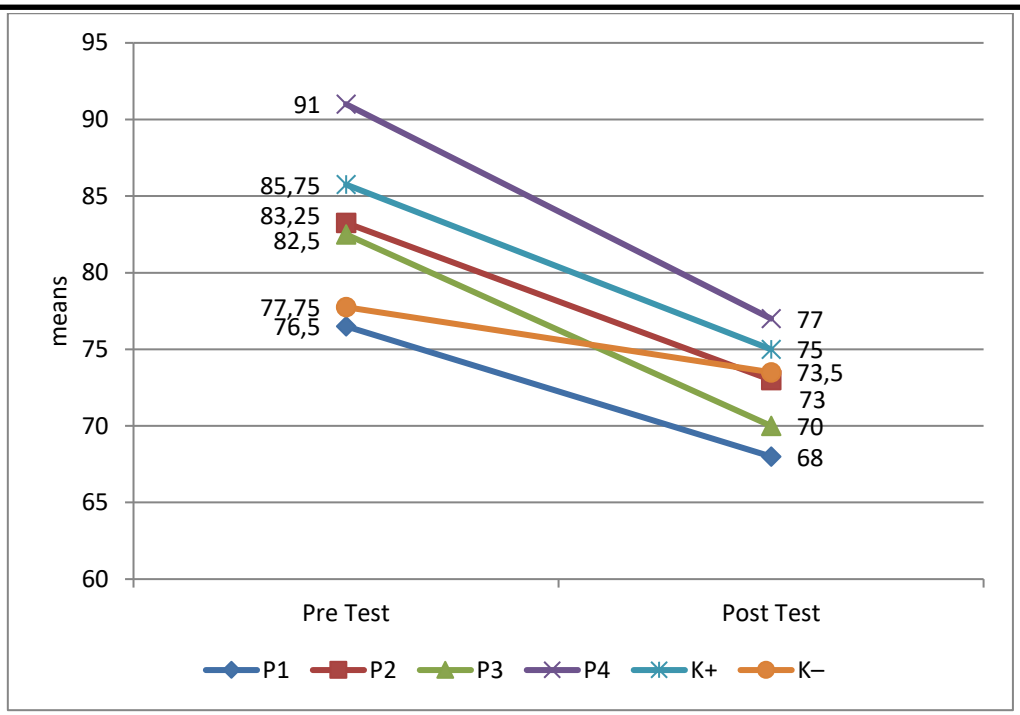

Figure 1. Diagram of treatment group means
(K- (aquades), K+ (chlorhexidine), Hibiscus extract (*H. rosa sinensis* L.) 6,25% (P1), 12,5% (P2), 25% (P3), 40% (P4)

DISCUSSION

The results of the research that has been done prove that *H. rosa sinensis* L. extract has an inhibitory power against *Streptococcus sp*. This is indicated by the difference in the number of bacterial colonies in the oral cavity before and after rinsing using *H. rosa sinensis* L extract. This inhibitory activity can occur because hibiscus flowers contain active antimicrobial compounds such as flavonoids, saponins, tannins and alkaloids.[19-21]

Flavonoid phenol groups with their antibacterial mechanism, namely protein denaturation and damage to cell membranes.[15] Saponins have an antibacterial mechanism by reducing surface tension, resulting in increased permeability and release of intracellular compounds.[16] Tannins can inhibit bacteria by binding to bacterial proteins and disrupting membranes.[17] Alkaloid compounds heterocyclic nitrogen and has groups of carbon, hydrogen and oxygen compounds and has an antibacterial mechanism by inhibiting nucleic acid synthesis.[18]

The results showed that the use of *H. rosa sinensis* L. extract at concentrations of 6.25%, 12.5%, 25%, and 40% which was used to rinse the mouth for 7 days was able to inhibit the growth of *Streptococcus sp* in the oral cavity, with an average each inhibited by 8.5 x 10^2 CFU/ml for a concentration of 6.25%, 10.25 x 10^2 CFU/ml for 12.5%, 12.5 x 10^2 CFU/ml for 25%, and 14 x 10^2 CFU/ml for 40 %. In this study, the difference in the average of *H. rosa sinensis* L. extract at a concentration of 25% proved to be the most effective in inhibiting the growth of *Streptococcus sp*, which was 12.5% when compared to K+ (0.2% Chlorhexy). At this concentration, there was a decrease in the growth of the number of *Streptococcus sp* colonies in the oral cavity and this was comparable to the content in the extract of *H. rosa sinensis* L.

In the treatment group of *H. rosa sinensis* L. extract mouthwash concentration of 40%, from the four sample repetitions there was 1 sample that showed no significant difference between *pretest* and *post test*, with *pretest* results of 90 x 10^2 CFU/ml and *post test* 90 x 10^2CFU/ml. The same thing happened in the control group (+), of the four sample repetitions there was 1 sample that experienced an increase in the number *Streptococcus sp* of bacteria colonies in the *post test*, with *pretest* results of 68 x 10^2 CFU/ml and *post test* ≥ 300 x 10^2 CFU /ml. This is probably due to various factors, namely the method of using and storing mouthwash that is not quite right, causing

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contamination in the mouthwash and results not being optimal, and contamination during the bacterial culture process carried out in the laboratory.

CONCLUSION

Extract of *H. rosa sinensis* L. in various concentrations can inhibit the growth of *Streptococcus sp*, and at a concentration of 25% is proven effective in inhibiting *Streptococcus sp* by 12.5%.

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