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COMPARISON OF ANTIBACTERIAL EFFECTIVENESS OF HERBAL TOOTHPASTE AND FLUORIDE ON THE GROWTH OF *STREPTOCOCCUS MUTANS*

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ABSTRACT

Background: *Streptococcus mutans* was the most dominant bacteria causing caries. Caries could be prevented by chemical plaque control methods using toothpaste. Toothpaste had antibacterial properties which help prevent bacteria from colonized. There were two antibacterial ingredients in toothpaste, fluoride and herbal ingredients. **Aim:** To determine the antibacterial effect of toothpaste with herbal and fluoride content on the growth of *Streptococcus mutans*. **Methods:** This study was an experimental study with a post-test only controlled group design. This research method was a diffusion method by means of wells. The sample used was the *Streptococcus mutans* colony. The study was divided into four groups, namely herbal toothpaste, fluoride toothpaste, positive control (*chlorhexidine*), and negative control (aquadest sterile) which were replicated 16 times in each group. The result of this study was the diameter of the resistance zone. **Results:** The average diameter of the zone of inhibition results obtained 12,50 ± 0,24 (herbal toothpaste), 14,67 ± 0,27 (fluoride toothpaste), 21,71 ± 0,02 (*chlorhexidine*) and aquadest sterile there were no inhibition zones. There was a significant difference with the p value <0.001 tested by the unpaired T test. **Conclusion:** Fluoride toothpaste had a better antibacterial effect than herbal toothpaste on the growth of *Streptococcus mutans*

Key words: herbal toothpaste, fluoride toothpaste, antibacterial effect

BACKGROUND

Caries was one of the cause of dental and oral health problems that often occur in the community.¹ Caries was a multifactorial disease with the cause of bacteria, host tissue, substrate and time, where bacteria are the main cause.² *Streptococcus mutans* was the most dominant bacteria caused of caries. *Streptococcus mutans* can synthesize acids from sugar quickly and produce polysaccharide. *Streptococcus mutans* produced polysaccharides, especially from sucrose, thus facilitated initial colonization and caused plaque formation.³

Caries can be prevented by plaque control methods, the method can be done mechanically and chemically.⁴ Chemical plaque control can be done by antiseptics in the form of mouthwash. Plaque control mechanically can be done by professionals or done by individuals through tooth brush. Tooth brush was not effective in prevented caries, it is necessary to brush teeth with toothpaste in order to prevented bacteria from colonized.⁵

Toothpaste manufacturers produce various types of toothpastes with the addition of different ingredients, for example the addition of herbal ingredients. Many herbal toothpastes claim to had an antibacterial effect.⁶ Herbal toothpaste on the market contains selected herbal ingredients as anti-bacterial

ingredients, for example, siwak wood, betel extract, gambier extract, and clove oil. The active ingredient of siwak wood, namely *benzyl isothiocyanate*, was identified as the main antimicrobial component.^{7,8} Betel extract was known to had several substances included volatile oil. The mechanism of essential oils in inhibited bacterial growth is by interfered with the process of formation of membranes or cell walls.⁹ Other antibacterial ingredients were fluoride, works by inhibitor the metabolism of plaque bacteria, fluoride can be bacteriostatic or bactericidal.¹⁰

The purpose of this study was to find out whether there were differences in the antibacterial effect of herbal toothpaste and fluoride toothpaste, and if there was, what type of toothpaste had greater antibacterial effect in inhibit the growth of *Streptococcus mutans* as the main bacteria that causes caries in the oral cavity.

MATERIAL AND METHODS

Research design

This research was an experimental study with a post test only controlled group design that was carried out at the Microbiology Laboratory of the Faculty of Medicine, Diponegoro University, Semarang in September to November 2019.



Material

The material in this study used the bacteria *Streptococcus mutans*, toothpaste with herbal contents, fluoride, chlorhexidine gluconate 0.2%, aquadest sterile and blood agar. The tools in this study used sterile ose, test tubes, test tube racks, petri dishes, incubators (Labnet, USA), micropipets, cotton sticks, candles and lighters, candle caps, autoclaves (Sanyo, Japan), digital calipers (Krisbow, China), sterile holes, analytic balance.

Procedure

Preparation of the device by sterilization used an autoclave, followed by made a suspension of *Streptococcus mutans* bacteria. Make a suspension is done in less than 10 minutes in accordance with McFarland 0.5 standards. Test of toothpaste antibacterial activity against *Streptococcus mutans* using the diffusion method of wells. The wells were made by 4 terile holes with a diameter of 6 mm totaled 4 holes in each cup of blood agar.

The bacterial suspension was flattened on the media using a squeezed cotton stick. Each toothpaste amounted to 0.01 grams, chlorhexidine gluconate 02% and aquadest sterile placed in the wells accorded to the group. Then all the petri dishes were put into a candle lid with a temperature of 370C for 24 hours. Clear area that points to the inhibitory area was measured using digital slide. Observations were made by 3 observers.

Data analysis

Data obtained from the study conducted a normality test used the *Saphiro-Wilk* test to see the distribution of data distribution. Data comparison were made using unpaired T test with a P value <0.05 which is interpreted statistically as the difference.

Research Ethics

Ethical clearance had requested for research with No. 116 / EC / H / KEPK / FK-UNDIP / VIII / 2019.

RESULT

The results of statistical data processing obtained the mean and median value of the inhibition zone in each group, can be seen in table 1.

Table 1. Research group data

Group	N	Mean ± SD	Median
Herbal toothpaste	16	12,50 ± 0,24	12,53
Fluoride toothpaste	16	14,67 ± 0,21	14,64
Chlorhexidine	16	21,71 ± 0,02	21,7
Aquadest sterile	16	0	0

The effectiveness of antibacterial herbal toothpaste and fluoride on the growth of *Streptococcus mutans* was known by conducted a normality test using *Shapiro Wilk* test, from the normality test the results obtained with a probability value (p> 0.05) where it shows that the data distribution was normal.

1. Effects of Herbal Toothpaste on Growth of Streptococcus mutans

Table 2. Comparison of the antibacterial effects of herbal toothpaste with positive and negative controls

Group	Mean ± SD	p
Herbal Toothpaste	12,50 ± 0,24	<0,001*
Control positive (Chlorhexidine)	21,71 ± 0,02	
Control negative (Aquadest sterile)	0	

The diameter measurement results in the herbal toothpaste group had a significant difference with a p value <0.05. This shows that herbal toothpaste has an antibacterial effect on the growth of *Streptococcus mutans*. The results of measured the diameter comparison found significant differences between herbal toothpaste with chlorhexidine, this shows that the antibacterial effect of herbal toothpaste was no better than chlorhexidine.

2. Effects of Fluoride Toothpaste on Growth of Streptococcus mutans

Table 3. Comparison of the antibacterial effects of fluoride toothpaste with positive and negative controls

Group	Mean ± SD	p
Pasta Gigi Fluoride	14,67 ± 0,21	<0,001*
Control positive (Chlorhexidine)	21,71 ± 0,02	
Control negative (Aquadest steril)	0	

These results indicate that fluoride toothpaste had an antibacterial effect on the growth of *Streptococcus mutans*. The results of measured the diameter comparison found a significant difference between fluoride toothpaste with chlorhexidine, this shows that the antibacterial effect of fluoride toothpaste was no better than chlorhexidine.



3. Comparison Antibacterial Effectiveness of the Herbal Toothpaste and Fluoride on the Growth of *Streptococcus mutans*

Comparisons of the herbal and fluoride toothpaste groups were known used the unpaired T test. Analysis of unpaired T test results obtained $p < 0.05$, this shows that there were significant differences between herbal toothpaste compared to fluoride toothpaste in inhibited the growth of *Streptococcus mutans*. Fluoride toothpaste has a better antibacterial effect when compared to herbal toothpaste.

DISCUSSION

This research aims to find out the type of toothpaste that had an antibacterial effect on the growth of *Streptococcus mutans*. The method used in this research was the diffusion method by means wells. This method was strongly influenced by several factors such as the environment, length of incubation, the interaction between bacteria and the media used, it must be protected with extreme care to prevent contamination.¹¹ This research compared herbal toothpaste and fluoride, so that the herbal toothpaste used in this research was chosen because this toothpaste did not contain fluoride content in its composition, while the fluoride toothpaste chosen in this research was a toothpaste with a composition similar to the herbal toothpaste that had been selected. In addition, the toothpaste used in this research was toothpaste which was widely circulated and used by the public.

To find out the antibacterial effect in each toothpaste group, it was done by looking at the zone of inhibition determined from the clear zone on the agar media, then compared with the negative control in example aquadest sterile. Observations obtained no inhibitory zones on negative controls.

The results of this research indicated that herbal toothpaste had an antibacterial effect on the growth of *Streptococcus mutans*. Herbal toothpaste in this research had active ingredients from siwak wood, namely benzyl isothiosianate, betel extract was known to have several substances including essential oils which have an antibacterial effect by interfering with the process of forming bacterial walls, gambir extract had an active ingredient namely catechins, and eugenol containing oil cloves had an antibacterial effect by inhibiting bacterial cell wall synthesis.^{9,12}

Fluoride toothpaste had an antibacterial effect on the growth of *Streptococcus mutans*. It was because fluoride toothpaste had fluoride content which played a role in the antibacterial effect by making it resistant to acids and inhibiting bacteria in producing acids, thus greatly inhibiting bacterial metabolic activity as a whole.^{10,11}

Subsequent observations were made by comparing the herbal toothpaste group and the fluoride toothpaste group with chlorhexidine as a positive control. Significant results were obtained in each group. The results obtained were that the diameter of the chlorhexidine inhibitory zone was greater when compared to the herbal toothpaste and fluoride toothpaste groups. It was because chlorhexidine as a positive control group had a large antibacterial effect, with an antibacterial mechanism that caused changed in the permeability of bacterial cell membranes that would interfere with cell survival, caused the release of cytoplasm and cell components from the cell through the cell membrane causing bacterial death.¹³

The antibacterial effect of fluoride toothpaste was greater when compared to herbal toothpaste. This result was caused by fluoride toothpaste which had two mechanisms in inhibiting *Streptococcus mutans*. The mechanism of fluoride antibacterial strength was by inhibiting the overall bacterial metabolic activity. Besides fluoride also had another mechanism by influencing the accumulation of protons. Thus reduced the ability of bacteria to grow and reduced the rate of metabolism in an acidic environment.¹⁴

CONCLUSION

Based on the results of this research, it could be concluded that toothpaste with herbal and fluoride content had antibacterial effect on the growth of *Streptococcus mutans*. Although the antibacterial effect on the content of herbal toothpaste and fluoride was no better than chlorhexidine. Fluoride toothpaste had better antibacterial effect when compared to herbal toothpaste on the growth of *Streptococcus mutans*.

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