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INHIBITORY POWER OF TOOTHPASTE CONTAINS *KENCUR* (*KAEMPFERIA GALANGA*) TO THE GROWTH OF *STREPTOCOCCUS MUTANS* (SM) BACTERIA

Wahyu Jati Dyah Utami[1*], Rahma Rizki Hutami[2], Diyah Fatmasari[1]

1) *Applied Science Program in Oral Health Therapist, Poltekkes Kemenkes Semarang, Semarang, Indonesia,*
2) *Postgraduate Program of Sultan Agung University, Semarang, Indonesia*

*Corresponding author's e-mail : wahyujatidyah@gmail.com

ABSTRACT

Background: Dental caries is a disease caused by the interaction between microorganisms, diet, and teeth (host). *Streptococcus mutants* is most common microorganisms which has a role in the process Microorganisms whose role is *Streptococcus mutants*. *Kencur* (*Kaempferia galanga*) has bactericidal properties because it contains essential oils, flavonoids, polyphenols, and saponins that can inhibit bacterial growth.
Aims: The purpose of this study was to determine the ability of *kencur* extracts 20% in toothpaste to inhibit the growth of *Streptococcus mutants*.
Methods: This was a l*aboratory experimental research* with *post-test control group design*. The sample was divided into 2 groups, toothpaste without *kencur extract* as group A and a toothpaste group containing *kencur* extract. Replication is done 12 times from each group. Incubation was performed for 24 hours at 27 0 C. The results are measured with calipers and the data were analysed by *Independent t-test*.
Results:The results showed that the average of toothpaste A inhibitory zone was 2.95 mm and the toothpaste containing *kencur* extract was 18.1 mm. *Independent test* results obtained t-test significant value of 0.000 $p<0.05$ which means there are differences in the average zone of inhibition significantly between groups *kencur* extract toothpaste and toothpaste brands A.
Conclusion: It can be concluded that, although *kencur* extracts toothpaste has inhibitory zone against the bacteria *Streptococcus mutants* however, toothpaste A has a larger inhibition zone.

Keywords: kencur extract toothpaste, Streptococcus mutans, inhibitory zone

INTRODUCTION

Dental caries is a dental disease commonly found in today's society. The result of basic health research (Riskesdas) in 2013 showed an increased prevalence of dental caries in Indonesia from 43.4% in 2007 to 53.2% in 2013, which is approximately 93,998,727 people in Indonesia are suffering from dental caries [1]. Dental caries is a dental disease of the tooth hard tissue, characterized by the destruction of enamel and dentine caused by bacterial metabolism activity in plaque. The destruction of enamel is causing demineralization resulting from interaction between microorganism products and parts of food as well as tooth enamel[2]. Caries is a dynamic process that is characterized by episodes of demineralization and re-

mineralization that occurs over time. If the higher demineralization happens, the mineral component failures will cause cavities [3].

Streptococcus mutans is a bacterium that is cariogenic and can thrive in acidic conditions. The presence of food in the mouth (carbohydrates) is a substrate that bacteria will ferment to get energy. Sucrose and glucose are fermented in such a way that extracellular polysaccharides will form. Extracellular polysaccharide is mainly composed of a glucose's polymer that would cause the plaque matrix to have a gelatin-like consistency, so that helped the bacteria to attach to the tooth surface[4].

One way to prevent caries is to inhibit plaque growth. Mechanical cleaning and the use of anti-microbial materials, mainly to suppress *Streptococcus mutans,* are plaque controlling methods. Brushing teeth can help eliminate meals leftovers on the teeth and helps to control dental caries [5]. Therefore, ways to inhibit plaque growth are brushing and rinsing with a mouthwash containing antimicrobial ingredients.

The ingredients used in tooth brushing is toothpaste. The main function of a toothpaste is to help the toothbrush to clean the teeth surface from food and beverage scraps, to maintain teeth and gums health, and also to eliminate odors caused by oral bacterial activity while providing a comfortable aroma and taste at the same time [5]. In modern times, many toothpaste producers develop toothpaste content by adding other substances that are beneficial to dental health. The addition of certain ingredients in toothpaste can reduce the amount of bacteria that cause caries [6]

Antimicrobial agents commonly used to inhibit bacterial growth include phenol, hexetidine, fluorine and chlorhexidine. Chlorhexidine is one of the most effective formula, however its long term use may cause adverse effects. Alternative ingredients from essential oils and plant extracts (herbs) are natural choices for anti-germicidal ingredient in toothpaste. In the market, toothpaste with herbal ingredients such as Aloe vera, eucalyptus, Siwak, and betel leaf is now widely circulating [5]

Kencur (*Kaempferia galanga*) is one plant that is widely used as a traditional medicine. *Kencur* is sporadically used throughout Indonesia because it is easy to find, cheap and often used as spices of cooking. Benefits of *kencur* includes anti-nausea, swelling treatment, cough relief, and antibacterial drugs. Based on existing research, the content of *kencur* rhizome such as essential oil is more or less (2,4% -3,9%). *Kencur* rhizome also contains other compounds such as flavonoids, polyphenols, and saponins. Efficacious substances as anti-bacteria in essential oils are cineol and borneol. *Kencur* contains substances that are bacteriostatic and bactericide, or have the ability to inhibit growth and kill bacteria (anti-bacterial effect) [7]. The inhibition of microbial processes is caused by bioactive compounds that have a hydroxyl group (OH) reacting with components in the material of the microorganism cells, so that these microbes could no longer have the activity and eventually die [7].

According to Pelczar and Chan (2005), as antibacterial, flavonoids in *kencur* plants work by inhibiting the development of microorganisms because they can form complex compounds with proteins through hydrogen bonds. The mechanism of action by denaturizing molecules of proteins and nucleic acids that causes coagulation and proteins clotting will eventually impaired metabolism and physiological functions of bacteria. If the metabolism of the bacteria is disturbed, then the bacteria could not produce sufficient energy, resulting in permanent destruction of bacterial cells, which ultimately leads to the death of bacteria and damage the cell membranes without repair, and reduce the risk of caries[8]. According to Almaida (2013), the red ginger extracts for antibacterial activity on Staphylococcus aurous at concentrations of 20% showed the most extensive formation inhibition zone [9]. Red Ginger extracts also contain other compounds such as namely flavonoids, polyphenols, and saponins. Based on the above description, the researcher wanted to examine the bacterial inhibition zone of 20% *kencur* extract in toothpaste on the growth of *Streptococcus mutans* as the main causes of caries in the oral cavity.

METHODS

This research is a laboratories experiment with post-test control group design. The independent variable is *kencur* extract toothpaste made by *kencur* rhizome. The dried rhizome was grinded and pulverized, then macerated with 96% ethanol for five days and stirred every day and concentrated with rotavator. After that, 20% *kencur* extract was mixed with the toothpaste. Brand "A" toothpaste that has been used by the wider community was used as a comparison. The dependent variable is the inhibition zone of bacteria *Streptococcus mutans*. Inhibition zone formed revealed that there is activity of *Streptococcus mutans* bacteria growth. Observations were made by measuring the clear zone formed using calipers with the ratio measurement scale.

The population in this study is the bacterium Streptococcus *mutans* from a collection of the Laboratory of Microbiology, Faculty of Medicine of Sultan Agung Islamic University, Semarang. The sample of this research is bacterial culture with McFarland 1.0 standard. The sample size is 12 repetitions.

The procedure of making *kencur* extract toothpaste is a combination of formula 9, entitled Cosmetics Science and Technology [10]

The procedures of making extract kencur toothpaste are as follows: prepare a solution of McFarland 1.0, and take a bacterial colony using ose. Put it into a tube containing 0.9% NaCl sterile. Mix and stir it until evenly distributed and compare the turbidity with McFarland 1.0 solution. If less turbid, more bacteria will be added while if it is too turbid, NaCl will be added. Take each of suspense with a cotton swab then squeeze the tube wall dab of cotton on each suspense on Mueller Hinton media evenly. Leave for 5-10 minutes. After that, take toothpaste with *kencur* extracts and toothpaste brand 'A' for 5-10 ml each and put them into the Bekker glass. Next, put in or soak in a blank disc on the *kencur* extract toothpaste. For 'A' brand toothpaste, leave for 5-10 minutes and take a blank disc using tweezers. Put it on Mueller Hinton media or petri dish containing Mueller Hinton media, incubate for 24-48 hours at 37^0C, observe, and measure the inhibition zone. The formation of inhibition zone revealed that there is growth activity of *Streptococcus mutans* bacteria. Observation was made by measuring the clear zone formed using the calipers, so it can be called inhibition zone, dispose of research waste by inserting petri dish containing bacteria into autoclave for two hours with pressure 121 degree Celsius. The plate is removed from the autoclave and the bacteria is dumped into the wastewater duct.

Kencur extract toothpaste is stored in a refrigerator and tested on the bacterium *Streptococcus mutans* within three days after the production. Storage at room temperature and/or the testing over three days after the making may affect the ability of extracts kencur in toothpaste in inhibiting the growth of *Streptococcus mutans*.

The data obtained is then analyzed using SPSS. Data were tested for normality by the Shapiro-Wilk and homogeneity with Levene's Test. If the distribution of data between the group is normal and data variation is homogeneous, then parametric Independent sample t-test for comparing two groups of unpaired samples will be performed.

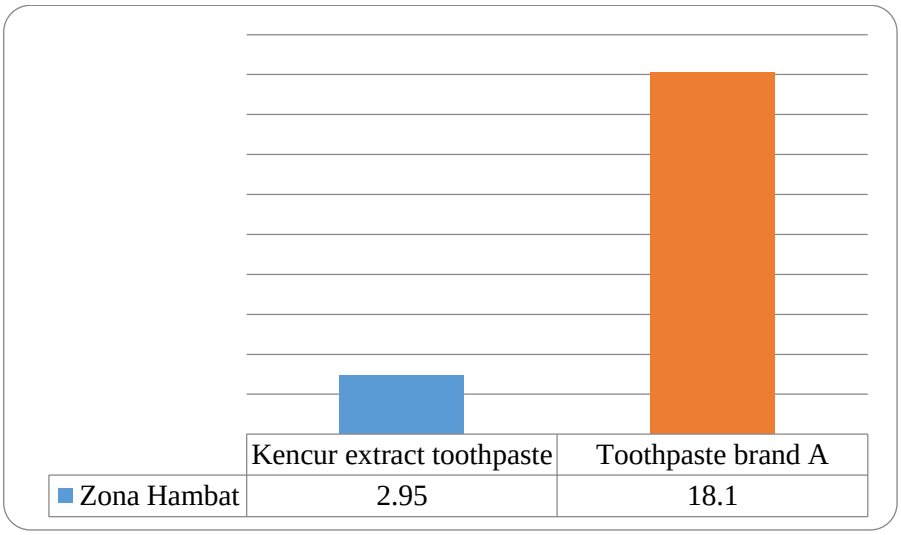
RESULTS

The measurement of inhibition zone on *Streptococcus mutans* bacteria growth in the two groups yielded the following results:

Table 1. Data on average zone of inhibition of bacteria *Streptococcus mutans* on *kencur* extract toothpaste group and brand

Group	**Inhibition Zone**
	Mean ±Deviation Standard
Kencur extract toothpaste	2,95 ±0,65
Toothpaste brand 'A'	18,1 ± 1,75

From table 1 above, it is discovered that the average zone of inhibition on the *kencur* extract toothpaste group is 2.95 mm lower than group toothpaste brands 'A' by 18.1 mm.



Graphic1. The average inhibitory zone of *kencur* extract toothpaste and toothpaste brand A

From the graph above, it can be seen that the average inhibition zone of *kencur* extract toothpaste group is lower. To prove the effectiveness of inhibitory zone on *kencur* extract toothpaste to brand 'A' toothpaste against the growth of bacteria *Streptococcus mutans*, then statistical tests was performed. The results of the normality test data are as follows:

Table 2. Result of data normality and homogeneity test

Group		**Sig**	**Note**
Test of normality	Toothpaste brand A	0,188	Data distributed normally
	Kencur extract toothpaste	0,538	Data distributed normally
Test of Homogeneity	Inhibition zone	0,004	Data not homogenous

Based on normality test result on toothpaste brand 'A' group and *kencur* extract toothpaste group, the researchers obtained normal data distribution (p> 0,05). Homogeneity test results indicate that the data in the two groups are not homogeneous (p <0.05), thus, the Independent t-test requirements can be met (data distributed normal). The Independent t-test results are as follows:

Table 3. *Independent t-test* result

Group	***Mean ± Std Dev***	***Sig***	**Note**
Kencur extract toothpaste	2,95 ±0,65	0,000	Significantly different
Toothpaste brand A	18,1 ± 1,75		

Based on those results, it can be seen that toothpaste containing extracts *kencur* and toothpaste brand 'A' both have antibacterial activity against *Streptococcus mutans*. However, the mean diameter of the brand 'A' toothpaste inhibition zone is much greater than the 20% *kencur* extract toothpaste zone on the gelatin medium.

DISCUSSION

Toothpaste brand 'A' is much more effective as compared to extract *kencur* toothpaste because it contains other compositions that play a role in inhibiting the growth of Streptococcus *mutans* and other chemicals that are more influential in terms of efficacy resulting in the formation of inhibitory zone. The composition and the chemicals are phenol, hexetidine, fluorine and chlorhexidine. Chlorhexidine is one of the most effective formula. The more active ingredients in a dosage, the greater the effect it produces[11].

Brand 'A' toothpaste is a mass-produced commercial toothpaste. One of toothpaste 'A' 's compositions that can inhibit bacterial growth is *sodium monofluorophosphate*, which has a therapeutic nature means to kill bacteria. Fluorine ion on *monofluorophosphate* can inhibit bacterial cell glycolysis and interrupt the formation of energy and phosphate ions that can cause agglutination or adhesion and binding of the bacteria [12]. Toothpaste also contains fluoride which serves as an anti caries and serves as initial caries re-mineralization [13].

Kencur extract toothpaste also has antibacterial activity against *Streptococcus mutans*, albeit in much smaller amount than toothpaste brands 'A'. *Kencur* has an active compound, such as caemferol, that is able to inhibit growth and kill bacteria (anti-bacterial effect). The process of inhibition of the microbes is because the bioactive hydroxyl group compound (OH) reacts with the material components in the cell of the microorganism, hence the microbes are left with no activity and eventually die [14]

Kencur also contains essential oils that serve as an anesthetic and antiseptic. Antiseptic is medicine that counteract or prevent sepsis circumstances, these substances can kill or prevent the growth of microorganisms [10].

Kencur also contains flavonoids as an antibacterial, which inhibits the development of microorganisms. Flavonoids can form complex compounds with proteins through hydrogen bonds by denaturing the protein molecules and nucleic acids that cause coagulation and freezing of proteins. The process will eventually cause metabolic disorders and disruption in bacterial physiological functions. If the bacterial metabolism is disrupted, the bacteria's energy needs are not fulfilled; resulting in permanent bacterial cells damage that ultimately leads to death [8].

Kencur extract toothpaste was stored in a refrigerator and tested on the bacterium *Streptococcus mutans* within three days after the production. Storage at room temperature and/or testing over three days after the making may affect the ability of extracts kencur in toothpaste in inhibiting the growth of *Streptococcus mutans*.

In previous studies on antimicrobial test on the compound ethyl p-methoxy cinnamic extracts against *Bacillus subtilis*, showed that the compound ethyl p-methoxy cinnamic on *kencur* extract (concentration of 0.1 M, 0.2 M, 0.3 M) has inhibitory power of 12 mm; 13.66 mm; 15.66 mm [15]. The aforementioned result is different from this study because this test used *Streptococcus mutans* rather than *Bacillus subtilis.*

CONCLUSION

Kencur extract toothpaste has an inhibition to the growth of SM bacteria of 2.95mm. Compared with toothpaste brand 'A', there is a difference in inhibitory effect on bacteria SM. Toothpaste brand "A" has a greater inhibition compared with *kencur* toothpaste. Our study showed that *kencur* extract toothpaste has an antibacterial effect against *Streptococcus mutans*. However, it is not as effective as regular toothpaste.

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