

THE POTENTIAL OF LIME PEEL EXTRACT MOUTHWASH AS A NON-PHARMACOLOGICAL PREPARATION INHIBITS CARIES BACTERIA

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ABSTRACT

Dental caries is a disease of the hard tissue of the teeth that attacks the enamel, dentin and cementum which is characterized by the demineralization of the hard tissue of the teeth. Streptococcus mutans bacteria itself is one of the bacteria found and lives in the oral cavity. One of the infectious diseases caused by the process of removing minerals due to bacteria on the surface of the teeth is called caries. An alternative choice as an effort to solve the problem of inhibiting caries-causing bacteria is by using herbal mouthwash. One of them is lime peel. Lime peel has the potential to inhibit Streptococcus mutans bacteria as indicated by the formation of an inhibition zone, which is formed against Streptococcus mutans bacteria. Making a mouthwash preparation of lime peel extract (*Citrus Aurantifolia*) which is feasible and effective in inhibiting the growth of Streptococcus mutans bacteria. This research is a true experiment with post test only control group design. This design was chosen because it was grouped into treatment and control groups. The intervention was given in the form of lime peel extract mouthwash with concentrations of 15%, 25% and 50%, while the administration of the UNISSULA Faculty of Medicine Biomedical Laboratory Mouthwash Formula was the control group. Based on the average inhibition graph within 24 hours, the concentration of extract mouthwash The lowest lime peel was 15% = 8.83 mm, the highest inhibition zone was at a concentration of 50% = 10.50 mm, 25% concentration = 9.80 mm, the control group Formula Mouthwash Biomedical Laboratory Concoction of the Faculty of Medicine UNISSULA = 10, 13mm. Lime peel extract mouthwash is feasible and effective in inhibiting streptococcus mutans which causes caries.

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INTRODUCTION

Dental and oral disease is a community disease that can affect all age groups (Wahyuningsih et al., 2020). Dental and oral disease is the most complained disease by the people of Indonesia, even ranking first out of 10 diseases. This condition deserves attention, because it illustrates the perception and behavior of Indonesian people towards dental and oral health is still bad, one of which is dental caries (Supriyanto et al., 2019).

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According to WHO (World Health Organization) data in 2016 states caries occurs in school children by 60-90% and almost 100% of caries is found in adults (Putri et al., 2020). While the results of Basic Health Research (Riskesdas) in 2018, the percentage of Indonesian people who experience dental caries is 45.3%. this shows that the average Indonesian population has 4-5 teeth per person who have problems with dental caries (Safela et al., 2021). The prevalence of caries in Central Java province at the age of 12 years and over is 67.8% and 43.1% is active dental caries, with the highest prevalence in Semarang City as much as 74% (Pratiwi et al., 2016).

Therefore, there is a need for alternatives in inhibiting bacteria that cause dental caries, one of which is by using mouthwash from natural ingredients (Hawwa et al., 2021). The use of natural ingredients can reduce the use of synthetic ingredients in medicine, one of which is lime peel (Usman & Rambung, 2020). Lime peel is a waste that is widely wasted and underutilized. Oranges have many antibacterial activities. The leaves and skin of lime fruits have activity to inhibit the growth of pathogenic bacteria in the mouth such as *streptococcus* and *lactobacillus*. The content in thin skin that can be useful as an antibacterial includes flavonoids, tannins, saponins, alkaloids and essential oils (Widyastuti et al., 2019).

Flavonoids are the largest group of polyphenolic compounds that can work as antioxidants and also as antibacterial by denaturing bacterial cell proteins and damaging bacterial cells.

Tannins have a role to inhibit reverse transcriptase enzymes and DNA topoisomerase so that bacterial cells cannot form, inactivate microbial cell adhesion, interfere with cell protein transport, and damage cell wall polypeptides so that cell wall formation becomes less perfect.

The mechanism of action of alkaloids as antibacterial by disrupting the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death. Alkaloids are also known as DNA interchelators and inhibit the *topoisomerase enzyme* of bacterial cells.

The mechanism of action of saponins is to cause leakage of proteins and enzymes from within the cell. Saponins damage membrane permeability and interfere with bacterial survival and diffuse through the outer membrane and cell wall and bind to the cytoplasmic membrane thereby disrupting and destabilizing the cell membrane, causing cytoplasm to exit the cell and result in cell death.

The content of essential oils can inhibit and kill microorganisms is associated with its ability to hydrophobic microorganisms. This causes oil to be partitioned on the cell membrane of the lipid bilayer, which affects the respiratory chain and causes leakage of bacterial cell contents. Weaknesses in bacterial enzyme systems can also be a potential mechanism of action. Various components of essential oils can increase the permeability of bacterial cells and increase the penetration of antibiotics.

In the research of Luthfia Dyah Puspita, Wulansari et al 2020, the antibacterial efficiency of lime extract (*Citrus Aurantifolia*) and stevia leaves as a non-ethanol mouthwash. Using mouthwash 40 ml, 50 ml and 60 ml with weak categories So

researchers are interested in examining the same study but using lime peel with dose variants increased to 15%, 25% and 50%.

Based on the description of his background, the researcher is interested in conducting research on "the potential of lime peel extract mouthwash (*Citrus aurantifolia*) as a non-pharmacological alternative ingredient to inhibit caries-causing bacteria."

Research Objectives

Developing a mouthwash of lime peel extract (*Citrus Aurantifolia*) which is feasible as a non-pharmacological preparation to inhibit the growth of *streptococcus mutans* bacteria.

Research Benefits

The results of this research can be used as reference material in developing research activities or used as a literature review for Poltekkes Kemenkes Semarang students and add insight and knowledge that is expected to improve the quality of education.

RESEARCH METHODS

This research is an analytic observational study using a case-control design, namely an epidemiological study design that studies the relationship between exposure (research factors) and disease by comparing the case group and the control group based on exposure status. This research was conducted at the NTB Provincial General Hospital in January 2023. The sampling technique used was the total sampling technique with total data on the incidence of uterine atony in mothers giving birth for five years, namely 2017-2021. Samples were taken through secondary data from medical record data of 60 samples with a group of cases of mothers who experienced uterine atony of 30 samples compared to the control group of mothers who did not experience uterine atony of 30 samples who had fulfilled the inclusion and exclusion criteria, so that from these data will be analyzed univariately and bivariately to then see the distribution of each sample and assess the relationship between research variables. The data obtained were analyzed using the chi-square correlation test.

RESULTS AND DISCUSSION

Results

Based on research on the inhibition of lime peel extract mouthwash against *Streptococcus mutans* bacteria, the results obtained were the inhibition of *Streptococcus mutans* bacteria, carried out in each treatment group, namely lime peel extract mouthwash with a concentration of 15, a concentration of 25%, a concentration of 50% and a control group.

Table 1 Resistance Test

The Potential of Lime Peel Extract Mouthwash as A Non-Pharmacological Preparation
Inhibits Caries Bacteria

Treatment	Resistance Zone (mm)						Mean	Inhibitory Zone Categories
	1	2	3	4	5	6		
Mouthwash Formula Concoction of the Biomedical Laboratory, UNISSULA Faculty of Medicine	10,20	9,10	12,0	10,0	7,50	12,0	10,13	strong
Mouthwash 15%	9,90	9,0	9,0	8,70	8,20	8,20	8,83	currently
Mouthwash 25%	9,10	9,10	9,10	10,50	11,0	10,0	9,80	currently
Mouthwash 50%	11,20	10,20	10,40	10,50	9,20	11,50	10,50	strong

The table above shows that the average inhibitory power of the lime peel extract mouthwash treatment group and the control group had inhibition power at various concentration variants on the growth of streptococcus mutans bacteria in repetition or replication time. In the lime peel extract mouthwash treatment group, the lowest inhibitory zone was at a concentration of 15% = 8.83, and the highest inhibitory zone was at a concentration of 50% = 10.50 mm.

Tabel 12. Uji Normalitas dan homogenitas

Bacteria	Group	Shapiro- Wilk	Levene	Sig.(p)
<i>Streptococcus Mutans</i>	Mouthwash 15%	0.546	1.943	0.157
	Mouthwash 25%	0.358		
	Mouthwash 50%	0.117		
	Medicinal Formula Gargle Blend Laboratory UNISSULA Faculty of Medicine Biomedicine	0.768		

Based on the table above shows the sig. on the Shapiro-Wilk test (> 0.05) in all treatment groups and the control group, so that it can be concluded that all data are normally distributed and the variance of the data is homogeneous. It can be seen from the Lavene statistical test that the value is sig. = 0.000 (> 0.05), which means that the data variance between treatment groups and the control group on the inhibition of *Streptococcus mutans* bacteria is homogeneous.

Table 3. Anova Test

Group	Mean±SD	P-value
Concentration mouthwash 15%	8.833±0.634	
Concentration mouthwash 25%	9.800±0.829	0.082
Concentration mouthwash 50%	10.500±0.809	
Formulated Mouthwash Formula	10.133±1.731	

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Based on the table above, the results of the anova statistical test obtained a value of ($p > 0.05$) which showed no significant difference between the lime kulilt extract mouthwash treatment group and the control group of the mouthwash formula concocted by the Biomedical Laboratory of the Faculty of Medicine UNISSULA on the measure of bacterial *inhibitionstreptococcus mutans*.

Table 4. Regression Test

Concentration	Average Diameter of the Inhibitory Zone (mm)	Regression Test <i>R square</i>
Concentration mouthwash 15%	8,83 mm	0,039
Concentration mouthwash 25%	9.80 mm	
Concentration mouthwash 50%	10,50 mm	
Formulated Mouthwash Formula	10,13 mm	

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The coefficient determination of R square of 0.039 means the effect of mouthwash of lime peel extract with various concentration variants on the diameter of the growth inhibition zone of *streptococcus mutans* bacteria by 3.9%.

Discussion

Observations are made by measuring the clear zone formed using a caliper, so it can be called the inhibitory zone. In the results of collecting data on the inhibitory power of bacteria *streptococcus mutans* Conducted with the ANOVA test which showed that the ability of the mouthwash formulation of lime peel extract concentration of 15%, concentration of 25% and concentration of 50% did not have a significant difference with the control group $p = 0.082$. This concentration is classified as antibacterial which is good in inhibiting bacterial growth *streptococcus mutans*. This is in accordance with Alfia Fitriani's 2016 research where lime juice combined with honey was able to inhibit the growth of *streptococcus mutans* bacteria at a concentration of 15%, a concentration of 25% and a concentration of 50%.

At concentrations of 15% and 25%, they were no better than the control group, the mouthwash formula concoction of the UNISSULA Faculty of Medicine Biomedical

Laboratory. This is because the mouthwash formula concoction of the Biomedical Laboratory, Faculty of Medicine, UNISSULA is influenced by the mechanism of action of compounds in the form of benzoic acid found in the formulary control group. The mechanism of action of benzoate compounds is to absorb benzoic acid in the cells, then the intracellular pH of the cells becomes acidic, then the anaerobic fermentation of glucose decreases drastically which causes cell growth and development of microorganisms to be inhibited.

The lime fruit extract which was analyzed using the one way ANOVA test showed that the lime peel extract had a large area of inhibition zone. The average inhibition zone at concentrations of 40%, 60% and 80% was 14.2 mm, 19.6 mm, 22.6 mm but these results were smaller than the positive control group which was 36.6 mm (Parama et al., 2019).

The concentration of lime peel extract mouthwash starting from a concentration of 15%, 25% and 50% was able to create an inhibition zone against the growth of *Streptococcus mutans* bacteria. One of the determining factors is the size of its ability to inhibit the bacteria tested with several categories. The category in determining the inhibition zone that is formed ≥ 20 mm is considered to have a strong inhibitory activity, 10-20 mm is declared to have a strong inhibitory activity, 5-10 mm is declared to have moderate inhibitory activity and ≤ 5 mm is declared to have a moderate inhibition activity weak. (Sinta et al., 2020)

The inhibitory power of lime peel extract mouthwash against the growth of *streptococcus mutans* bacteria is most effective at a concentration of 50%. This means that the greater the concentration of lime peel extract mouthwash given, the greater the percentage of inhibitory power produced, with a concentration of 65% able to inhibit the formation of dental plaque (Kamila et al., 2021). As for some compounds contained in thin skin that can be useful as antibacterials, including *lavonoid*, *tannin saponin*, *alkaloid*.

Flavonoid compounds cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA (*Deoxyribonucleic Acid*) (Hakim et al., 2018). Flavonoids inhibit cell membrane function by forming complex compounds with extracellular and dissolved proteins so that they can disrupt the integrity of bacterial cell membranes, damage bacterial cell membranes and followed by the release of intracellular compounds also inhibit cell membrane function by disrupting cell membrane permeability and inhibit enzyme binding also inhibit energy metabolism by inhibiting the use of oxygen by bacteria, Bacteria need energy for macromolecule biosynthesis, so if their metabolism is inhibited, bacterial molecules cannot develop so that bacterial cells will experience death (Agatha et al., 2021a).

Alkaloids are substances that have a tendency to inhibit bacterial growth, contain one or more nitrogenous atoms that are alkaline and are active substances (Fazil et al., 2017). The mechanism of action of alkaloids as antibacterial by disrupting the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed

intact and causes cell death. Alkaloids are also known as DNA interchelators and inhibit the topoisomerase enzyme of bacterial cells (Ngajow et al., 2013).

The mechanism of action of saponins is to cause leakage of proteins and enzymes from within the cell. Saponins damage membrane permeability and interfere with bacterial survival and diffuse through the outer membrane and cell wall and bind to the cytoplasmic membrane thereby disrupting and destabilizing the cell membrane, causing cytoplasm to exit the cell and resulting in cell death.(Agatha et al., 2021b). Saponin compounds are complex glycoside compounds, which are compounds resulting from sugar condensation with hydroxyl compounds which if hydrolyzed will produce glycon and non-sugar. In addition, saponins are active compounds that have soap-like properties, are strong surface active compounds, which cause foam when shaken in water.

Tannins are astringent compounds that have an astringent taste from their polyphenol groups that can bind and precipitate or shrink proteins. Tannins can dissolve in water, their solubility is large and will bind when dissolved in hot water, tannins will dissolve in organic solvents such as methanol, ethanol, acetone, and other organic solvents. Tannins are able to inhibit reverse transcriptase enzymes and DNA topoisomerase so that bacterial cells cannot form, inactivate microbial cell adhesion, interfere with cell protein transport, and damage cell wall polypeptides so that cell wall formation becomes less perfect (Ngajow et al., 2013).

CONCLUSION

Lime peel extract mouthwash is feasible and effective in inhibiting streptococcus mutans which causes caries. The formulation of lime peel extract mouthwash is effective in inhibiting the growth of Streptococcus mutans at a concentration of 15% in the moderate category (5-10 mm). The formulation of lime peel extract mouthwash is effective in inhibiting the growth of Streptococcus mutans at a concentration of 25% in the moderate category (5-10 mm). The formulation of lime peel extract mouthwash is effective in inhibiting the growth of Streptococcus mutans at a concentration of 50% in the strong category (10-20 mm).

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