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Comparison Analysis of Temperature Treatment on Cutleaf Groundcherry's Leaf and Stem

Lilis Supratman[1*] and Raden Teti Rostikawati[2]

[1,2]Biology Education Study Program, Universitas Pakuan, Bogor, Indonesia

**Email: lilissupratman74@gmail.com*

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Abstract

The mouth has a normal microflorane of bacteria as the body's defense. Streptococcus mutans bacteria are bacteria that colonize the surface of teeth which have a role in the formation of dental caries. The leaves of ciplukan (Physalis angulata L.) are rich in polyphenols, alkaloids, and flavonoids which are reported to have quite good antimicrobial activity. The aim of this research was to determine the effect of ethanol extract of ciplukan leaves and stalks mouthwash on Streptococcus mutans bacteria. This research method is purely laboratory experimental using Streptococcus mutans bacteria culture. The antibacterial power test used the liquid dilution method which was then followed by solid dilution with an incubation period of 28 ° C. The statistical analysis test used a descriptive test. The results showed that the ethanol extract of the leaves and stems of ciplukan (Physalis angulata L.) had a minimum inhibitory level (MIC) and a minimum kill rate (MBC) at the same concentration, namely 15%. The temperature of the human oral cavity in cold places tends to change because the mouth is very close to the outside environment, so this mouthwash can be recommended to minimize bacterial growth in cold areas..

Keywords*:* cutleaf groundcherry; mouthwash; *Streptoccus mutans*

INTRODUCTION

Dental caries is a hard tissue disease involving enamel, dentin and cementum. Caries is caused by an activity of microorganisms in a dispersible carbohydrate characterized by demineralization of tooth hard tissue. Based on the classification of streptococcal bacteria, *Streptococcus mutans* is a group of *Streptococcus viridans* bacteria. *Streptococcus mutans* colonizes the tooth surface in the supragingiva and is a complex bacteria consisting of 700 species.

The use of mouthwash as an aid in oral hygiene is relatively new to developing countries in the world. In addition, all herbal mouthwash solutions do not use alcohol and sugar, both of which are food for microorganisms. The cutleaf groundcherry plant is an annual herbal plant found in various tropical regions of the world. This plant can be found in tropical continents including Africa, Asia and America. The cutleaf groundcherry plant grows to 1 meter with small cream-colored flower stems and yellowish

orange fruit color. Based on phytochemical research, it is known that the roots and stems of the cutleaf groundcherry plant contain saponins and flavonoids (Fitrianti, 2011).

Cutleaf groundcherry leaves are rich in polyphenols, alkaloids and flavonoids. The active ingredient is reported to have fairly good antimicrobial activity. Based on the above background, it is important to conduct research on the effectiveness of the antibacterial power of cutleaf groundcherry leaves ethanol extract mouthwash to determine the antibacterial power of the active compounds contained in cutleaf groundcherry leaves and stems against the bacteria *Streptococcus mutans* which causes caries. Meanwhile, according to Susilowati (2020), cutleaf groundcherry roots and stems contain saponins and flavonoids. Medical benefits can be used as a drug for diabetes mellitus and high blood pressure.

METHOD

This research is a purely laboratory experimental study conducted in vitro. There were 5 concentration groups of cutleaf groundcherry leaves and stem (*Physalis angulata* L.) ethanol extract mouthwash, namely 5%, 10%, 15%, 20%, and 25% and a positive control group, namely the basic formula of mouthwash and 0.2% Chlorhexidine gluconate. as a negative control. Isolation of *Streptococcus mutans* bacteria was carried out by subculturing it in Tryptic Soy Agar (TSA) media for 18-24 hours at 28 ° C. Then using sterile ose several bacterial colonies were selected and put in 1-2 ml of NaCl solution. Then incubated for 2–4 hours at 28 ° C. Then diluted by adding BHI (Brain Heart Infusion) to obtain the number of germs in accordance with the Standard Brown III solution identified with a germ concentration of 108 CFU / ml. Then the *Streptococcus mutans* bacteria were diluted again using BHI liquid medium so that the concentration of bacteria became 106 CFU / ml.

Table 1. Composition of cutleaf groundcherry leaves and stems of ethanol extract mouthwash (*Physalis angulata* L.)

Material	Formula I	Formula II	Formula III	Formula IV	Formula V
	5%	10%	15%	20%	25%
Ethanol extract	2.5	5	7.5	10	12.5
Peppermint oil (ml)	0.5	0.5	0.5	0.5	0.5
Na-Saccharine (gr)	0.3	0.3	0.3	0.3	0.3
Benzoat acid (gr)	0.025	0.025	0.025	0.025	0.025
Aquades ad (ml)	50	50	50	50	50
End volume (ml)	50	50	50	50	50

The leaves of cutleaf groundcherry (Physalis angulata L.) which have been extracted in the laboratory are made in the form of 5 mouthwash formulas which can be seen in the table. 1.

The test for the antibacterial power of ciplukan leaf extract (Physalis angulata L.) is by using the tube dilution method. 28 sterile tubes were provided with 4 repetitions, each dilution in one repetition using 5 tubes and 2 tubes were used for the remaining dilution, germ growth control (positive control) and media control (negative control). The first dilution is to test for the minimum inhibitory levels and the minimal kill levels of the ciplukan leaves and stems. 7 sterile test tubes (2 for control) were prepared:

a. Tube I is filled with 1 ml formula 1 + 1 ml bacterial suspension 106 CFU / ml.
b. Tube II is filled with 1 ml formula 2 + 1 ml bacterial suspension 106 CFU / ml.
c. Tube III is filled with 1 ml of formula 3 + 1 ml of bacterial suspension 106 CFU / ml.

d. The IV tube is filled with 1 ml of formula 4 + 1 ml of bacterial suspension 106 CFU / ml.
e. Tube V was filled with 1 ml of formula 5 + 1 ml of bacterial suspension 106 CFU/ml.
f. Tube VI filled with 1 ml Chlorhexidine gluconate 0.2% + 1 ml bacterial suspension (control -)
g. Tube VII was filled with 1 ml of basic formula of mouthwash with ethanol extract of leaves and stems of ciplukan with a concentration of 0% + 1 ml of bacterial suspension 106 CFU / ml (control +).

All tubes were incubated at 37 ° C for 18-24 hours. Observations were made on the presence or absence of germ growth by comparing the turbidity level with a positive control. The minimum inhibitory level is obtained by observing the tube which does not show the growth of germs with the lowest concentration. Subcultures that did not show any germ growth were then planted using ose on Tryptic Soy Agar (TSA) media which was incubated at 37 ° C for 18-24 hours. The minimum kill rate is indicated by no bacterial growth in the medium for the lowest concentration of nutrients.

KHM readings were determined by looking at the turbidity in the liquid in the test tube compared to standard controls. Value readings are based on:

a. Negative sign (-): by looking at the clarity of the tube, it shows the absence of the growth of *Streptococcus mutans* bacteria so that the ciplukan leaf extract mouthwash can inhibit bacterial growth.
b. Positive sign (+): by looking at the turbidity in the tube, it shows the growth of *Streptococcus mutans* bacteria so that the ciplukan leaf extract mouthwash cannot inhibit bacterial growth.

The reading of KBM can be determined by testing the smallest concentration of test material that can still kill bacteria. This is indicated by the presence or absence of the growth of *Streptococcus mutans* bacteria colonies on Tryptic Soy Agar (TSA) media.

The research data were analyzed descriptively in the form of research results tables. The results are then discussed by looking at the minimum inhibitory levels (MIC) and minimal kill levels (KBM) of the antibacterial power of the ethanol extract of ciplukan leaves (*Physalis angulata* L.) mouthwash against *Streptococcus mutans* bacteria.

RESULT AND DISCUSSION

Based on the results of the liquid test, the formula for mouthwash with ethanol extract of ciplukan leaves and stems (Physalis angulata L.) (Table 3) at a concentration of 15% shows a clear formula meaning there is no bacterial growth. Further tests were carried out by growing the formula on solid media and there was no bacterial growth.

Table 3. The results of testing the liquid dilution of ciplukan leaf extract (*Physalis angulata* L.) in the form of a mouthwash against *the Streptococcus mutans* bacteria

Tube	Materials	I	II	III	IV
1	Formula I	-	-	-	-
2	Formula II	-	-	-	-
3	Formula III	-	-	-	-
4	Formula IV	-	-	-	-
5	Formula V	TT	TT	TT	TT
6	Negative Control	-	-	-	-
7	Positive Control	+	+	+	+

The results of the solid dilution test in table 4 show that at the lowest concentration, there is no bacterial growth on solid BHI media so that the minimum kill rate (KBM) of ciplukan leaf and stem extract (*Physalis angulata* L.) mouthwash against *Streptococcus mutans* bacteria is present at a concentration of 15%.

Table 4. Descriptive results of the liquid dilution test

Observation component	Formula I 5%	Formula II 10%	Formula III 15%	Formula IV 20%	Formula V 25%
Color of the solution before incubation	clear	clear	clear	clear	clear
Color of the solution after incubation	clear	clear	clear	clear	clear
Odor	peppermint	peppermint	peppermint	peppermint	peppermint
Sediment/no	no	no	no	no	yes
Sediment color	no	no	no	yes	white
Gas bubbles/no	yes	yes	no	no	no

This research was conducted to determine the effect of the cutleaf groundcherry leaf and stem extract test (Physalis angulata L.) in the form of a mouthwash against *Streptococcus mutans* bacteria in vitro using the liquid dilution method and followed by inoculation tests on Tryptic Soy Agar (TSA) media to determine inhibition levels. minimum (KHM) and minimal kill rate (KBM).

In this study, a temperature of 28 ° C was used, this temperature is related to the temperature of the human mouth organs in highlands or cold areas where the temperature reaches 5 ° C. The results showed that 15% concentration was quite effective in the formulation. It is suspected that this formula is suitable for reducing bacteria that can live optimally at a temperature of 20 ° -40 ° C. Sunatmo (2009) states that this group of bacteria is often known as the psychrotolerant group of bacteria. These bacteria are often found in environments with constant cold temperatures and will die immediately at room temperature so that when they are examined, transported, isolated and given other treatments. This is because these bacteria prefer constant cold temperatures than very cold temperatures. The formulation of mouthwash with a concentration of 15% is thought to minimize the number of harmful bacteria in the oral cavity that inhabit cold areas so that it can be recommended to do further research with other endemic bacteria in the oral cavity.

Based on the observations, it shows that the extracts of the leaves and stems of cutleaf groundcherry (*Physalis angulata* L.) in the form of mouthwash have an antibacterial effect against *Streptococcus mutans* bacteria. The minimum inhibitory level (MIC) is the lowest concentration which indicates the absence of bacterial growth that can inhibit bacterial growth and is observed based on the level of turbidity in the tube. The minimum kill rate is the lowest concentration that can kill bacterial growth and is observed based on the absence of bacterial colonies on the agar medium.

The minimum inhibitory level (MIC) was observed by the liquid dilution method which was determined based on the turbidity level in the tube where at a concentration of 15% showed clarity in the tube or there was no bacterial growth then inoculated on agar medium and incubated for 18-24 hours. The observations of solid dilution at a concentration of 15% have shown that there is no bacterial growth on the agar medium so that the possibility of the minimum inhibitory content (MIC) is at a concentration below 15%. Observation of the solid dilution test results showed that the concentration of 15% is the minimum kill rate (KBM).

The solid dilution test is a test carried out on agar media to determine the minimum kill rate (MBC) and to confirm the results of the liquid dilution test. The minimum kill rate (MBC) against *Streptococcus mutans* bacteria at a concentration of 15% was obtained by observing the growth of bacteria resulting from liquid dilution which was inoculated on the media so that it was marked by no growth of *Streptococcus mutans* bacteria on agar media.

One of the main bacteria that causes dental caries is *Streptococcus mutans* which produces the enzyme glucosyltransferase (GTF), so that these bacteria can form colonies that are firmly attached to the tooth surface. Octrian (2018) which states that *Streptococcus* can cause dental plaque and can further cause dental caries. *Streptococcus mutans* produces sticky extracellular polysaccharides from dietary carbohydrates and is able to ferment carbohydrates to acids. The bacterial cell wall protects the cytoplasmic membrane, maintains cell shape, and prevents lysis due to osmotic pressure. If the cell wall is damaged or not formed, the cell will lysis or cannot divide. Cell lysis occurs because the surrounding hypoosmotic fluid diffuses into the cell causing swelling (swell) followed by lysis. In addition to producing polysaccharides, these endemic bacteria can also produce acids, which form acids that can damage teeth because they can be used by other bacteria as an energy source to demineralize the surface of tooth enamel (Susanti, 2013).

The flavonoids contained in the ethanol extract of ciplukan leaves and stems have the ability to form complexes with extracellular and dissolved proteins, and with cell walls, and have lipophilic properties. This activity causes damage to the cytoplasmic membrane so that the bacterial cells will be damaged and die, as well as the cell membrane will be damaged. Alkaloids are the end products of detoxification reactions which are the final metabolites of components that are harmful to plants. Alkaloids have an antibacterial function by disrupting the components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes bacterial death.

Aneja (2010) stated that the difference in the sensitivity of bacterial cell walls to antibacterials is influenced by the structure of the cell wall of gram-positive bacteria which is simpler than the structure of the cell wall of gram-negative bacteria, making it easier for antibacterial compounds to enter into gram-positive bacterial cells. The cell wall of Gram positive bacteria contains (teichoic acid) (Aneja, 2010). Teichoic acid is a water-soluble polymer that functions as a positive ion transport. This solubility indicates that the cell wall of gram-positive bacteria is more polar. Aneja (2010) explained that polar flavonoids are easier to penetrate polar peptidoglycan than nonpolar lipid layers, causing the inhibitory activity of gram-positive bacteria to be greater than gram-negative. The mechanism of action of flavonoids as bactericides against the growth of *Streptococcus mutans* is to interfere with the function of the cell wall as a protector from osmotic lysis, resulting in bacterial cell death. This is in line with Jawetz's (1996) statement, which states that flavonoids as antibacterial compounds inhibit bacterial protein and nucleic acid synthesis, by means of antibacterial compounds destroying nucleic acids and denaturing proteins, causing disruption of protein and nucleic acid synthesis processes, resulting in damage to cells in total. Molan (1992) stated that antibiotics can withstand the attack of disease-causing pathogens. Saponins found in cutleaf groundcherry stems can act as antibiotics. According to Sulistiowati (2020), antibiotics in saponins can significantly minimize bacterial growth. This strengthens the recommendation that the extract and stem of the cutleaf groundcherry can be used as a qualified mouthwash.

The polyphenol content in ciplukan leaves has a mechanism of action on microorganisms as enzyme inhibitors by oxidized compounds, possibly through reactions with sufhydryl groups or through reactions with sufhydryl groups or through non-specific interactions with proteins (AS, Noorhamdani, 2014). The inhibition of the enzyme will interfere with the function of the enzyme and its substrate. If the function of enzymes and substrates is disrupted, it will gradually result in cell death (AS, Noorhamdani, 2014). Phenols bind to proteins through hydrogen bonds, causing the protein structure to be damaged. Because most of the structure of the bacterial cell wall and cytoplasmic membrane contains protein and fat, phenol is thought to also have the ability to denature proteins and bacterial cell membranes. The instability of the bacterial cell wall and cytoplasmic membrane causes the function of selective permeability, active transport function, and control of the protein structure of bacterial cells to be disturbed (Aneja, 2010).

CONCLUSION

Based on the results of research regarding the comparative analysis of temperature treatment on the formulation of mouthwash with extracts of cutleaf groundcherry leaves and stems as a source of biology learning, it can be concluded that the 15% formulation can be recommended for further research so that it can be used by people who live in cold temperature areas.

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