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Formulation of Mouthwash Honje Laka (*Etlingera Hemisphaerica* (Blume) R.M.Sm.) Flower Extract Against *Streptococcus Mutans*

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ABSTRACT

Dental and oral health is an important part of physical health that requires our attention. *Streptococcus mutans* (*S. mutans*) is known as a cariogenic microorganism because it breaks down sugar and forms tooth substances for energy production. One of the preventive measures against the occurrence of chemical dental plaque is the use of mouthwash. Therefore, a mouthwash formulation from natural ingredients was developed. The potential plant for natural mouthwash is the honje laka flower (*Etlingera hemisphaerica* (Blume) R.M.Sm.) which contains flavonoids, polyphenols, quinones, monoterpenoids, and sesquiterpenoids as antibacterial. This study aims to determine the potential of honje laka flower extract in inhibiting the growth of *S. mutans* and as an ingredient in mouthwash. The procedure of this research started from the extraction of honje laka flower using 96% ethanol solvent followed by phytochemical screening test and antimicrobial test with a well diffusion method. The concentration of honje laka flower extract used for MIC test were 5%, 10%, and 15%. Evaluation of honje laka extract mouthwash preparations were organoleptic test, pH test, centrifugation test, cycling test, and bacterial inhibition zone test. The results showed the ethanol extract of honje laka flowers has activity against *S. mutans* with a MIC at a concentration of 5% and inhibition zone at 9.22±0.115 mm, while the mouthwash formula at a concentration of 15% has an inhibitory zone at 9.35±0.02 mm. As a conclusion, ethanol extract from honje laka flower can be used as a mouthwash and can inhibit the activity of *S. mutans*.

Keywords: Activity; Honje Laka flower; Mouthwash; *Streptococcus mutans*

INTRODUCTION

Dental and oral health is an important parameter in physical health that we need to take care of. According to World Health Organization (WHO) data in The World Oral Health Report 90% of the Indonesian population still suffers from dental and oral diseases, but this is far from estimates. Based on data from the National Basic Health Research (RIKESDAS) in 2018, around 57,6% of the Indonesian population has dental and oral problems. The largest proportion of dental and oral health problems affecting the Indonesian population is gums swelling or boils (abscesses) 14% (Skripsa et al., 2021).

Streptococcus mutans (*S. mutans*) is a microorganism that plays an important role in the development of tooth decay. *S. mutans* bacteria are cariogenic bacteria that are strongly attached to the tooth surface and from a colony. These colonies ferment the remaining sucrose or carbohydrates into acid, thereby lowering the pH of the mouth and tooth surfaces (Suryani et al., 2019).

An antibacterial activity test was held determine whether a certain compound on plant can inhibit bacterial growth by measuring the response of bacterial growth to its compound. Antibacterial activity can be studied by various methods, namely the solid dilution method, the disc diffusion method, and the liquid dilution method. The activity of plant compounds was observed by measuring a clear area around the disc paper which indicated an inhibition zone on bacterial growth (Balouiri et al., 2016). Another method is to make wells on the surface of the solid agar which had been inoculated with the test bacteria. The location of the well holes can be cracked or damaged, this can interfere with the absorption of antibiotics into the media which affects the formation of clear zone diameters when carrying out a sensitivity test (Nurhayati et al., 2020). The antibacterial activity power was categorized based on the diameter of clear zone i.e 20 mm or more indicates very strong, 10-20 mm indicates strong, 5-10 mm indicates moderate and 5 mm or less indicates weak (Willia, 2016).

The use of mouthwash is one method of preventing the development of chemical dental plaque. To clean the respiratory tract and freshen the breath, mouthwash is a solution that also contains emulsions, surfactants, antibacterial agents, and astringents. Mouthwash is used to maximize tooth cleaning and has antiseptic properties. The advantage of mouthwash is that it's easy to carry everywhere. The mouthwash was practical when used compared to other oral preparations, eg. toothpaste. There are several types of mouthwashes, including mouthwashes containing 0.2% Clorhexidin and those containing essential oils, the main ingredient of which is methyl salicylate. This type of mouthwash can effectively reduce plaque formation, reduce periodontal abnormalities and reduce symptoms of gingivitis (NS, 2010).

Honje laka (*Etlingera hemisphaerica* (Blume) R.M.Sm.) is one of the natural active ingredients in mouthwash. All parts of the flowering plant honje containing flavonoids, alkaloids, phenols, tannins, steroids, saponins, glycosides, and triterpenoids. Previous study has shown that honje laka flowers has antibacterial and antioxidant properties (Ahmad et al., 2015). The antibacterial substance of the ethanolic extract from the honje laka flower can inhibit various bacteria such as *Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli, Salmonella typhimurium, Listeria monocytogenes* (Soemarie et al., 2019) and *Staphylococcus aureus* (Binugraheni & Larasati, 2020)

The compound of the honje laka flower contains several active substances such as alkaloids, flavonoids, polyphenols, steroids, and essential oils (Sukandar et al., 2010). Based on the research conducted, the content of secondary metabolites in honje laka flowers includes phenolic compounds consisting of gallic acid, caffeine tannic, and chlorogenic; while the content of flavonoids include quercetin, apigenin, kaempferol, luteolin, and myricetin and have biological activities such as antiasthmatic, anticancer, antipyretic, antiinflammatory, anticarcinogenic, antimutagenic, antimicrobial and antioxidant (Ghasemzadeh et al., 2015). Flavonoid compounds are compounds that have antibacterial effects.

Flavonoids function as free radical scavengers and are beneficial to increase the effectiveness of vitamin C, as well as antibiotics by interfering with the function of microorganisms such as bacteria and viruses (Malinggas & Mariati, 2015). The mechanism of action of flavonoids as antibacterials is to form complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane and be followed by the release of intracellular compounds (Warganegara & Restina, 2016). Polyphenols can damage the bacterial cell wall (Komala et al., 2020).

Honje laka also known as honje, kantan, or torch ginger, is a plant from the Zingiberaceae family (Jackie et al., 2011). Pink flowering honje plants are easier to find in the countryside or forests than red or white flowers. In Australia, Hong Kong, Brazil, the United States, and Thailand it is used as a wreath (Choon & Ding, 2016).

Research on the activity test of the honje laka flower has never been studied before. This gives interest to researchers in reviewing the antibacterial potential of honje laka flower extract mouthwash against *S. mutans* bacteria.

MATERIAL AND METHODS

Materials

The materials used are Honje laka (*Etlingera hemisphaerica* (Blume) R.M.Sm.) flower was collected from Cisangkal village, Pangandaran West Java, Indonesia. The solvent for this experiment was aquadest, 96% ethanol (Quadrat), and chloroform (Smartlab). The mouthwash formulation used were tween 80, glycerin, sodium saccharine, and sodium benzoate (Merck). The bacterial test were *S. mutans* (ATCC 25175). The Muller Hinton Agar (Oxoid) over Nutrient Agar (Oxoid) and antibiotic test, clindamycin HCl, and DMSO as positive and negative control.

Tool

The tools used in the research were steam dish, glass, macerator, rotary vacuum evaporator (IKA HB10 basic, Germany), analytical balance (Excellent, China), thermometer, pH meter (Ohaus, USA), oven (Memmert, Germany), furnace (WiseTerm, China), autoclave (Biobase, China), and incubator (Memmert, Germany).

Methods

The honje laka flowers were collected and dried under the sun. The sample was powdered and sieved with mesh no. 40 to obtain dry powder sample (Soemarie et al., 2019). The extraction process of honje laka was carried out by maceration method using 96% ethanol solvent. As much as 1000 g was put in the macerator and added with 10 L of 96% ethanol. The filtrate was evaporated using a rotary evaporator and concentrated in a water bath until a thick extract was obtained (Gustandy & Soegiharjo, 2013).

Test of Parameter Quality

The test for parameter quality of plant extract includes macroscopic and microscopic examination, determination of water content, determination of total ash content, determination of acid-insoluble ash content, drying shrinkage, and phytochemical Screening. The extract and the powder of honje laka flower were then subjected to phytochemical tests to detect the presence of alkaloids, flavonoids, steroids, polyphenols, tannins, terpenoids, quinones, monoterpenoids, saponins, and sesquiterpenoids (Baitariza et al., 2020).

The Mouthwash Preparations of Honje Laka Flower Extract

Each ingredient in Table 1 is weighed as needed in the mouthwash formula. Tween 80 was dissolved in distilled water (M1). Dissolve sodium benzoate, sodium bicarbonate, sodium saccharin, and glycerin in aquadest, add the ethanol extract of the honje laka flower and stir until the oleum methane dissolves (M2). Mix M1 and M2 until homogeneous (Nurdianti et al., 2020).

Evaluation of Mouthwash Formula

Organoleptic testing includes an examination of color, shape, aroma, and taste. pH checks are carried out with a pH meter. Formulations that meet the oral pH criteria have a pH in the range of 6-7. In the centrifugation test, 2 mL of mouthwash was put in a centrifuge tube which was then centrifuged at a speed of 3000 rpm for a period of 30 minutes. The results of the process can be seen from the occurrence of separation or not.

Table 1. Honje Laka Flower Extract Mouthwash Formula

Material Name	F0	F1	F2	F3	Function
Honje Laka Flower Extract	-	5%	10%	15%	Active Pharmaceutical Ingredient (API)
Tween 80	10%	10%	10	10%	Surfactant
Glycerin	2.5%	2.5%	2.5%	2.5%	Humectants
Saccharin Sodium	0.1%	0.1%	0.1%	0.1%	Sweetener
Sodium Benzoate	0.5%	0.5%	0.5%	0.5%	Preservative
Oleum menthae	0.2%	0.2%	0.2%	0.2%	Freshener
Sodium Bicarbonate	-	1%	1%	1%	Source of Base
Aquadest ad	100%	100%	100%	100%	Solvent

Antibacterial Activity Test (MIC Test) of Honje Laka Flower Ethanol Extract

The test was carried out using well diffusion method. As much as 0.2 mL of bacterial suspension and 20 mL of Mueller Hinton Agar (MHA) are placed in a sterile petri dish. The petri dish was rotated until well blended and allowed to harden. Four holes were made using cork borer on agar surface in each petri dish. As much as 50 µL Ethanol of honje laka flower extract of was inserted into the hole in each petri dish. Tests were carried out at various concentrations from 10% to 100%. Then incubated for 24 hours at 37°C. The diameter of clear zone formed on agar surface was measured as the inhibition zone. that would later be formed and determine the MIC from the lowest concentration that was still able to inhibit the growth of *S. mutans*.

Honje laka Flower Mouthwash Preparations Activity Test

A total of 0.2 mL of bacterial suspension and ± 20 mL of MHA were placed in a sterile petri dish. Rotate the petri dish until well blended, allow it to harden, and then make 4 holes in each petri dish. A p 50 µL of ethanol extract of honje laka flower mouthwash was prepared into the hole in each petri dish. Then incubated for 24 hours at 37°C. Measure the diameter of the inhibition zone formed (Handayani et al., 2016).

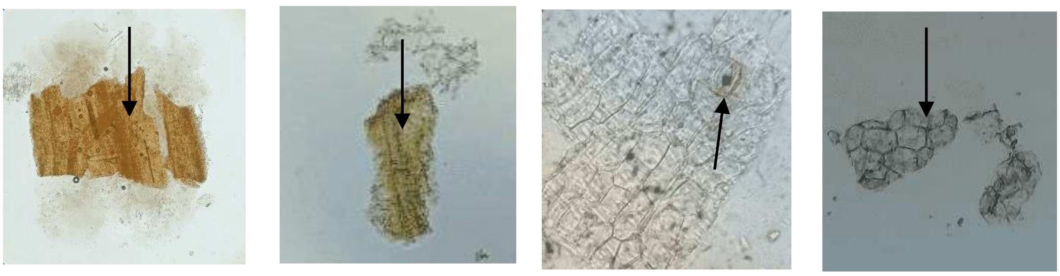
RESULT

Characteristics of Honje Laka

Honje laka flower powder (1000 g) was macerated with 96% ethanol as solvent. Obtained 31.66 grams of a thick extract with a yield of 3.16%. The simple macroscopic examination of the honje laka flower is red, has a distinctive and slightly sour aroma, the flower stalk is long and hard, the base of the flower is hump-shaped, the petals are tubular split, the flower crown is trumpet-shaped, the stamens are curved and the pistil is triangular (Lestari et al., 2014).

The honje laka flower dry powder has a distinctive aromatic aroma, red in color, and has a bitter taste (Figure 1). Microscopic observation of honje laka flower dry powder taken using a monocular microscope with magnification 40x (Figure 2). The results showed the presence of four fragments as shown below. The observation results confirmed refer to by Indonesian Herbal Pharmacopoeia (Depkes, 2017).

Figure 1. Macroscopic of Honje Laka Flower



a. **b.** **c.** **d.**

Figure 2. Microscopic results of honje laka flower powder in 40x magnification, a. Upper epidermis, b. Carrier beam with thickened ladder shape, c. Epidermis with stomata, d. Collenchyma.

Table 2. The Parameter Quality of Honje Laka Flower Extract

Parameter	Result (%) ± SD	Indonesian Herbal Pharmacopoeia Terms (2017)
Total ash content	9.40±0.76	10.6%
Acid insoluble ash content	0.29±0.13	4.7%
Water content	7.97±0.005	10 %
Water soluble juice content	19.50±0.40	11.6%
Ethanol soluble extract content	12.4±0.19	16.5%
Drying shrink	8.37±0.71	10 %

The Results of Parameter Quality test

The results of the study showed the fulfillment of the requirements of the Indonesian Herbal Pharmacopoeia regarding water content, total ash content water-soluble extract content, drying pressure, and acid-insoluble ash content (Depkes, 2017). The honje laka flower extract yield was 19.50%. The content of ethanol-soluble extract content of the average yield was 12.4%. The small amount of honje laka flower extract compounds that have dissolved in ethanol is not the same as that dissolved in water. This does not comply with the requirements of the Indonesian Herbal Pharmacopoeia (Depkes, 2017). The reason is that polar compounds such as tannins, saponins, and flavonoids are soluble in water (Supriningrum et al., 2019). The factors that greatly affect the quality of materials such as temperature, light, water, oxygen, humidity, nutrients, and the presence of maintenance affect the content of compounds in the honje laka flower plant. The determination of the specific gravity was obtained by the results of the specific gravity of the ethanol extract of 0.825gr/ml. The specific gravity value gives an idea of the dissolved chemical content (Depkes, 2000).

The Results of Antibacterial Activity Test

The microbial activity of Honje laka flower extracts was carried out using well diffusion methods. *S. mutans* was used as bacterial test. From the test (Table 4), the ethanol extract of honje laka flower with a concentration of 10%-100% can inhibit *S. mutans* bacteria in the strong category. This result is different from the research (Syahrani et al., 2021) where there is an inhibition zone of 11 mm at a concentration of 100%. This means that the sample under study has a higher activity than previous studies. The difference in the results occurred because of the different media and methods used, and the differences in source materials that differ from place and location. External factors such as temperature, light, oxygen, and moisture greatly affect, especially the quality of the material, where the honje laka flower in the area is cultivated and planted on a large area of land and the existence of maintenance affects the content of compounds in the honje laka flower plant.

Based on the results of the formation of an inhibition zone from the ethanol extract of the honje laka flower, the compounds present in the flower can be interconnected. DMSO is a solvent that is applied with extracts from the honje laka flower. Clindamycin HCl was used as a positive control. The inhibition zone in this study resulted in 39.51±0.01 indicating a very strong category. The results showed that the honje laka flower extract had almost the same inhibiting ability as the concept of the antibiotic clindamycin HCl on the growth of *S. mutans* bacteria, but when compared with this potential, the size of the inhibition zone produced had a slight difference as seen in Figure 3. This is because of the concentration of the honje laka flower extract, the magnitude of the inhibition zone formation is not proportional to the inhibition zone formation by the antibiotic clindamycin which is the positive control (Malinggas & Mariati, 2015).

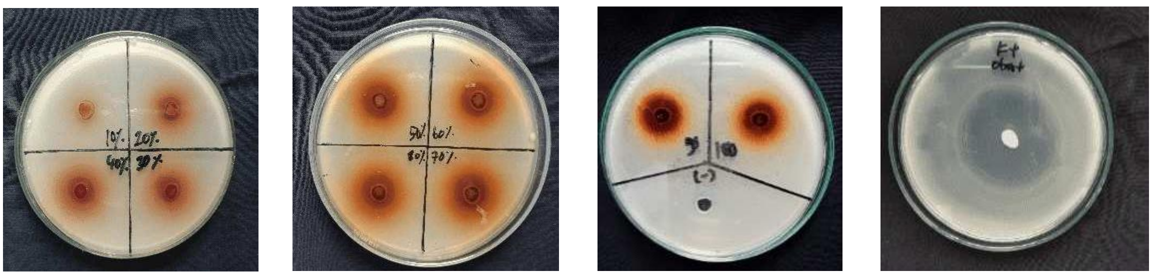
Table 3. Phytochemical Screening Results of Honje Laka Flower

Compound	Raw Powder	Extract
Alkaloids	-	-
Flavonoids	+	+
Saponins	-	-
Tannins	-	-
Polyphenol	+	+
Quinone	+	+
Steroids and Triterpenoids	-	-
Monoterpenoids and sesquiterpenoids	+	+

Information:
(+) Positive = the presence of the compound is detected
(-) Negative = no compound is detected

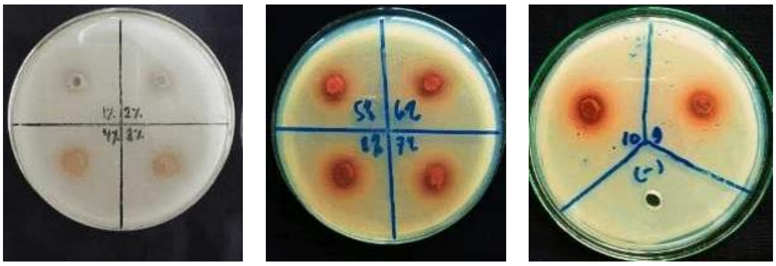
Table 4. Antibacterial Activity Test of Honje Laka Flower Ethanol Extract

Concentration (%)	Inhibition zone (mm) mean±SD
Control (-)	-
10	11.10±0.110
20	12.17±0.062
30	13.93±0.055
40	14.6±0.060
50	14.65±0.075
60	15.83±0.122
70	15.91±0.117
80	16.59±0.109
90	16.94±0.261
100	17.90±0.115
Control (+)	39.51±0.01



a. b. c. d.

Figure 3. The antibacterial activity test of honje laka flower extract, a. Extract concentrations of 10%, 20%, 30%, and 40%, b. Extract concentrations of 50%, 60%, 70%, and 80%, c. Extract concentration of 90%, 100%, Negative control, d. Positive control



a. b. c.

Figure 4. The results of the MIC activity test of honje laka flower extract, a. Extract concentration of 1%,2%,3%, and 4%, b. Extract concentrations of 5%, 6%, 7%, and 8%, c. Extract concentrations of 9%, 10%, and negative control.

The MIC test results

Based on the test results, it is known that the MIC value of the ethanol extract of the honje laka flower still has activity up to a concentration of 5% in the medium category. At a concentration of 9% to 10% honje laka flower extract produced activity in the moderate category. Meanwhile, at concentrations of 1% to 4%, there is no activity. The smaller the concentration, the inhibition zone obtained will decrease. The cause ingredient active in the content of the extract is a little bit effective in preventing the growth of *S.mutans*.

Table 5. The MIC test of honje laka flower ethanol extract

Concentration (%)	Inhibition zone (mm) mean±SD
Control(-)	-
1	-
2	-
3	-
4	-
5	9.22±0.115
6	9.43±0.020
7	9.49±0.060
8	9.59±0.060
9	11.19±0.079
10	11.48±0.040
Control(+)	39.51±0.010

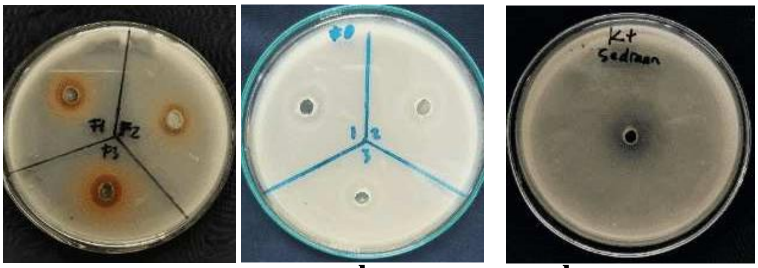
Antibacterial Activity of Mouthwash Formula

The activity test results for honje laka flower extract mouthwash were presented in Table 6. The results showed that the inhibitory power in Formula 1-3 is included in the medium category. The results of the preparation experienced a decrease in activity and reduced inhibitory power. Several factors cause the inhibition to decrease maybe when the preparation is made because additional formulas are added which can reduce the extract's stability and affect its activity.

Table 6. Antibacterial Activity of Honje Laka Flower Extract Mouthwash Against *S. mutans*

Formula	Average±SD(mm)
0	-
1	8.61±0.03
2	8.70±0.01
3	9.35±0.02
Positive Control	15.42±0.02

The decrease in the antibacterial power in the extract after the mouthwash was made due to a chemical reaction that occurs when the ingredients in the preparation with a strong concentration are mixed, the preparation can be used to inhibit the growth of *S.mutans* bacteria. Factors that affect physical, chemical, and microbiological degradation are temperature. where high temperatures make the drug unstable. High temperatures have high antibacterial activity when compared to low temperatures. This is because the heating that occurs can give the active ingredients and additives to the preparation decreased so the smaller the levels obtained. For this reason, it is important to store mouthwash at room temperature between 23-29^{o}C (Tampoliu et al., 2021).



a. b. b.

Figure 5. The activity test results of honje laka flower extract mouthwash, a. Preparation of mouthwash with extract concentrations of 5%, 10%, and 15%, b. Mouthwash formula without extract, c. Preparations of mouthwash in the market.

Organoleptic Test Results

After preparing this mouthwash, it is necessary to do several tests on days 0, 7, 14, and 21 so that there is no change in shape, taste, color, or smell with organoleptic tests. Based on the results of organoleptic testing of the preparation of honje laka flower extract mouthwash for formula 0, the base without extract is colorless (clear), has a mint smell, tastes spicy, and is in liquid form. As for formulas 1, 2, and 3, they have a brownish and thick color, this is due to the addition of extract from the honje laka flower, which has a mint smell and also a fairly distinctive smell from the honje laka flower, the taste is spicy due to the addition of oleum methane in it and its shape fluid.

It can be seen that the high concentration of honje laka flower extract that will be added will make the distinctive aroma obtained will also be stronger. As for the color in the formulation of the honje laka flower mouthwash tends the darker and darker the resulting product. Based on the check results, there is no change during the storage period.

Test the pH using a pH meter. The results of the evaluation of the pH (Table 7) shows the range of the formula pH limit values in the range of trade standards seen from the Quality Standards for herbal mouthwashes, namely pH between 5-7 (Hidayanto et al., 2017). The pH limit of the honje laka flower mouthwash formulation was obtained with various concentrations ranging from 6.22 to 7.45. This mouthwash formulation has the lowest pH of 6.22 at F3 and the highest pH is found in F1 which has a pH value of 7.45. The aim is that there is no acidic nature in the mouthwash that can cause corrosiveness to the teeth and if it is alkaline it can also have a disturbed sense of taste. The four formulations produce a pH that meets the normal requirements of the mouth.

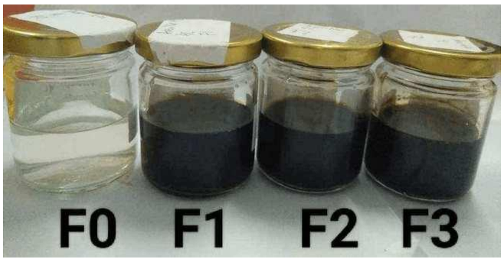


Figure 5. The organoleptic of honje laka flower extract mouthwash. Base formula without extract (F0), mouthwash with extract concentrations of 5% (F1), 10% (F2), and 15% (F3)

Table 7. pH Test Results of Mouthwash Formula

Formula	Week-				Average ± SD
	0	1	2	3	
0	7.45	7.46	7.37	7.31	7.39±0.07
1	7.61	7.58	7.54	7,10	7.450±0.24
2	7.27	7.59	7.21	7.31	7.34±0.16
3	6.19	6.24	6.22	6.23	6.22±0.02

The centrifugation test at 3000 rpm for 30 minutes showed that there was no separation for formula 0 in the 1st cycle to the 6th cycle. Formula 1 with a concentration of 5%, formula 2 with a concentration of 10%, and formula 3 with a concentration of 15% of the cycle. 1st to 4th cycle there is no separation. Meanwhile, from the 5th cycle to the 6th cycle, the extract precipitated and phase separation occurred. This change can be caused by one of the factors that can affect the oxidation of preparation, namely extreme temperatures.

The purpose of this accelerated test is that the preparation stays stably during the specified storage time (Rusmin, 2020). The test is carried out by keeping the preparations in different storage temperatures and a predetermined period so that changes that occur can be analyzed and occur under normal conditions. The results showed that there was no change in color, aroma, or shape when observed before and after the 6-cycle test (Suryani et al., 2017).

CONCLUSIONS

The ethanolic extract of the honje laka flower had activity against *S.mutans* with a MIC of 9.22±0.115 at a concentration of 5% and the mouthwash formula of 15% extract can inhibit 9.35±0.02. The ethanol extract of honje laka flower can be used as a mouthwash and can inhibit the activity of *S. mutans.*

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