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MOUTHWASH FORMULATION OF ONION (ALLIUM CEPA L.) METHANOL EXTRACT FOR INHIBITING THE GROWTH OF STREPTOCCUS MUTANS BACTERIA

Sugeng Supriyanto, Naelaz Zukhruf Wakhidatul Kiromah, Ery Purwanti, Titi Pudji Rahayu

Department of Pharmacy, Universitas Muhammadiyah Gombong, Kebumen 54411, Indonesia

naelaz.zukhruf@unimugo.ac.id

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ABSTRACT

Dental and oral problems experienced a significant increase from 2007 to 2018. Based on the results of basic health research, dental and oral problems increased from 23.2% to 57.6%. Dental caries is ranked sixth with a prevalence of 60% to 80%. The main cause of dental caries is Streptococcus mutans. The use of mouthwash with synthetic active ingredients can cause side effects. In addition, only a few mouthwashes were able to inhibit the growth of Streptococcus mutans bacteria. The purpose of this study was to make a mouthwash formulation from onion methanol extract and to determine its ability to inhibit the growth of Streptococcus mutans bacteria. Three mouthwash formulas were made with 10%, 20% and 30% extract concentrations respectively, then physical evaluation was carried out for 14 days on days 0, 7 and 14. Physical evaluation included stability tests (odor, taste, turbidity and precipitate), pH and diameter of inhibition. The results of the formula stability evaluation on day 14 there was a change in formulas 2 and 3 color, but not in formula 1. This was due to differences in the concentration of extracts and the sappans color stability in the formulas. The pH test results for each formula are in the range of 6.0-6.3. A good mouthwash has a pH close to neutral like the pH of the mouth, which is 6-7. The results of the diameter inhibition test ranged from 6-8 mm. A significant difference was seen between the positive controls with formulas 1 and 3, but there was no significant difference between formulas 1 and 3. The conclusion of this study was that the mouthwash of onion methanol extract had the ability to inhibit the growth of Streptococcus mutans bacteria with moderate strength and the best formula was mouthwash with 10% extract concentration.

Keywords: Dental Caries; Mouthwash; Allium cepa; Streptococcus mutans

1. INTRODUCTION

Teeth and mouth are the gateway for bacteria and germs to enter the body, but unfortunately, some people tend to overlook their dental and oral health, resulting in the disturbance of other organs' health. The results of the Basic Health Research in 2007 and 2013 showed an increase in dental and oral problems from 23.2% to 25.9% (Kementerian Kesehatan RI, 2014), even rising dramatically to 57.6% in 2018 (Kementerian Kesehatan RI, 2018). Dental caries are one of the most common dental and oral problems experienced by the community in all age groups, with a prevalence above 70% (Kusuma & Taiyeb, 2020).

Dental plaque has been proven to be the main cause of dental caries. Therefore, one way to prevent dental caries is by limiting the formation of dental plaque (Kaligis et al., 2017). Dental plaque is a thin layer consisting of various microorganisms that form on the surface of teeth shortly after they come into contact with saliva (Rezki & Pawarti, 2014). The composition of dental plaque consists of microorganisms, with more than 400 species, one of which is Streptococcus mutans, which plays a crucial role in the formation of dental plaque (Ristianti et

al., 2015). The mechanism of this bacteria in the formation of dental plaque is by fermenting sucrose into acid, causing a decrease in pH on the surface of the teeth, resulting in tooth mineralization (Anastasia et al., 2017).

Mouthwash is one of the ways that can be used to control the formation of dental plaque. However, long-term use can cause various side effects such as tooth pigmentation, changes in taste sensation, and the formation of tartar on the upper part of the gum, causing the oral cavity to turn red, mucosal damage resulting in pain, and thyroid gland function impairment (Kasuma et al., 2016). The community, in general, is also unaware that only a few brands of mouthwash are capable of inhibiting the growth of Streptococcus mutans bacteria, the cause of dental plaque, thus requiring an alternative mouthwash with antibacterial active ingredients derived from herbal ingredients (Hasanah, 2013).

One of the herbs that have antibacterial properties is shallots. Shallots are one of the main food sources containing flavonoids. The flavonoids contained in shallots are anthocyanins and quercetin. The quercetin content in shallots reaches 85-95%, while anthocyanins are around 10%, which is not dependent on the size or weight of the bulb (Rodrigues et al., 2017).

Flavonoids, as antibacterials, work by denaturing proteins and disrupting the lipid layer, resulting in damage to the cell wall (Kono et al., 2018). Flavonoids have a polar nature; therefore, the selection of solvents in extraction needs to be considered to obtain optimal results. The results of the selection of solvents in the extraction of shallots to obtain optimal total phenol content (TPC) and total flavonoid content (TFC) found that 70% methanol is the most optimal of several polar solvents used for testing (Singh et al., 2017).

The antibacterial activity of red onion methanol extract against Streptococcus mutans indicates that red onion has a strong ability to inhibit the activity of Streptococcus mutans, the cause of dental caries, both in the form of thick extract and nanoparticles (Gomaa, 2017; Shukla et al., 2013). The development of red onion into a form of medication to address the problem of dental plaque in Indonesia is still very limited. The results of literature studies on the use of red onion in Indonesia are limited to its bioactivity research (Hatijah, 2013). On the other hand, the development of red onion into a form of medication to address the problem of dental plaque is currently in the form of antibacterial dental chewing gum (Ika, 2017). Research on red onion to be developed into a mouthwash form of medication is still lacking. Therefore, it is expected that this research can produce a new product to address the problem of dental plaque from red onion extract.

2. METHODS

2.1. Materials

The equipment used in this research were glassware (Pyrex,) blender (Miyako), rotary evaporator (Biobase,) waterbath (Biobase), refrigerator (Panasonic), TLC chamber (Pyrex), petri dish (Pyrex), UV lamp, oven (Memmert), autoclave (All American), Laminar Air Flow (Biobase), incubator (Memmert), hotplate magnetic stirrer (Biobase), and a caliper.

The materials used in this research were red onion purchased from Gombong market, 70% technical grade methanol, aquadest, glycerin, propylene glycol, menthol, liquid stevia, secang wood bark, HCl, Mg powder, FeCl3, 10% NaOH, glacial acetic acid, butanol, GF254 silica TLC plate, quercetin, Mueller Hinton Agar (MHA), *Streptococcus mutans*, Whatman No. 1 paper, and 0.9% NaCl.

2.2. Procedure

2.2.1. Preparation of Simplisia

The sample used was red onion (Allium cepa L.) obtained from Gombong city. The red onion was sorted wet to remove unwanted dirt or parts, then washed with clean running water, and thinly sliced to speed up the drying process. After that, it was dried by airing it without direct

sunlight exposure until it was completely dry. The dried red onion was then sorted again to separate any remaining dirt or foreign objects.

2.2.2. Preparation of Methanol Extract of Red Onion

The method used for extracting red onion was maceration. A 50-gram sample of the dried red onion powder was added to a maceration container and 500 mL of 70% methanol solvent was added, or in a 1:10 ratio, for 3 days with occasional stirring. Next, it was filtered and the filtrate was evaporated with a rotary evaporator at 50°C, followed by using a waterbath at 60°C until a concentrated extract was obtained.

2.2.3. Qualitative Test of Flavonoid Extract

a) Tube Test

1) Alkaline Reagent Test

The extract was dissolved in aquadest and a few drops of 10% NaOH were added. The mixture turned yellow, and the yellow color faded when a dilute HCl solution was added. This indicates the presence of flavonoids in the extract (Talukdar et al., 2017).

2) Wilstater Test

4 mL of the extract solution was mixed with 1.5 mL of 50% methanol. The solution was heated and added to Mg metal. The addition of 5-6 drops of diluted HCl changed the solution to yellow, orange, or red, indicating the presence of flavonoids in the extract (Ergina et al., 2014; Jayashree et al., 2016).

b) Thin-Layer Chromatography (TLC) Test

Flavonoids in the methanol extract of red onion were detected using TLC method with quercetin as the comparator. The stationary phase used in the TLC test was GF254 silica gel, while the mobile phase used was a combination of n-butanol:acetic acid:water with a ratio of 3:1:1 (Andersen & Markham, 2006). The TLC plate was cut into a size of 4x10 cm and activated in an oven at 110°C for 30 minutes (Dewi et al., 2018). The extract and quercetin were then spotted on the plate close to each other, with a distance of 1 cm between spots. Next, it was eluted using the prepared mobile phase.

2.2.4. Formulation Design

The mouthwash formula was adapted from the formula created by Kono (Kono et al., 2018), with some modifications to the oleum menthae, calcium lactate, potassium thiocyanate, and 70% sorbitol, as shown in **Table 1**. The formula was divided into three, namely formula 1 (F1), formula 2 (FII), and formula 3 (FIII).

Table 1. The mouthwash formula

Ingredient	FI	FII	FIII
Red onion extract (Allium cepa. L) (g)	10	20	30
Propylene glycol (mL)	15	15	15
PEG-40 hydrogenated castor oil (g)	1	1	1
Menthol (g)	0.25	0.25	0.25
Benzoic acid (mg)	5	5	5
Sodium benzoate (g)	2	2	2
Flavoring	qs	qs	qs
Secang 3% (g) (Simplicia)	3	3	3
Stevia (liquid)	qs	qs	qs
Aquadest (mL)	Ad 100	Ad 100	Ad 100

Where, FI: Formula 1, FII: Formula 2, FIII: Formula 3

2.2.5. Production of Mouthwash

Secang is added to 100 mL of distilled water until the water changes color to red. This water is then used to dissolve the extract of red onion (Allium cepa. L) (water-soluble phase). Benzoic acid and menthol are dissolved with some propylene glycol (non-aqueous phase) using

a separate container and emulsified with PEG-40 hydrogenated castor oil. The remaining propylene glycol is then slowly poured and stirred until homogeneous, then slowly pour the water-soluble phase while stirring until homogeneous. Finally, add the sodium benzoate solution in water until the pH becomes 6-7 (use a pH meter to measure the pH). Aroma and Stevia are added as desired after the mouthwash is ready.

2.2.6. Physical Evaluation of Mouthwash Formulation

Formula stability includes color, odor, taste, turbidity, and sedimentation observed on days 0, 7, and 14. Observations on these days are aimed at determining the effect of time on the stability of the mouthwash formulation that has been made. The formula stability test refers to previous research conducted by Anastasia with modifications of observation time only up to 14 days (Anastasia et al., 2017).

2.2.7. Formula pH Test

The pH test uses a pH meter on days 0, 7, and 14.

2.2.8. Zone of Inhibition Test

The method used is the paper disk diffusion using Mueller Hinton Agar (MHA) media (Handayani et al., 2016). The paper disks are made using Whatmann No. 1 filter paper. Paper disks to be used for antibacterial tests are inserted into porcelain cups, each containing mouthwash formulations (FI, FII, and FIII), positive controls (total care antiseptic mouthwash), and negative controls (mouthwash formula without red onion extract). The paper disks are soaked for 30 minutes, then drained and placed into petri dishes containing MHA media that has been solidified and swabbed with the test bacteria suspension. The petri dishes are then incubated at 37°C for 24 hours, and the zone of inhibition is measured by the clear zone around the paper disk using a caliper. The zone of inhibition test is performed three times for each formula. The purpose of replication is to verify the accuracy of the obtained results.

2.2.9. Data Analysis Technique

Data analysis includes qualitative and quantitative data. Qualitative data are presented in the form of tables or graphs for easy reading and understanding. This data includes the results of stability formula tests, pH tests, and qualitative flavonoid tests (Handayani et al., 2017). Meanwhile, quantitative data will be analyzed using statistics (SPSS version 16) with the One-way ANOVA method to determine the effect of red onion extract concentration on the formula (Anastasia et al., 2017).

3. RESULTS AND DISCUSSION

3.1. The Production of Shallot Simplisia and Methanol Extract

Shallots are first prepared in the form of simplisia due to the higher content of flavonoids in shallot simplisia compared to fresh shallots (Awouafack et al., 2017). The preparation of shallot simplisia is done naturally by drying the shallots in the shade, away from direct sunlight. This is done to prevent the flavonoid content in the shallots from evaporating and becoming damaged during the drying process, which can be affected by temperature, light, and duration of the simplisia production (Aires, 2016). The obtained yield of simplisia is 16.26%.

The shallot simplisia is then blended to reduce the particle size, which will allow for more effective extraction by increasing the contact surface area between the solvent and the simplisia (Awouafack et al., 2017). The selection of solvent is also important to ensure maximal extraction of the active compounds in shallot simplisia. The solvent used in the extraction process must be chosen based on the characteristics of the active compounds.

The main active compound in shallot, quercetin, is highly soluble in polar solvents. Therefore, polar solvents are the preferred choice based on the principle of "like dissolve like" (Awouafack et al., 2017). Quercetin is also categorized as a low molecular weight polyphenol,

making methanol a more efficient solvent for the extraction process (Do et al., 2013; Fromm et al., 2012).

Another important factor to consider during the extraction process is temperature. The use of excessively high temperatures should be avoided to prevent the degradation or loss of flavonoids in the shallot extract. The recommended temperature range to maintain flavonoid content during the extraction process is 20-50°C. The use of temperatures above 70°C should be avoided as it can quickly degrade and significantly reduce the flavonoid content (Brglez Mojzer et al., 2016). This is also the basis for choosing the maceration extraction method and air-drying in the production of simplisia. The yield of the obtained extract is 60.52%. The flavonoid content of the extract was qualitatively tested.

3.2. Qualitative Flavonoid Test Results

The qualitative test of the flavonoid extract using the tube test method showed positive results, indicated by a color change in the extract sample (**Table 2**). The base reagent test on the extract also showed a positive result, as the addition of 10% NaOH to the extract resulted in a yellow color, which faded when HCl was added. The Wilstater test also showed a positive result, with a color change to orange in the sample.

Table 2. Results of flavonoid qualitative test

Test	Method	Result
Base reagent	Sample +10% NaOH changes to yellow, +HCL yellow color fades	+
Wilstater	Sample +50% methanol, heated +Mg powder+Dilute HCl changes to yellow, orange to red	+
TLC	Stationary phase Silica gel GF254, Mobile phase n-butanol: acetic acid: water (3:1:1)	+

TLC: Thin Layer Chromatography

Qualitative testing for the presence of flavonoid content in the red onion extract using TLC also showed a positive result. This is indicated by the Rf value in the extract that is parallel to the reference quercetin. Dark spots observed using a UV lamp at 254 nm, and blue fluorescence observed using a UV lamp at 365 nm also indicate the presence of flavonoid content in the extract (**Figure 1**) (Andersen & Markham, 2006).

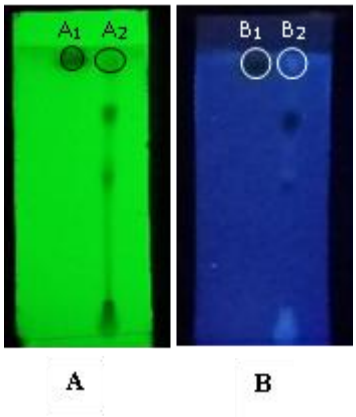

Figure 1. Results of TLC test. A. Observation at UV 254 nm B. Observation at UV 365 nm, A1 B1 Rutin Reference Kuersetin Rf 0.95, A2 B2 Red Onion Extract Rf 0.9

Red onion extract, which has been qualitatively proven to contain flavonoids, is formulated into a mouthwash preparation. Generally, the composition of mouthwash consists of ethanol and other solvents, humectants, solubilizers, flavoring agents, preservatives, and pH regulators (alkaline) (Rachma, 2010). Red onion extract functions as the main active antibacterial agent against Streptococcus mutans, which causes dental plaque. Propylene glycol, which is present at

a concentration of 15% in the formula, functions as a humectant to prolong the contact time with bacteria when used in the mouth (Anastasia et al., 2017; Rowe et al., 2009). Propylene glycol is commonly used in topical and systemic preparations and food formulations because it is generally safe to use. However, studies on the effects of routine use of propylene glycol on oral tissue and saliva properties have not yet been conducted (Radzki et al., 2022). However, a study on the use of propylene glycol in electronic cigarettes for 90 days showed no signs of toxicity in users (Phillips et al., 2017). Based on this information, the potential toxicity of using propylene glycol in mouthwash is expected to be small because mouthwash is not ingested like electronic cigarettes, which are inhaled and enter the organs in the body, such as the lungs. In addition to its role as a humectant, propylene glycol, at a concentration of 15%, also functions as a cosolvent, which is useful for dissolving phenolic compounds, and as a preservative (Anastasia et al., 2017; Sheskey et al., 2017). PEG-40 hydrogenated castor oil functions as an emulsifying agent and solubilizer, and the recommended concentration in mouthwash formula can reach 2% (Kementerian Kesehatan R.I, 2012; Rowe et al., 2009). Menthol in the formula acts as a flavoring agent to reduce the strong flavor of red onions, and the recommended concentration for mouthwash preparations is 0.1-2% (Rowe et al., 2009). Benzoic acid, together with sodium benzoate, is generally known and used as a preservative, but in this formula, both substances act as pH regulators to control the pH of the formula (Kono et al., 2018). The pH of the mouthwash solution must be controlled to match the pH of the mouth, which is 6-7. This is intended to prevent mouthwash from being corrosive to teeth due to being too acidic or interfering with the sense of taste due to being too basic (Kono et al., 2018; Mumpuni et al., 2019). Raspberry aroma helps reduce the strong aroma of red onions, secang functions as a natural red coloring agent to make it more visually appealing, and stevia is a natural sweetener that is safe to use even for people with diabetes (Raini & Isnawati, 2011; Sari & Suhartati, 2016).

3.3. Stability Test Results of the Formula

The stability test results (**Table 3**) generally indicate that all formulas remained stable in terms of odor, taste, turbidity, and sedimentation throughout the evaluation period. The only observed instability was related to color, especially for formulas 2 and 3 on day 14, while the color of formula 1 tended to remain stable during the evaluation period. Observations were conducted on days 0, 7, and 14 to assess the physical stability of the preparation. The color instability observed in secang-based formulas primarily stems from pH levels. At low pH (2-5), secang imparts a yellow color, while at pH 6-7, it exhibits a vibrant and bright red color. At alkaline pH levels, it appears purplish-red due to the presence of a compound called brazilin (Kurniati et al., 2012). The color change in secang-based formulas can be influenced by various factors, including pH, oxidizing agents, sunlight exposure, storage conditions (room temperature and cold), storage duration, and the addition of zinc metal (Kurniati et al., 2012). The stability of secang color during storage depends on the solvent used. When ethanol is used as the solvent, the color stability can last up to 9 days at room temperature, while water as the solvent provides stability for up to 8 days (Kurniati et al., 2012; Padmaningrum et al., 2012).

3.4. pH Evaluation Results

The pH evaluation results for each formula during the evaluation period also showed stability (**Table 4**). This can be observed from the pH measurements taken using a pH meter on days 0, 7, and 14, which indicated that the pH remained relatively constant for each formula, with a minimum pH of 6.0 for formulas 2 and 3, and a pH of 6.3 for formula 1. A good mouthwash generally has a pH close to neutral, similar to the pH of the mouth, which is 6-7 (Rachma, 2010). Mouthwashes with a pH $\leq$ 5.5 can cause tooth demineralization, tooth erosion, and significant enamel loss within the first few minutes of contact with the acidic mouthwash (Vivek & Shwetha, 2015). One way to maintain the pH stability of a mouthwash is by using a buffer solution (Rachma, 2010).

Table 3. Results of formula stability test

Formula	Observation	Day-		
		0	7	14
F1	Color	Light Pink	Light Pink	Light Pink
	Odor	Menthol	Menthol	Menthol
	Taste	Sweet	Sweet	Sweet
	Turbidity	Clear	Clear	Clear
	Sediment	None	None	None
F2	Color	Red	Dark Red	Dark Red
	Odor	Menthol	Menthol	Menthol
	Taste	Sweet	Sweet	Sweet
	Turbidity	Clear	Clear	Clear
	Sediment	None	None	None
F3	Color	Dark Red Purple	Dark Red Purple	Dark Red Purple
	Odor	Onion Menthol	Onion Menthol	Onion Menthol
	Taste	Sweet	Sweet	Sweet
	Turbidity	Intense Clarity	Intense Clarity	Intense Clarity
	Sediment	None	None	None

FI: Formula 1, FII: Formula 2, FIII: Formula 3

Table 4. pH evaluation results of the formula

Formula	pH on Day-		
	0	7	14
F1	6,3	6,3	6,3
F2	6,0	6,1	6,0
F3	6,0	6,0	6,0

FI: Formula 1, FII: Formula 2, FIII: Formula 3

The buffer used in this study is a combination of benzoic acid and sodium benzoate. Benzoic acid and sodium benzoate become inactive as preservatives at pH >5, and they are optimal preservatives when they are at a pH range of 2-5 for sodium benzoate and <4.5 for benzoic acid (Rowe et al., 2009). In addition to the buffer solution, the concentration of red onion extract in each formula also influences the formula's pH. This can be observed in the decreasing pH values of the formulas as the concentration of red onion extract increases. Based on the references mentioned above, it can be concluded that all formulas have good pH values, with the best pH level found in formula 1, which is 6.3. This is because formula 1 has the highest pH among the formulas.

3.5. Evaluation of Inhibition Zone Diameter Results

The evaluation of inhibition zone diameters showed that all formulas demonstrated the ability to inhibit the growth of Streptococcus mutans bacteria, with diameters ranging from 6-8 mm throughout the evaluation period. The positive control, Total Care antiseptic mouthwash, also exhibited similar results (**Table 5**). Total Care antiseptic mouthwash was chosen as the positive control due to its antibacterial properties. The assessment of inhibition zones is categorized into several groups: very strong (> 20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (< 5 mm) (Kono et al., 2018). Based on these evaluation criteria, it can be concluded that both the positive control and red onion extract mouthwash have a moderate inhibitory effect.

Table 5. The results of the inhibition zone diameter test

Formula	Mean Inhibition Zone Diameter (mm) on Day-		
	0	7	14
F1	8.407	7.042	5.880
F2	7.000	6.493	6.617
F3	8.167	6.900	6.683
K+	7.200	6.533	6.500

F1 refers to Formula 1, F2 refers to Formula 2, F3 refers to Formula 3, and K+ refers to the Positive Control. The inhibition zone diameter values already include the diameter of the paper disk.

The evaluation results of the inhibitory diameter of the red onion extract mouthwash formula fell far short of expectations. Previous studies on the inhibitory effect of red onion extract on Streptococcus mutans bacteria have shown strong inhibitory activity, with inhibitory diameters ranging from 10-20 mm (Gomaa, 2017; Shukla et al., 2013). The methanol extract of red onion at a concentration of 25% exhibited an inhibitory diameter of 10.4 mm. However, in the mouthwash with a concentration of 30%, the inhibitory diameter was only 8.2 mm. The difference in inhibitory diameter results is likely due to the different test methods used. The previous studies employed the well diffusion method, while this study utilized the paper disk method. Research comparing the well diffusion and paper disk methods has shown that the well diffusion method yields better and wider inhibitory diameters compared to the paper disk method (Haryati et al., 2017; Khusuma et al., 2019; Prayoga, 2013). This is because the well diffusion method allows for a more comprehensive and homogeneous osmolar process, as each well is filled with extract according to the concentration being tested (Haryati et al., 2017).

3.6. Statistical Test Results

The inhibitory diameter data of the red onion extract mouthwash on day 0 were analyzed using SPSS 16 to determine whether there were significant differences in the concentrations of each formula compared to the positive control. The analysis used was one-way ANOVA, as there was only one variable being analyzed, which was the concentration in each formula. The one-way ANOVA test yielded a significance value of 0.001 < 0.05, indicating significant differences in inhibitory diameters among the formulas. Post hoc tests are needed because the one-way ANOVA test yielded significant results. This is done to identify which groups differ significantly. One of the post hoc tests that can be used for homogenous data variance is Tukey's test, while for non-homogeneous data, Games-Howell test can be employed. In this case, since the data variance is homogenous, Tukey's test was chosen for the post hoc analysis (**Table 6**) (Suliyono, 2010).

Table 6. Post hoc test results of One-Way ANOVA

Formula	Significance Values between Formulas (P-Value)			
	F1	**F2**	**F3**	**K+**
F1	-	0.001	0.717	0.003
F2	0.01	-	0.04	0.810
F3	0.717	0.04	-	0.011
K+	0.003	0.810	0.011	-

F1: Formula 1, F2: Formula 2, F3: Formula 3, K+: Positive Control

The results of the Tukey post hoc test revealed significant differences between the positive control and F1 as well as F3. F1 and F3 exhibited better inhibitory efficacy compared to the positive control, as indicated by the post hoc test with significance values <0.05. However, there was no significant difference between F1 and F3 (significance value >0.05), indicating that F1 and F3 have similar abilities in inhibiting the activity of Streptococcus mutans bacteria. On the other hand, there was no significant difference between F2 and the positive control, suggesting that F2 is equally effective as the positive control. However, there was a significant difference between F2 and F1 as well as F3, indicating that F1 and F3 have better inhibitory capabilities than F2. Based on this data, it can be concluded that both F1, F2, and F3 are effective in inhibiting the growth of Streptococcus mutans bacteria, with F1 and F3 having similar but superior efficacy compared to the positive control and F2.

Observations of the inhibitory zone diameter were also conducted on days 7 and 14. The results showed a decrease in the inhibitory efficacy against Streptococcus mutans bacteria, as indicated by the reduction in the diameter of the inhibitory zones. This decrease is likely due to the decrease in the quercetin content, which is the active antibacterial compound against Streptococcus mutans, in the extract caused by storage duration (Millet et al., 2012). The stability

of quercetin in red onions is influenced by temperature, pH, and storage conditions (Wang et al., 2016). Storage at a temperature of 20 °C for 28 weeks can degrade quercetin up to 100%. In this study, the extract was stored at room temperature, which potentially accelerates the degradation of quercetin due to the storage temperature exceeding 20 °C. The decrease in quercetin content will certainly affect the antibacterial activity of the red onion extract.

4. CONCLUSION

Red onion methanol extract mouthwash has the ability to inhibit the growth of Streptococcus mutans bacteria with moderate strength, and the best formula based on physical evaluation results is F1 with a 10% extract concentration. Recommendations for further researchers include conducting inhibition zone diameter tests using the well diffusion method, adding glycerin to the formula, and performing co-pigmentation when using natural dyes

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6. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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