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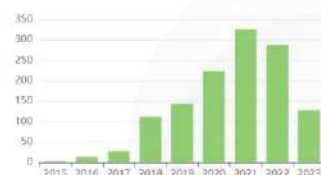
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RESEARCH ARTICLE

Evaluation of viscosity and pH of *Zingiber officinale* var. *officinale* juice in mouthwash formulation

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ABSTRACT

Zingiber officinale var. *officinale* juice has many benefits, including as a mixture of mouthwash. This research aims to analyze the differences in pH and viscosity values of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% on days 0, 14, 28, 42, and 56. *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% were mixed with propylene glycol, PEG-40 hydrogenated castor oil, oleum menthae, benzoic acid, sodium benzoate, calcium lactate, sorbitol 70% and calcium thiocyanate. The formulation was measured with a pH meter (Jenway, United Kingdom) and a viscometer (Brookfield, Massachusetts) for 56 days. pH data was analyzed using GLM repeated measures ANOVA and one-way ANOVA ($p < 0.05$). Viscosity data was analyzed using GLM repeated measured ANOVA and the Kruskal-Wallis test ($p < 0.05$). The pH value of the 3.125% formulation showed significant difference for 56 days in the range of 5.50-5.61. The viscosity value showed no significant difference for 56 days in the range of 3.03-3.09 cP. The pH and viscosity value of the 6.25% formulation showed no significant difference for 56 days with pHs of 5.78-5.83, while the viscosity is 3.12-3.13 cP. The pH and viscosity value of the 12.5% formulation showed no significant difference for 56 days with pHs of 4.90, while the viscosity was 3.43-3.50 cP. The *Zingiber officinale* var. *officinale* mouthwash formulation with a concentration of 3.125% was unstable, but it had the lowest viscosity value and was stable for 56 days. The pH and viscosity value of the 6.25% and 12.5% formulations were stable for 56 days. The highest pH value was in the 6.25% formulation.

Keywords: herbal mouthwash; pH; viscosity; *Zingiber officinale* var. *officinale*

INTRODUCTION

The prevalence of oral disease is increasing gradually with growing urbanization and changing living conditions in most countries with low to middle income.¹ Dental caries, considered as the most common dental and oral disease, is associated with biofilm or dental plaque,² which can be removed with mouthwash.³ Mouthwash can also improve oral health, reduce plaque or biofilm accumulation, prevent dental caries, and freshen the breath.^{3,4}

There are two types of mouthwashes: chemical mouthwashes and herbal mouthwashes.⁵ Although chemical mouthwashes have a good effect on the oral cavity, they tend to cause discoloration of the teeth and can produce other side effects on the oral cavity. Herbal mouthwashes can eradicate oral

pathogens, relieve pain instantly, and have fewer side effects compared to chemical mouthwashes.⁶

Each ingredient of mouthwash has its own role in establishing mouthwash formulation.⁷ Benzoic acid and sodium benzoate act as preservatives and buffer solutions.^{8,9} Propylene glycol (humectant) helps prevent evaporation of active ingredients, thus maintaining the stability and duration of mouthwash contact on the teeth.¹⁰ Surfactant (PEG-40 hydrogenated castor oil) lowers the surface tension of the solution, so the substances contained become more soluble and enhance hydrophilic penetration.^{11,12} Oleum menthae acts as a solvent and flavoring.⁹ Calcium lactate and potassium thiocyanate act as therapeutic agents.¹³ The function of sorbitol 70% is as an artificial sweetener.⁹

Most herbal extracts contain antimicrobial properties.⁵ *Zingiber officinale* var. *officinale* is one of them. *Zingiber officinale* var. *officinale* is less spicy than *Z. officinale* var. *Rubrum* and also has a better antibacterial property than *Z. officinale* var. *Amarum*. Thus, it may offer excellent potential as mouthwash ingredient.¹⁴ Evaluation of mouthwash formulations must be carried out before they undergo clinical trials. The tests include organoleptic tests (odor, color, taste, and consistency), pH (acidity), viscosity, sedimentation, and density.¹⁵ The purpose of this study is to analyze the differences of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% on days 0, 14, 28, 42, and 56.

MATERIALS AND METHODS

This study used a true experimental research design with a posttest-only control group design. Mouthwash formulations were produced at the Dental Material and Testing Center of Research and Education Laboratory (DMTCORE), Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia. pH and viscosity tests were carried out at the Pharmaceutical Physics Laboratory, Faculty of Pharmacy, University of Indonesia, Depok, Indonesia. Equipment used in this study were analytical balances (Fujitsu, Japan), measuring cups (Pyrex, China), beakers

(Pyrex, China), magnetic stirrers (Toption, China), Whatman filter paper no. 1 (Midland Scientific, USA), pipettes (Bomex, China), an autoclave (Tomy, Japan), a pH meter (Jenway, United Kingdom), a viscometer (Brookfield, Massachusetts, USA), and grated ginger. The materials used in this study were propylene glycol (Dow Chemical Pacific, Singapore), PEG-40 hydrogenated castor oil (Lambertispa, Italy), oleum menthae (Anhui Province Yifan Spice Co., LTC, China), benzoic acid (Wuhan Youji Industries Co., LTD, China), sodium benzoate (Wuhan Youji Industries Co., LTD, China), calcium lactate (Galactic, Belgium), potassium thiocyanate (Merck Millipore, Germany), sorbitol 70% (Sorini Towa Berlian Corporindo, Indonesia), rhizome of *Zingiber officinale* var. *officinale* (Balitro, Indonesia), chlorhexidine gluconate 0.2% (Minosep, Indonesia), Enkasari mouthwash herbalcare protection fresh mint flavor (Enkasari, Indonesia), and aquadest.

One kilogram of 10-month-old *Zingiber officinale* var. *officinale* was cleaned, peeled, and washed with sterile aquadest. It was then mashed and filtered with Whatman filter paper no.1. The *Zingiber officinale* var. *officinale* juice was diluted to reach concentrations of 3.125%, 6.25%, and 12.5%. The *Zingiber officinale* var. *officinale* mouthwash was formulated by dissolving calcium lactate (50 mg) and potassium thiocyanate (100 mg) in aquadest (M1). Next, benzoic acid

Table 1. The formulation of juice of *Zingiber officinale* var. *officinale*

Materials	Function	F1	F2	F3
<i>Zingiber officinale</i> var. <i>officinale</i> juice	Active materials	3.125%	6.25%	12.5%
Propylene glycol (mL)	Humectant	5	5	5
PEG-40 hydrogenated castor oil (g)	Detergent	1	1	1
Oleum menthae (drops)	Solvent	10	10	10
Benzoic acid (mg)	Preservative	5	5	5
Sodium benzoate (g)	Preservative	2	2	2
Calcium lactate (mg)	Therapeutic agent	50	50	50
Sorbitol 70% (mL)	Flavoring agent	15	15	15
Potassium thiocyanate (mg)	Therapeutic agent	100	100	100



Figure 1. *Zingiber officinale* var. *officinale* juice mouthwash formulation

(5 mg) and ginger juice with a predetermined concentration were dissolved with ten drops of oleum menthae (M2). In the third step, M2 was emulsified with PEG-40 hydrogenated castor oil (1 gram) and propylene glycol (5 mL) was added gradually and stirred using a magnetic stirrer until the solution was homogeneous (M3). M1 was added little by little to the M3 solution and stirred until it was homogeneous. Fifteen mL of sorbitol 70% was added gradually and stirred until homogeneous. Finally, sodium benzoate (2 grams) was dissolved in aquadest and added to the mouthwash solution to obtain the appropriate pH of the mouthwash. The formulation was presented in Table 1. The formulations of the mouthwashes were stored in glass bottles at room temperature (Figure 1).

The pH values were measured by a pH meter (Jenway, United Kingdom), and the values were the average of three consecutive measurements. pH measurements were taken on days 0, 14, 28, 42 and 56. The viscosity value was measured by a viscometer (Brookfield, Massachusetts). Similar to the pH values, the results of the viscosity measurement were the average of three consecutive measurements. Viscosity measurements were taken on days 0, 14, 28, 42 and 56. Data normality was tested using the Shapiro-Wilk test. pH value data was analyzed using GLM repeated measures ANOVA and one-way ANOVA ($p < 0.05$). Viscosity value data was analyzed using GLM repeated measures ANOVA and the Kruskal-Wallis test ($p < 0.05$). Data was

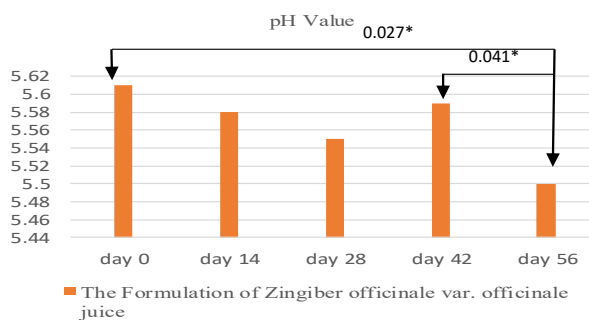
analyzed using SPSS version 25.0 (IBM SPSS Statistics, United States of America).

RESULTS

The pH value of the mouthwash formulations ranged from 5.46 to 5.83 (Table 2). The data was normally distributed ($p > 0.05$), and the results of GLM repeated measures ANOVA test of 0%, 6.25% and 12.5% mouthwash formulations were not significantly different in 56 days ($p > 0.05$). In contrast, the 3.125% mouthwash formulation showed a significant difference ($p < 0.05$). The results of the post-hoc paired wise comparison test of the 3.125% mouthwash formulation group is displayed in Figure 2.

The positive controls (chlorhexidine and Enkasari) were tested using a paired sample t-test because the test was only carried out on days 0 and 56. The paired sample t-test showed that there was no significant difference in the pH value of chlorhexidine and Enkasari on the day of the test ($p > 0.05$). One-way ANOVA test showed there were significant differences in pH values between the mouthwash groups with one-time measurement on days 0, 14, 28, 42, and 56 ($p < 0.05$). The results can be seen in Table 2.

The viscosity value of the mouthwash formulations ranged from 3.03 to 3.50 cP (Table 3). The data was normally distributed ($p > 0.05$) and the results of GLM repeated measures ANOVA test of 0%, 3.125%, 6.25%, and 12.5% mouthwash formulations were not significantly



*: significantly different ($p < 0.05$)

Figure 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation 3.125% (post-hoc paired wise comparison ($p < 0.05$)).

Table 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n = 3)	Mean ± Standard deviation					p
	day 0	day 14	day 28	day 42	day 56	
Chlorhexidine (positive control)	4.89	-	-	-	4.88	0.478
Enkasari (positive control)	4.77	-	-	-	4.76	0.225
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	5.48 ± 0.005	5.47	5.46 ± 0.005	5.47 ± 0.008	5.47	0.366
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3.125%	5.61 ± 0.005	5.58 ± 0.008	5.55 ± 0.011	5.59 ± 0.005	5.50	0.004*
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6.25%	5.78 ± 0.008	5.81 ± 0.008	5.78 ± 0.011	5.83 ± 0.005	5.80	0.081
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12.5%	4.97 ± 0.011	4.95 ± 0.005	4.93 ± 0.005	4.93 ± 0.005	4.90 ± 0.005	0.064
p	0.001†	0.001†	0.001†	0.001†	0.001†	

*GLM Repeated Measures ANOVA test (p < 0.05)

†One-way ANOVA test (p < 0.05)

Table 3. The viscosity value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n = 3)	Mean ± Standard deviation					p
	day 0	day 14	day 28	day 42	day 56	
Chlorhexidine (positive control)	4.42	-	-	-	4.34 ± 0.010	0.005*
Enkasari (positive control)	5.17	-	-	-	5.03 ± 0.231	0.423
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	1.91	1.93 ± 0.012	1.95 ± 0.021	1.96 ± 0.015	1.96 ± 0.008	0.180
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3.125%	3.03	3.09 ± 0.055	3.06 ± 0.025	3.06 ± 0.025	3.08 ± 0.021	0.588
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6.25%	3.12	3.12 ± 0.008	3.12 ± 0.008	3.12 ± 0.008	3.13 ± 0.008	0.491
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12.5%	3.50 ± 0.033	3.44 ± 0.074	3.43 ± 0.071	3.50 ± 0.011	3.43 ± 0.044	0.482
p	0.005*	0.024*	0.017*	0.019*	0.006*	

* Kruskal Wallis test (p < 0.05)

* Paired sample t-test (p < 0.05)

different ($p > 0.05$). The paired sample t-test was carried out to analyze the viscosity values of the positive controls (chlorhexidine and Enkasari) because the tests were only taken on days 0 and 56. The test showed that there was a significant difference in the chlorhexidine viscosity values on days 0 and 56 ($p < 0.05$). Meanwhile, the results of the Enkasari test showed that there was no significant difference on days 0 and 56 ($p > 0.05$). Table 3 shows the results of the analysis.

Based on the results of the normality test, the data was not normally distributed ($p < 0.05$); therefore, one-way ANOVA test could not be performed. As an alternative, the data could be analyzed using the Kruskal-Wallis test which is a non-parametric test. The Kruskal-Wallis test showed that there was a significant difference in pH values of the mouthwash groups on days 0, 14, 28, 42, and 56 ($p < 0.05$). The results of the analysis are presented on Table 3.

DISCUSSION

In this research, the mouthwash formulation contained *Zingiber officinale* var. *officinale* juice with concentrations of 3.125%, 6.25%, and 12.5%. The mouthwash formulations used in this study contain various ingredients that can maintain oral health. Propylene glycol functions as a humectant, antimicrobial, disinfectant, cosolvent, and stabilizer.^{16,17} *Oleum menthae* is used as a flavoring agent, solvent, anti-inflammatory, anti-spasmolytic, and antibacterial agent which acts as a bactericidal against pathogens such as *Escherichia coli* and *Staphylococcus aureus*.¹⁸ Sorbitol functions as a sugar substitute sweetener which has calorific value, low glycemic index, and free of carcinogenic effects. In addition, sorbitol prevents the formation of plaque and tooth decay owing to resistance to the metabolism of oral bacteria.¹⁹ The preservatives are sodium benzoate and benzoic acid,^{8,9} which have an activity as antifungal and antibacterial agents. Benzoic acid and sodium benzoate act as buffer in mouthwash.²⁰ Polyoxyl 40 (PEG-40) hydrogenated castor oil is used as surfactant

to lower the surface tension in the solution, solubilize fat-soluble components, and increase hydrophilic penetration.¹² The therapeutic agents are calcium lactate and potassium thiocyanate. The functions of calcium lactate are to strengthen tooth structure, and as antibacterial agents, antitartar agents, and bone substitute materials.¹³ Potassium thiocyanate acts as an antifungal and anti-caries agent in teeth.²¹

Zingiber officinale var. *officinale* is a natural ingredient which has antimicrobial and antifungal effects to prevent caries and oral candidiasis; therefore, ginger may help maintain oral health.²² Phytochemical test of *Zingiber officinale* var. *officinale* shows that it contains alkaloids, flavonoids, quinones, saponins, monoterpenoids, and sesquiterpenoids.²³ Previous studies have demonstrated that mouthwashes containing natural ingredients are safe and have fewer side effects.²⁴

Stability tests were performed to evaluate changes in the mouthwash preparation to determine the mouthwash suitability. The pH test showed the value was in the range of 4.90-5.83. The pH of quality standard of herbal mouthwash ranges from 5 to 7.²⁵ In this study, the mouthwash formulations with 0%, 3.125% and 6.25% concentrations of ginger met the quality standards of herbal mouthwash with a pH in the range of 5.46-5.83. The 12.5% mouthwash formulation had a pH from 4.90 to 4.97, which was the lowest, and it may indicate that it did not meet the quality standards of herbal mouthwashes because it was too acidic. When mouthwash solution proven too acidic, it makes the bacteria grow faster and may irritate to the oral cavity.^{3,26} Surprisingly, when the 12.5% mouthwash formulation was compared to commercial mouthwash, which is a positive control in this study, it showed that the pH of the mouthwash was higher than the commercial mouthwash. The pH values of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 0%, as a negative control, 6.25%, and 12.5% were stable for 56 days. However, the pH value of the mouthwash formulation with a concentration of ginger of 3.125% was unstable for 56 days. In

addition, the mouthwash formulations across the three different percentages showed different pHs, both in negative and positive control at one-time measurement. It is highly likely that differences in the mouthwash ingredients could affect the pH value of the mouthwash.

The viscosity test showed that the value was in the range of 3.03-3.50 cP. The best mouthwash viscosity is the one that is closest to the viscosity of water, which is 0.89 cP.²⁷ It is mainly by considerations of convenience when patients rinse their mouth. The viscosity values of mouthwash formulations containing *Zingiber officinale* var. *officinale* juice were found higher than the viscosity value of standard mouthwash. This could be caused by the relatively high content of *Zingiber officinale* var. *officinale* and sorbitol in the mouthwash. In comparison with commercial mouthwashes, which were the positive controls in this study, it was found that the viscosity values of the mouthwash formulations with concentrations of ginger of 3.125%, 6.25%, and 12.5% were closer to the viscosity of water than that of the commercial ones. The mouthwash formulation in this test showed a stable viscosity for 56 days. In addition, there were differences in the viscosity values between the positive controls (Enkasari and chlorhexidine), negative controls, and the three mouthwash formulations (3.125%, 6.25%, and 12.5%) at one-time measurement tested on days 0, 14, 28, 42, and 56. Taken together, differences in mouthwash ingredients could affect the viscosity value of the mouthwash. This study found that the higher the concentration of *Zingiber officinale* var. *officinale*, the higher the viscosity of the mouthwash. One unanticipated result in this study was that the mouthwash formulations had cloudy colors, whereas mouthwash formulations should have clear color to appeal the public. Mouthwash formulations have to pass various tests such as organoleptic, density, sedimentation and toxicity tests. These tests, however, had not been carried out in this study. Further research, which incorporates these tests to determine the feasibility of the mouthwash, is therefore recommended.

CONCLUSION

Based on the results of this research, it can be concluded that the pH of the *Zingiber officinale* var. *officinale*-containing mouthwash with a concentration of 3.125% was unstable for 56 days with pHs in the range of 5.50-5.61. The viscosity value, however, was stable for 56 days. It had lower viscosity value compared to those with concentrations of 6.25% and 12.5%, which was between 3.03 and 3.09 cP. Mouthwash formulation containing 6.25% concentration of *Zingiber officinale* var. *officinale* juice had a stable pH and viscosity value for 56 days. It showed a higher pH value compared to pHs of mouthwashes with ginger concentrations of 3.125% and 12.5%, which was in the range of 5.78-5.83, while the viscosity value ranged from 3.12 to 3.13 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice with a concentration of 12.5% had a stable pH value for 56 days and had lower pH value compared to the pHs of those with the concentrations of 3.125% and 6.25%, which was between 4.90 and 4.97. The viscosity value was stable and higher compared to those of the mouthwashes with ginger concentrations of 3.125% and 6.25%, which was in the range of 3.43-3.50 cP.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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VISCOSITY AND pH EVALUATION OF Zingiber officinale var. officinale JUICE IN MOUTHWASH FORMULATION

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VISCOSITY AND pH EVALUATION OF *Zingiber officinale* var. *officinale* JUICE IN MOUTHWASH FORMULATION

INTRODUCTION

The prevalence of oral disease gradually grows for the increasing urbanization and changing living conditions in most countries with low to middle income.¹ Dental caries, considered as the most common dental and oral disease, is associated with biofilm or dental plaque.² Biofilm or dental plaque can be eradicated by mouthwash.³ Mouthwash improve oral health, reduce plaque or biofilm accumulation, prevents dental caries, and freshens breath.^{3,4}

There are two types of mouthwashes, chemical mouthwashes and herbal mouthwashes.⁵ Although chemical mouthwashes have a good effect on the oral cavity, they tend to cause discoloration of the teeth and can produce other side effects on the oral cavity. Herbal mouthwashes can eradicate oral pathogens, relieve pain instantly, and have fewer side effects compared to chemical mouthwashes.⁶

Each mouthwash ingredient has its own role in establishing the mouthwash formulation.⁷ Benzoic acid and sodium benzoate act as preservatives and buffer solutions.^{8,9} Humectants (propylene glycol) prevents active ingredients evaporation, so they can maintain the stability and duration of mouthwash contact on the teeth.¹⁰ Whilst surfactant (PEG-40 hydrogenated castor oil) lowers the surface tension of the solution, so the substances contained become more soluble.^{11,12} *Oleum menthae* act as a solvent and flavoring.⁹ Calcium lactate and potassium thiocyanate act as therapeutic agents.^{12,13} The function of sorbitol 70% is an artificial sweetener.⁹

Most herbal extracts contain antimicrobial properties.⁵ *Zingiber officinale* var. *officinale* is one of them. *Zingiber officinale* var. *officinale* is less spicy than *Z. officinale* var. *Rubrum* and also has a better antibacterial property than *Z. officinale* var. *Amarum*. This leads to consider it for mouthwash ingredient.¹⁴ Evaluation must be carried out on mouthwash formulations before they are undergoing clinical trials. The tests that must be performed including organoleptic tests (odor, color, taste, and consistency), pH (acidity), viscosity, sedimentation, and density.¹⁵ The purpose of this study is to analyse the differences of *Zingiber officinale* var. *officinale* mouthwashes 3.125%, 6.25%, and 12.5% on pH and viscosity values on day 0, 14, 28, 42, and 56.

MATERIALS AND METHODS

This is true experimental study with a posttest-only control group design. Mouthwash formulations are produced at the Dental Material & Testing Center of Reserch and Education Laboratory (DMTCORE)¹⁰ Faculty of Dentistry, Trisakti University, Jakarta, Indonesia. pH and viscosity tests were carried out at ¹⁰ Pharmaceutical Physics Laboratory, Faculty of Pharmacy, University Indonesia, Depok, Indonesia. The tools that used in this study are analytical balances (Fujitsu, Japan), measuring cups (Pyrex, China), beaker glass (Pyrex, China), magnetic stirrers (Toption, China), Whatman filter paper no. 1 (Midland Scientific, USA), pipette (Bomex, China), autoclave (Tomy, Japan), pH meter (Jenway, United Kingdom), viscometer (Brookfield, Massachusetts, USA), and grated ginger. The materials that used in this study are propylene glycol (Dow Chemical Pacific, Singapore), PEG-40 hydrogenated castor oil Lambertispa, Italy), oleum menthae (Anhui Province Yifan Spice Co., LTC, China), benzoic acid (Wuhan Youji Industries Co., LTD, China), sodium benzoate (Wuhan Youji Industries Co., LTD, China), calcium lactate (Galactic, Belgium), potassium thiocyanate (Merck Millipore, Germany), sorbitol 70% (Sorini Towa Berlian Corporindo, Indonesia), the rhizome of *Zingiber officinale* var. *officinale* (Balitro,

Indonesia), chlorhexidine gluconate 0.2% (Minosep, Indonesia), enkasari mouthwash herbalcare protection fresh mint flavor (Enkasari, Indonesia), and aquadest.

1. *Zingiber officinale* var. *officinale* Juice in 3.125%, 6.25% and 12.5% concentration

One kilograms of 10-month-old *Zingiber officinale* var. *officinale* is cleaned, peeled, washed with sterile aquadest, mashed, filtered with Whatman filter paper no.1, diluted to reach 3.125%, 6.25% and 12.5% concentrations.

2. Production of *Zingiber officinale* var. *officinale* Juice Mouthwash

The juice production of *Zingiber officinale* var. *officinale* mouthwash formulation is made by dissolving calcium lactate (50 mg) and potassium thiocyanate (100 mg) in aquadest (M1). Next, benzoic acid (5 mg) and ginger juice with a predetermined concentration are dissolved with ten drops of oleum menthe (M2). The third step, M2 is emulsified with PEG-40 hydrogenated castor oil (1 gram) and added gradually propylene glycol (5 mL) and stirred using a magnetic stirrer until the solution is homogeneous (M3). M1 was added little by little to the M3 solution and stirred until it is homogeneous. 15 mL of sorbitol 70% is added gradually and stirred until it becomes homogeneous. Finally, sodium benzoate (2 grams) is dissolved with aquadest and added to the mouthwash solution to obtain the appropriate pH of the mouthwash. The formulation is in table 1. The formulations of the mouthwashes are stored in glass bottles at room temperature (Figure 1).

Table 1. The formulation of juice of *Zingiber officinale* var. *officinale*

Materials	Function	F1	F2	F3
<i>Zingiber officinale</i> var. <i>officinale</i> juice	Active materials	3.125%	6.25%	12.5%
Propylene glycol (mL)	Humectant	5	5	5
PEG-40 hydrogenated castor oil (g)	Detergent	1	1	1
Oleum menthae (drops)	Solvent	10	10	10
Benzoic acid (mg)	Preservative	5	5	5
Sodium benzoate (g)	Preservative	2	2	2
Calcium lactate (mg)	Therapeutic agent	50	50	50
Sorbitol 70% (mL)	Flavoring agent	15	15	15
Potassium thiocyanate (mg)	Therapeutic agent	100	100	100



Figure 1. *Zingiber officinale* var. *officinale* juice mouthwash formulation

3. pH Test

The pH value is measured by a pH meter (Jenway, United Kingdom). The pH measurement is taken three times at one-time measurement, then the results are averaged. pH measurements are taken on days 0, 14, 28, 42 and 56.

4. Viscosity Test

The viscosity value is measured by a viscometer (Brookfield, Massachusetts). Viscosity measurement is taken three times at one-time measurement, then the results are averaged. Viscosity measurements are taken on days 0, 14, 28, 42 and 56.

5. Data Analysis

Data normality is tested using the Shapiro-Wilk test. pH value data is analyzed using GLM Repeated Measures ANOVA and One-way ANOVA ($p < 0.05$). Viscosity value data is analyzed using GLM Repeated Measures ANOVA and Kruskal Wallis ($p < 0.05$). Data is analyzed using SPSS version 25.0 (IBM SPSS Statistics, United States of America).

RESULTS

a. The Result of pH Test

The pH value of the mouthwash formulations ranges from 5.46 to 5.83 (Table 2). The data is normally distributed ($p>0.05$) and the result of GLM Repeated Measures ANOVA test on 0%, 6.25% and 12.5% mouthwash formulations are not significantly different in 56 days ($p>0.05$). In contrast to the 3.125% mouthwash formulation group which shows a significant difference ($p<0.05$). The result of the post-hoc paired wise comparison test in the 3.125% mouthwash formulation group can be seen in Figure 2.

Table 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n=3)	Mean±Standard Deviation					p
	Day 0	Day 14	Day 28	Day 42	Day 56	
Chlorhexidine (positive control)	4.89	-	-	-	4.88	0.478
Enkasari (positive control)	4.77	-	-	-	4.76	0.225
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	5.48±0.005	5.47	5.46±0.005	5.47±0.008	5.47	0.366
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3,125%	5.61±0.005	5.58±0.008	5.55±0.011	5.59±0.005	5.50	0.004*
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6,25%	5.78±0.008	5.81±0.008	5.78±0.011	5.83±0.005	5.80	0.081
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12,5%	4.97±0.011	4.95±0.005	4.93±0.005	4.93±0.005	4.90±0.005	0.064
p	0.001†	0.001†	0.001†	0.001†	0.001†	

*GLM Repeated Measures ANOVA test ($p<0.05$)

†One-way ANOVA test ($p<0.05$)

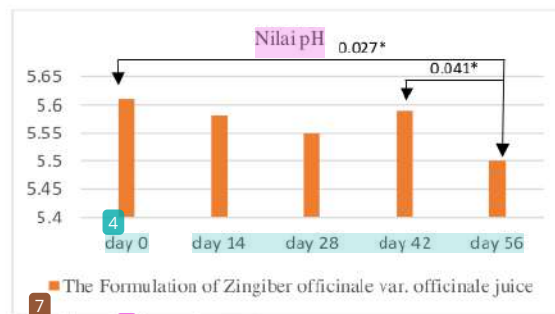


Figure 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation 3.125% (post-hoc paired wise comparison ($p<0.05$)).

The positive controls (chlorhexidine and enkasari) are tested using a *paired sample t-test* because the test is only carried out on day 0 and 56. The paired sample t-test shows that there is no significant difference in the pH value of chlorhexidine and enkasari on the day of test ($p>0.05$). *One-way ANOVA* test shows there are significant differences in pH values between the mouthwash groups at one-time measurement on day 0, 14, 28, 42 and 56 ($p<0.05$). The results can be seen in table 2.

b. The Result of Viscosity Tests

The viscosity value of the mouthwash formulations ranges from 3.03 to 3.50 cP (Table 3). The data is normally distributed ($p>0.05$) and the results of *GLM Repeated Measures ANOVA* test on 0%, 3.125%, 6.25% and 12.5% mouthwash formulations are not significantly different ($p>0.05$). The *paired sample t-test* is carried out to analyze the viscosity values of the positive controls (chlorhexidine and enkasari) in this study because the tests are only taken on day 0 and 56. The test shows that there is a significant difference in the chlorhexidine viscosity values on day 0 and 56 ($p<0.05$). Meanwhile, the results of the enkasari test shows that there is no significant difference on day 0 and 56 ($p>0.05$). The results of the analysis can be seen in Table 3.

Table 3. The viscosity value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n=3)	Mean±Standard Deviation					p
	Day 0	Day 14	Day 28	Day 42	Day 56	
Chlorhexidine (positive control)	4.42	-	-	-	4.34±0.010	0.005*
Enkasari (positive control)	5.17	-	-	-	5.03±0.231	0.423
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	1.91	1.93±0.012	1.95±0.021	1.96±0.015	1.96±0.008	0.180
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3,125%	3.03	3.09±0.055	3.06±0.025	3.06±0.025	3.08±0.021	0.588
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6,25%	3.12	3.12±0.008	3.12 ± 0.008	3.12 ± 0.008	3.13±0.008	0.491
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12,5%	3.50±0.033	3.44±0.074	3.43±0.071	3.50±0.011	3.43±0.044	0.482
p	0.005*	0.024*	0.017*	0.019*	0.006*	

* *Kruskal Wallis* test ($p<0.05$)

* *Paired sample t-test* ($p<0.05$)

Based on the normality test performed on the sample, it is found that the data is not normally distributed ($p<0.05$), so that *One-way ANOVA* test cannot be performed. As an alternative, data can be analyzed using *Kruskal Wallis* test which is a non-parametric test. *Kruskal Wallis* test shows that there is a significant difference in pH value between the mouthwash groups on day 0, 14, 28, 42 and 56 ($p<0.05$). The results of the analysis can be seen in Table 3.

DISCUSSION

In this research, the mouthwash formulation contains *Zingiber officinale* var. *officinale* juice in

3.125%, 6.25% and 12.5% concentration. The mouthwash formulations used in this study contain various ingredients that can maintain oral health. Propylene glycol functions as a humectant, antimicrobial, disinfectant, cosolvent, and stabilizer.^{16,17} *Oleum menthae* is used as a flavoring, solvent, anti-inflammatory, anti-spasmodic, and antibacterial agent which acts as a bactericidal against pathogens such as *Escherichia coli* and *Staphylococcus aureus*.¹⁸ Sorbitol functions as a sugar substitute sweetener which has caloric value, low glycemic index, and carcinogenic effects free. In addition, sorbitol prevents the formation of plaque and tooth decay owing to resistance to the metabolism of oral bacteria.¹⁹ The preservatives are sodium benzoate and benzoic acid.^{8,9} Sodium benzoate and benzoic acid has an activity as ⁸ antifungal and antibacterial. Benzoic acid and sodium benzoate act as buffer in mouthwash.²⁰ Polyoxyl 40 (PEG-40) hydrogenated castor oil is used as a detergent which lowers the surface tension in the solution, so the substances become more soluble and are able to kill bacteria in the oral cavity by destroying the bacterial cell walls.¹² The therapeutic agents are calcium lactate and potassium thiocyanate. The functions of calcium lactate are strengthening tooth structure, antibacterial agents, antitartar agents, and bone substitute materials.¹³ Potassium thiocyanate acts as an antifungal and anti-caries agent in teeth.²¹

Zingiber officinale var. *officinale* is natural ingredient which carries antimicrobial and antifungal effects to prevent caries and oral candidiasis, so that ginger influences to maintain oral health.²² Phytochemical test of *Zingiber officinale* var. *officinale* proves that it contains alkaloids, flavonoids, quinones, saponins, monoterpenoids, and sesquiterpenoids.²³ Based on previous studies, mouthwashes containing natural ingredients are safe for the body and have fewer side effects.²⁴

Stability tests are performed to evaluate changes of the mouthwash preparation that may ⁶ determine the mouthwash suitability. The pH test shows the value is in the range of 4.90-5.83. The quality standard of herbal mouthwash ⁶ is 5-7 pH value.²⁵ The mouthwash formulations in 0%, 3.125% and 6.25% concentration meet the quality standards of herbal mouthwash with a pH range of 5.46-5.83. The 12.5% mouthwash formulation reaches 4.90 to 4.97 pH value, which is the lowest, indicates it does not meet the quality standards of herbal mouthwashes in consideration of being too acidic. When mouthwash solution proven too acidic, it makes the bacteria grow faster and irritate to the oral cavity.^{3,26} Surprisingly, when the mouthwash formulation 12.5% is compared to commercial mouthwash which is a positive control in this study, it shows that the pH value of the mouthwash is higher than the mouthwash used by the public. The pH value of a mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 0% as a negative control, 6.25%, and 12.5% concentration ¹ have been stabled for 56 days. However, the pH value of the 3.125% concentration mouthwash formulation has found to be unstable for 56 days. In addition, there are the differences in pH values between mouthwash formulations in 3.125%, 6.25% and 12.5% concentration, negative and positive control at one-time measurement. This strongly indicates that the difference of the mouthwash ingredients will affects the pH value of the mouthwash.

The viscosity test shows that the value is in the range of 3.03-3.50 cP. The best mouthwash viscosity is one that closest to the viscosity of water for convenient reason when patients rinse their mouths. The water viscosity value is 0.89 cP.²⁷ The viscosity values of a mouthwash formulations containing *Zingiber officinale* var. *officinale* juice are found higher than the standard value of mouthwash. This can be caused by the content of *Zingiber officinale* var. *officinale* and sorbitol which is quite high. In fact, when these mouthwash formulations are compared with commercial mouthwashes which are the positive controls in this study, it prove ⁶ that the viscosity values of the mouthwash formulations 3.125%, 6.25% and 12.5% are closer to the viscosity of

water than the commercial ones. The mouthwash formulation in this test shows a stable viscosity for 56 days. In addition, there are differences in the viscosity values between the positive controls (enkasari and chlorhexidine), negative controls, and mouthwash formulations 3.125%, 6.25% and 12.5% at one-time measurement which is tested on day 0, 14, 28, 42 and 56. Based on these results, it can be concluded that the difference of mouthwash ingredients will affect the viscosity value of the mouthwash. The higher the concentration of *Zingiber officinale* var. *officinale*, the higher the viscosity of the mouthwash will be.

9 CONCLUSION

Based on the research that has been done, it can be concluded that the pH of *Zingiber officinale* var. *officinale* juice mouthwash in 3.125% concentration has been unstable for 56 days with 5.50-5.61 of pH value. The viscosity value has been stabled for 56 days. It has the lowest viscosity value compared to concentrations of 6.25% and 12.5%, that is 3.03-3.09 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 6.25% concentration has a stable pH and viscosity value for 56 days. It has the highest pH value compared to concentrations of 3.125% and 12.5%, that is 5.78-5.83, while the viscosity value ranges from 3.12-3.13 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 12.5% concentration has a stable pH value for 56 days with the lowest pH value compared to concentrations of 3.125% and 6.25%, that is 4.90-4.97. The viscosity value has been stabled with the highest viscosity value compared to concentrations of 3.125% and 6.25%, that is 3.43-3.50 cP.

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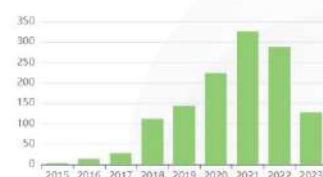
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RESEARCH ARTICLE

Evaluation of viscosity and pH of *Zingiber officinale* var. *officinale* juice in mouthwash formulation

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ABSTRACT

Zingiber officinale var. *officinale* juice has many benefits, including as a mixture of mouthwash. This research aims to analyze the differences in pH and viscosity values of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% on days 0, 14, 28, 42, and 56. *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% were mixed with propylene glycol, PEG-40 hydrogenated castor oil, oleum menthae, benzoic acid, sodium benzoate, calcium lactate, sorbitol 70% and calcium thiocyanate. The formulation was measured with a pH meter (Jenway, United Kingdom) and a viscometer (Brookfield, Massachusetts) for 56 days. pH data was analyzed using GLM repeated measures ANOVA and one-way ANOVA ($p < 0.05$). Viscosity data was analyzed using GLM repeated measured ANOVA and the Kruskal-Wallis test ($p < 0.05$). The pH value of the 3.125% formulation showed significant difference for 56 days in the range of 5.50-5.61. The viscosity value showed no significant difference for 56 days in the range of 3.03-3.09 cP. The pH and viscosity value of the 6.25% formulation showed no significant difference for 56 days with pHs of 5.78-5.83, while the viscosity is 3.12-3.13 cP. The pH and viscosity value of the 12.5% formulation showed no significant difference for 56 days with pHs of 4.90, while the viscosity was 3.43-3.50 cP. The *Zingiber officinale* var. *officinale* mouthwash formulation with a concentration of 3.125% was unstable, but it had the lowest viscosity value and was stable for 56 days. The pH and viscosity value of the 6.25% and 12.5% formulations were stable for 56 days. The highest pH value was in the 6.25% formulation.

Keywords: herbal mouthwash; pH; viscosity; *Zingiber officinale* var. *officinale*

INTRODUCTION

The prevalence of oral disease is increasing gradually with growing urbanization and changing living conditions in most countries with low to middle income.¹ Dental caries, considered as the most common dental and oral disease, is associated with biofilm or dental plaque,² which can be removed with mouthwash.³ Mouthwash can also improve oral health, reduce plaque or biofilm accumulation, prevent dental caries, and freshen the breath.^{3,4}

There are two types of mouthwashes: chemical mouthwashes and herbal mouthwashes.⁵ Although chemical mouthwashes have a good effect on the oral cavity, they tend to cause discoloration of the teeth and can produce other side effects on the oral cavity. Herbal mouthwashes can eradicate oral

pathogens, relieve pain instantly, and have fewer side effects compared to chemical mouthwashes.⁶

Each ingredient of mouthwash has its own role in establishing mouthwash formulation.⁷ Benzoic acid and sodium benzoate act as preservatives and buffer solutions.^{8,9} Propylene glycol (humectant) helps prevent evaporation of active ingredients, thus maintaining the stability and duration of mouthwash contact on the teeth.¹⁰ Surfactant (PEG-40 hydrogenated castor oil) lowers the surface tension of the solution, so the substances contained become more soluble and enhance hydrophilic penetration.^{11,12} Oleum menthae acts as a solvent and flavoring.⁹ Calcium lactate and potassium thiocyanate act as therapeutic agents.¹³ The function of sorbitol 70% is as an artificial sweetener.⁹

Most herbal extracts contain antimicrobial properties.⁵ *Zingiber officinale* var. *officinale* is one of them. *Zingiber officinale* var. *officinale* is less spicy than *Z. officinale* var. *Rubrum* and also has a better antibacterial property than *Z. officinale* var. *Amarum*. Thus, it may offer excellent potential as mouthwash ingredient.¹⁴ Evaluation of mouthwash formulations must be carried out before they undergo clinical trials. The tests include organoleptic tests (odor, color, taste, and consistency), pH (acidity), viscosity, sedimentation, and density.¹⁵ The purpose of this study is to analyze the differences of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 3.125%, 6.25%, and 12.5% on days 0, 14, 28, 42, and 56.

MATERIALS AND METHODS

This study used a true experimental research design with a posttest-only control group design. Mouthwash formulations were produced at the Dental Material and Testing Center of Research and Education Laboratory (DMTCORE), Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia. pH and viscosity tests were carried out at the Pharmaceutical Physics Laboratory, Faculty of Pharmacy, University of Indonesia, Depok, Indonesia. Equipment used in this study were analytical balances (Fujitsu, Japan), measuring cups (Pyrex, China), beakers

(Pyrex, China), magnetic stirrers (Toption, China), Whatman filter paper no. 1 (Midland Scientific, USA), pipettes (Bomex, China), an autoclave (Tomy, Japan), a pH meter (Jenway, United Kingdom), a viscometer (Brookfield, Massachusetts, USA), and grated ginger. The materials used in this study were propylene glycol (Dow Chemical Pacific, Singapore), PEG-40 hydrogenated castor oil (Lambertispa, Italy), oleum menthae (Anhui Province Yifan Spice Co., LTC, China), benzoic acid (Wuhan Youji Industries Co., LTD, China), sodium benzoate (Wuhan Youji Industries Co., LTD, China), calcium lactate (Galactic, Belgium), potassium thiocyanate (Merck Millipore, Germany), sorbitol 70% (Sorini Towa Berlian Corporindo, Indonesia), rhizome of *Zingiber officinale* var. *officinale* (Balitro, Indonesia), chlorhexidine gluconate 0.2% (Minosep, Indonesia), Enkasari mouthwash herbalcare protection fresh mint flavor (Enkasari, Indonesia), and aquadest.

One kilogram of 10-month-old *Zingiber officinale* var. *officinale* was cleaned, peeled, and washed with sterile aquadest. It was then mashed and filtered with Whatman filter paper no.1. The *Zingiber officinale* var. *officinale* juice was diluted to reach concentrations of 3.125%, 6.25%, and 12.5%. The *Zingiber officinale* var. *officinale* mouthwash was formulated by dissolving calcium lactate (50 mg) and potassium thiocyanate (100 mg) in aquadest (M1). Next, benzoic acid

Table 1. The formulation of juice of *Zingiber officinale* var. *officinale*

Materials	Function	F1	F2	F3
<i>Zingiber officinale</i> var. <i>officinale</i> juice	Active materials	3.125%	6.25%	12.5%
Propylene glycol (mL)	Humectant	5	5	5
PEG-40 hydrogenated castor oil (g)	Detergent	1	1	1
Oleum menthae (drops)	Solvent	10	10	10
Benzoic acid (mg)	Preservative	5	5	5
Sodium benzoate (g)	Preservative	2	2	2
Calcium lactate (mg)	Therapeutic agent	50	50	50
Sorbitol 70% (mL)	Flavoring agent	15	15	15
Potassium thiocyanate (mg)	Therapeutic agent	100	100	100



Figure 1. *Zingiber officinale* var. *officinale* juice mouthwash formulation

(5 mg) and ginger juice with a predetermined concentration were dissolved with ten drops of oleum menthae (M2). In the third step, M2 was emulsified with PEG-40 hydrogenated castor oil (1 gram) and propylene glycol (5 mL) was added gradually and stirred using a magnetic stirrer until the solution was homogeneous (M3). M1 was added little by little to the M3 solution and stirred until it was homogeneous. Fifteen mL of sorbitol 70% was added gradually and stirred until homogeneous. Finally, sodium benzoate (2 grams) was dissolved in aquadest and added to the mouthwash solution to obtain the appropriate pH of the mouthwash. The formulation was presented in Table 1. The formulations of the mouthwashes were stored in glass bottles at room temperature (Figure 1).

The pH values were measured by a pH meter (Jenway, United Kingdom), and the values were the average of three consecutive measurements. pH measurements were taken on days 0, 14, 28, 42 and 56. The viscosity value was measured by a viscometer (Brookfield, Massachusetts). Similar to the pH values, the results of the viscosity measurement were the average of three consecutive measurements. Viscosity measurements were taken on days 0, 14, 28, 42 and 56. Data normality was tested using the Shapiro-Wilk test. pH value data was analyzed using GLM repeated measures ANOVA and one-way ANOVA ($p < 0.05$). Viscosity value data was analyzed using GLM repeated measures ANOVA and the Kruskal-Wallis test ($p < 0.05$). Data was

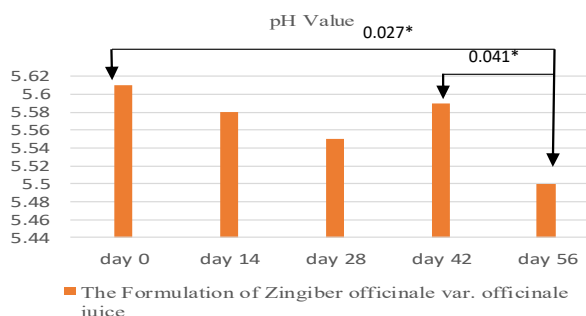
analyzed using SPSS version 25.0 (IBM SPSS Statistics, United States of America).

RESULTS

The pH value of the mouthwash formulations ranged from 5.46 to 5.83 (Table 2). The data was normally distributed ($p > 0.05$), and the results of GLM repeated measures ANOVA test of 0%, 6.25% and 12.5% mouthwash formulations were not significantly different in 56 days ($p > 0.05$). In contrast, the 3.125% mouthwash formulation showed a significant difference ($p < 0.05$). The results of the post-hoc paired wise comparison test of the 3.125% mouthwash formulation group is displayed in Figure 2.

The positive controls (chlorhexidine and Enkasari) were tested using a paired sample t-test because the test was only carried out on days 0 and 56. The paired sample t-test showed that there was no significant difference in the pH value of chlorhexidine and Enkasari on the day of the test ($p > 0.05$). One-way ANOVA test showed there were significant differences in pH values between the mouthwash groups with one-time measurement on days 0, 14, 28, 42, and 56 ($p < 0.05$). The results can be seen in Table 2.

The viscosity value of the mouthwash formulations ranged from 3.03 to 3.50 cP (Table 3). The data was normally distributed ($p > 0.05$) and the results of GLM repeated measures ANOVA test of 0%, 3.125%, 6.25%, and 12.5% mouthwash formulations were not significantly



*: significantly different ($p < 0.05$)

Figure 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation 3.125% (post-hoc paired wise comparison ($p < 0.05$)).

Table 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n = 3)	Mean ± Standard deviation					p
	day 0	day 14	day 28	day 42	day 56	
Chlorhexidine (positive control)	4.89	-	-	-	4.88	0.478
Enkasari (positive control)	4.77	-	-	-	4.76	0.225
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	5.48 ± 0.005	5.47	5.46 ± 0.005	5.47 ± 0.008	5.47	0.366
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3.125%	5.61 ± 0.005	5.58 ± 0.008	5.55 ± 0.011	5.59 ± 0.005	5.50	0.004*
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6.25%	5.78 ± 0.008	5.81 ± 0.008	5.78 ± 0.011	5.83 ± 0.005	5.80	0.081
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12.5%	4.97 ± 0.011	4.95 ± 0.005	4.93 ± 0.005	4.93 ± 0.005	4.90 ± 0.005	0.064
p	0.001†	0.001†	0.001†	0.001†	0.001†	

*GLM Repeated Measures ANOVA test (p < 0.05)

†One-way ANOVA test (p < 0.05)

Table 3. The viscosity value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n = 3)	Mean ± Standard deviation					p
	day 0	day 14	day 28	day 42	day 56	
Chlorhexidine (positive control)	4.42	-	-	-	4.34 ± 0.010	0.005*
Enkasari (positive control)	5.17	-	-	-	5.03 ± 0.231	0.423
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	1.91	1.93 ± 0.012	1.95 ± 0.021	1.96 ± 0.015	1.96 ± 0.008	0.180
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3.125%	3.03	3.09 ± 0.055	3.06 ± 0.025	3.06 ± 0.025	3.08 ± 0.021	0.588
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6.25%	3.12	3.12 ± 0.008	3.12 ± 0.008	3.12 ± 0.008	3.13 ± 0.008	0.491
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12.5%	3.50 ± 0.033	3.44 ± 0.074	3.43 ± 0.071	3.50 ± 0.011	3.43 ± 0.044	0.482
p	0.005*	0.024*	0.017*	0.019*	0.006*	

* Kruskal Wallis test (p < 0.05)

* Paired sample t-test (p < 0.05)

different ($p > 0.05$). The paired sample t-test was carried out to analyze the viscosity values of the positive controls (chlorhexidine and Enkasari) because the tests were only taken on days 0 and 56. The test showed that there was a significant difference in the chlorhexidine viscosity values on days 0 and 56 ($p < 0.05$). Meanwhile, the results of the Enkasari test showed that there was no significant difference on days 0 and 56 ($p > 0.05$). Table 3 shows the results of the analysis.

Based on the results of the normality test, the data was not normally distributed ($p < 0.05$); therefore, one-way ANOVA test could not be performed. As an alternative, the data could be analyzed using the Kruskal-Wallis test which is a non-parametric test. The Kruskal-Wallis test showed that there was a significant difference in pH values of the mouthwash groups on days 0, 14, 28, 42, and 56 ($p < 0.05$). The results of the analysis are presented on Table 3.

DISCUSSION

In this research, the mouthwash formulation contained *Zingiber officinale* var. *officinale* juice with concentrations of 3.125%, 6.25%, and 12.5%. The mouthwash formulations used in this study contain various ingredients that can maintain oral health. Propylene glycol functions as a humectant, antimicrobial, disinfectant, cosolvent, and stabilizer.^{16,17} *Oleum menthae* is used as a flavoring agent, solvent, anti-inflammatory, anti-spasmolytic, and antibacterial agent which acts as a bactericidal against pathogens such as *Escherichia coli* and *Staphylococcus aureus*.¹⁸ Sorbitol functions as a sugar substitute sweetener which has calorific value, low glycemic index, and free of carcinogenic effects. In addition, sorbitol prevents the formation of plaque and tooth decay owing to resistance to the metabolism of oral bacteria.¹⁹ The preservatives are sodium benzoate and benzoic acid,^{8,9} which have an activity as antifungal and antibacterial agents. Benzoic acid and sodium benzoate act as buffer in mouthwash.²⁰ Polyoxyl 40 (PEG-40) hydrogenated castor oil is used as surfactant

to lower the surface tension in the solution, solubilize fat-soluble components, and increase hydrophilic penetration.¹² The therapeutic agents are calcium lactate and potassium thiocyanate. The functions of calcium lactate are to strengthen tooth structure, and as antibacterial agents, antitartar agents, and bone substitute materials.¹³ Potassium thiocyanate acts as an antifungal and anti-caries agent in teeth.²¹

Zingiber officinale var. *officinale* is a natural ingredient which has antimicrobial and antifungal effects to prevent caries and oral candidiasis; therefore, ginger may help maintain oral health.²² Phytochemical test of *Zingiber officinale* var. *officinale* shows that it contains alkaloids, flavonoids, quinones, saponins, monoterpenoids, and sesquiterpenoids.²³ Previous studies have demonstrated that mouthwashes containing natural ingredients are safe and have fewer side effects.²⁴

Stability tests were performed to evaluate changes in the mouthwash preparation to determine the mouthwash suitability. The pH test showed the value was in the range of 4.90-5.83. The pH of quality standard of herbal mouthwash ranges from 5 to 7.²⁵ In this study, the mouthwash formulations with 0%, 3.125% and 6.25% concentrations of ginger met the quality standards of herbal mouthwash with a pH in the range of 5.46-5.83. The 12.5% mouthwash formulation had a pH from 4.90 to 4.97, which was the lowest, and it may indicate that it did not meet the quality standards of herbal mouthwashes because it was too acidic. When mouthwash solution proven too acidic, it makes the bacteria grow faster and may irritate to the oral cavity.^{3,26} Surprisingly, when the 12.5% mouthwash formulation was compared to commercial mouthwash, which is a positive control in this study, it showed that the pH of the mouthwash was higher than the commercial mouthwash. The pH values of *Zingiber officinale* var. *officinale*-containing mouthwashes with concentrations of 0%, as a negative control, 6.25%, and 12.5% were stable for 56 days. However, the pH value of the mouthwash formulation with a concentration of ginger of 3.125% was unstable for 56 days. In

addition, the mouthwash formulations across the three different percentages showed different pHs, both in negative and positive control at one-time measurement. It is highly likely that differences in the mouthwash ingredients could affect the pH value of the mouthwash.

The viscosity test showed that the value was in the range of 3.03-3.50 cP. The best mouthwash viscosity is the one that is closest to the viscosity of water, which is 0.89 cP.²⁷ It is mainly by considerations of convenience when patients rinse their mouth. The viscosity values of mouthwash formulations containing *Zingiber officinale* var. *officinale* juice were found higher than the viscosity value of standard mouthwash. This could be caused by the relatively high content of *Zingiber officinale* var. *officinale* and sorbitol in the mouthwash. In comparison with commercial mouthwashes, which were the positive controls in this study, it was found that the viscosity values of the mouthwash formulations with concentrations of ginger of 3.125%, 6.25%, and 12.5% were closer to the viscosity of water than that of the commercial ones. The mouthwash formulation in this test showed a stable viscosity for 56 days. In addition, there were differences in the viscosity values between the positive controls (Enkasari and chlorhexidine), negative controls, and the three mouthwash formulations (3.125%, 6.25%, and 12.5%) at one-time measurement tested on days 0, 14, 28, 42, and 56. Taken together, differences in mouthwash ingredients could affect the viscosity value of the mouthwash. This study found that the higher the concentration of *Zingiber officinale* var. *officinale*, the higher the viscosity of the mouthwash. One unanticipated result in this study was that the mouthwash formulations had cloudy colors, whereas mouthwash formulations should have clear color to appeal the public. Mouthwash formulations have to pass various tests such as organoleptic, density, sedimentation and toxicity tests. These tests, however, had not been carried out in this study. Further research, which incorporates these tests to determine the feasibility of the mouthwash, is therefore recommended.

CONCLUSION

Based on the results of this research, it can be concluded that the pH of the *Zingiber officinale* var. *officinale*-containing mouthwash with a concentration of 3.125% was unstable for 56 days with pHs in the range of 5.50-5.61. The viscosity value, however, was stable for 56 days. It had lower viscosity value compared to those with concentrations of 6.25% and 12.5%, which was between 3.03 and 3.09 cP. Mouthwash formulation containing 6.25% concentration of *Zingiber officinale* var. *officinale* juice had a stable pH and viscosity value for 56 days. It showed a higher pH value compared to pHs of mouthwashes with ginger concentrations of 3.125% and 12.5%, which was in the range of 5.78-5.83, while the viscosity value ranged from 3.12 to 3.13 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice with a concentration of 12.5% had a stable pH value for 56 days and had lower pH value compared to the pHs of those with the concentrations of 3.125% and 6.25%, which was between 4.90 and 4.97. The viscosity value was stable and higher compared to those of the mouthwashes with ginger concentrations of 3.125% and 6.25%, which was in the range of 3.43-3.50 cP.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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VISCOSITY AND pH EVALUATION OF Zingiber officinale var. officinale JUICE IN MOUTHWASH FORMULATION

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VISCOSITY AND pH EVALUATION OF *Zingiber officinale* var. *officinale* JUICE IN MOUTHWASH FORMULATION

INTRODUCTION

The prevalence of oral disease gradually grows for the increasing urbanization and changing living conditions in most countries with low to middle income.¹ Dental caries, considered as the most common dental and oral disease, is associated with biofilm or dental plaque.² Biofilm or dental plaque can be eradicated by mouthwash.³ Mouthwash improve oral health, reduce plaque or biofilm accumulation, prevents dental caries, and freshens breath.^{3,4}

There are two types of mouthwashes, chemical mouthwashes and herbal mouthwashes.⁵ Although chemical mouthwashes have a good effect on the oral cavity, they tend to cause discoloration of the teeth and can produce other side effects on the oral cavity. Herbal mouthwashes can eradicate oral pathogens, relieve pain instantly, and have fewer side effects compared to chemical mouthwashes.⁶

Each mouthwash ingredient has its own role in establishing the mouthwash formulation.⁷ Benzoic acid and sodium benzoate act as preservatives and buffer solutions.^{8,9} Humectants (propylene glycol) prevents active ingredients evaporation, so they can maintain the stability and duration of mouthwash contact on the teeth.¹⁰ Whilst surfactant (PEG-40 hydrogenated castor oil) lowers the surface tension of the solution, so the substances contained become more soluble.^{11,12} *Oleum menthae* act as a solvent and flavoring.⁹ Calcium lactate and potassium thiocyanate act as therapeutic agents.^{12,13} The function of sorbitol 70% is an artificial sweetener.⁹

Most herbal extracts contain antimicrobial properties.⁵ *Zingiber officinale* var. *officinale* is one of them. *Zingiber officinale* var. *officinale* is less spicy than *Z. officinale* var. *Rubrum* and also has a better antibacterial property than *Z. officinale* var. *Amarum*. This leads to consider it for mouthwash ingredient.¹⁴ Evaluation must be carried out on mouthwash formulations before they are undergoing clinical trials. The tests that must be performed including organoleptic tests (odor, color, taste, and consistency), pH (acidity), viscosity, sedimentation, and density.¹⁵ The purpose of this study is to analyse the differences of *Zingiber officinale* var. *officinale* mouthwashes 3.125%, 6.25%, and 12.5% on pH and viscosity values on day 0, 14, 28, 42, and 56.

MATERIALS AND METHODS

This is true experimental study with a posttest-only control group design. Mouthwash formulations are produced at the Dental Material & Testing Center of Reserch and Education Laboratory (DMTCORE)¹⁰ Faculty of Dentistry, Trisakti University, Jakarta, Indonesia. pH and viscosity tests were carried out at ¹⁰ Pharmaceutical Physics Laboratory, Faculty of Pharmacy, University Indonesia, Depok, Indonesia. The tools that used in this study are analytical balances (Fujitsu, Japan), measuring cups (Pyrex, China), beaker glass (Pyrex, China), magnetic stirrers (Toption, China), Whatman filter paper no. 1 (Midland Scientific, USA), pipette (Bomex, China), autoclave (Tomy, Japan), pH meter (Jenway, United Kingdom), viscometer (Brookfield, Massachusetts, USA), and grated ginger. The materials that used in this study are propylene glycol (Dow Chemical Pacific, Singapore), PEG-40 hydrogenated castor oil Lambertispa, Italy), oleum menthae (Anhui Province Yifan Spice Co., LTC, China), benzoic acid (Wuhan Youji Industries Co., LTD, China), sodium benzoate (Wuhan Youji Industries Co., LTD, China), calcium lactate (Galactic, Belgium), potassium thiocyanate (Merck Millipore, Germany), sorbitol 70% (Sorini Towa Berlian Corporindo, Indonesia), the rhizome of *Zingiber officinale* var. *officinale* (Balitro,

Indonesia), chlorhexidine gluconate 0.2% (Minosep, Indonesia), enkasari mouthwash herbalcare protection fresh mint flavor (Enkasari, Indonesia), and aquadest.

1. ¹ *Zingiber officinale* var. *officinale* Juice in 3.125%, 6.25% and 12.5% concentration

One kilograms of 10-month-old *Zingiber officinale* var. *officinale* is cleaned, peeled, washed with sterile aquadest, mashed, filtered with Whatman filter paper no.1, diluted to reach 3.125%, 6.25% and 12.5% concentrations.

2. Production of *Zingiber officinale* var. *officinale* Juice Mouthwash

The juice production of *Zingiber officinale* var. *officinale* mouthwash formulation is made by dissolving calcium lactate (50 mg) and potassium thiocyanate (100 mg) in aquadest (M1). Next, benzoic acid (5 mg) and ginger juice with a predetermined concentration are dissolved with ten drops of oleum menthe (M2). The third step, M2 is emulsified with PEG-40 hydrogenated castor oil (1 gram) and added gradually propylene glycol (5 mL) and stirred using a magnetic stirrer until the solution is homogeneous (M3). M1 was added little by little to the M3 solution and stirred until it is homogeneous. 15 mL of sorbitol 70% is added gradually and stirred until it becomes homogeneous. Finally, sodium benzoate (2 grams) is dissolved with aquadest and added to the mouthwash solution to obtain the appropriate pH of the mouthwash. The formulation is in table 1. The formulations of the mouthwashes are stored in glass bottles at room temperature (Figure 1).

Table 1. The formulation of juice of *Zingiber officinale* var. *officinale*

Materials	Function	F1	F2	F3
<i>Zingiber officinale</i> var. <i>officinale</i> juice	Active materials	3.125%	6.25%	12.5%
Propylene glycol (mL)	Humectant	5	5	5
PEG-40 hydrogenated castor oil (g)	Detergent	1	1	1
Oleum menthae (drops)	Solvent	10	10	10
Benzoic acid (mg)	Preservative	5	5	5
Sodium benzoate (g)	Preservative	2	2	2
Calcium lactate (mg)	Therapeutic agent	50	50	50
Sorbitol 70% (mL)	Flavoring agent	15	15	15
Potassium thiocyanate (mg)	Therapeutic agent	100	100	100



Figure 1. *Zingiber officinale* var. *officinale* juice mouthwash formulation

3. pH Test

The pH value is measured by a pH meter (Jenway, United Kingdom). The pH measurement is taken three times at one-time measurement, then the results are averaged. pH measurements are taken on days 0, 14, 28, 42 and 56.

4. Viscosity Test

The viscosity value is measured by a viscometer (Brookfield, Massachusetts). Viscosity measurement is taken three times at one-time measurement, then the results are averaged. Viscosity measurements are taken on days 0, 14, 28, 42 and 56.

5. Data Analysis

Data normality is tested using the Shapiro-Wilk test. pH value data is analyzed using GLM Repeated Measures ANOVA and One-way ANOVA ($p < 0.05$). Viscosity value data is analyzed using GLM Repeated Measures ANOVA and Kruskal Wallis ($p < 0.05$). Data is analyzed using SPSS version 25.0 (IBM SPSS Statistics, United States of America).

RESULTS

a. The Result of pH Test

The pH value of the mouthwash formulations ranges from 5.46 to 5.83 (Table 2). The data is normally distributed ($p>0.05$) and the result of GLM Repeated Measures ANOVA test on 0%, 6.25% and 12.5% mouthwash formulations are not significantly different in 56 days ($p>0.05$). In contrast to the 3.125% mouthwash formulation group which shows a significant difference ($p<0.05$). The result of the post-hoc paired wise comparison test in the 3.125% mouthwash formulation group can be seen in Figure 2.

Table 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n=3)	Mean±Standard Deviation					p
	Day 0	Day 14	Day 28	Day 42	Day 56	
Chlorhexidine (positive control)	4.89	-	-	-	4.88	0.478
Enkasari (positive control)	4.77	-	-	-	4.76	0.225
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	5.48±0.005	5.47	5.46±0.005	5.47±0.008	5.47	0.366
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3,125%	5.61±0.005	5.58±0.008	5.55±0.011	5.59±0.005	5.50	0.004*
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6,25%	5.78±0.008	5.81±0.008	5.78±0.011	5.83±0.005	5.80	0.081
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12,5%	4.97±0.011	4.95±0.005	4.93±0.005	4.93±0.005	4.90±0.005	0.064
p	0.001†	0.001†	0.001†	0.001†	0.001†	

*GLM Repeated Measures ANOVA test ($p<0.05$)

†One-way ANOVA test ($p<0.05$)

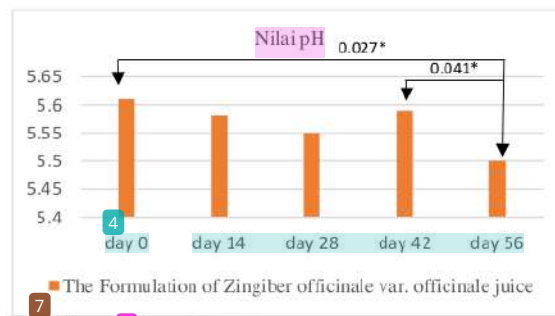


Figure 2. The pH value of juice of *Z. officinale* var. *officinale* mouthwash formulation 3.125% (post-hoc paired wise comparison ($p<0.05$)).

The positive controls (chlorhexidine and enkasari) are tested using a *paired sample t-test* because the test is only carried out on day 0 and 56. The paired sample t-test shows that there is no significant difference in the pH value of chlorhexidine and enkasari on the day of test ($p>0.05$). *One-way ANOVA* test shows there are significant differences in pH values between the mouthwash groups at one-time measurement on day 0, 14, 28, 42 and 56 ($p<0.05$). The results can be seen in table 2.

b. The Result of Viscosity Tests

The viscosity value of the mouthwash formulations ranges from 3.03 to 3.50 cP (Table 3). The data is normally distributed ($p>0.05$) and the results of *GLM Repeated Measures ANOVA* test on 0%, 3.125%, 6.25% and 12.5% mouthwash formulations are not significantly different ($p>0.05$). The *paired sample t-test* is carried out to analyze the viscosity values of the positive controls (chlorhexidine and enkasari) in this study because the tests are only taken on day 0 and 56. The test shows that there is a significant difference in the chlorhexidine viscosity values on day 0 and 56 ($p<0.05$). Meanwhile, the results of the enkasari test shows that there is no significant difference on day 0 and 56 ($p>0.05$). The results of the analysis can be seen in Table 3.

Table 3. The viscosity value of juice of *Z. officinale* var. *officinale* mouthwash formulation for 56 days

Groups (n=3)	Mean±Standard Deviation					p
	Day 0	Day 14	Day 28	Day 42	Day 56	
Chlorhexidine (positive control)	4.42	-	-	-	4.34±0.010	0.005*
Enkasari (positive control)	5.17	-	-	-	5.03±0.231	0.423
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 0% (negative control)	1.91	1.93±0.012	1.95±0.021	1.96±0.015	1.96±0.008	0.180
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 3,125%	3.03	3.09±0.055	3.06±0.025	3.06±0.025	3.08±0.021	0.588
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 6,25%	3.12	3.12±0.008	3.12 ± 0.008	3.12 ± 0.008	3.13±0.008	0.491
<i>Z. officinale</i> var. <i>officinale</i> mouthwash 12,5%	3.50±0.033	3.44±0.074	3.43±0.071	3.50±0.011	3.43±0.044	0.482
p	0.005*	0.024*	0.017*	0.019*	0.006*	

* *Kruskal Wallis* test ($p<0.05$)

* *Paired sample t-test* ($p<0.05$)

Based on the normality test performed on the sample, it is found that the data is not normally distributed ($p<0.05$), so that *One-way ANOVA* test cannot be performed. As an alternative, data can be analyzed using *Kruskal Wallis* test which is a non-parametric test. *Kruskal Wallis* test shows that there is a significant difference in pH value between the mouthwash groups on day 0, 14, 28, 42 and 56 ($p<0.05$). The results of the analysis can be seen in Table 3.

DISCUSSION

In this research, the mouthwash formulation contains *Zingiber officinale* var. *officinale* juice in

3.125%, 6.25% and 12.5% concentration. The mouthwash formulations used in this study contain various ingredients that can maintain oral health. Propylene glycol functions as a humectant, antimicrobial, disinfectant, cosolvent, and stabilizer.^{16,17} *Oleum menthae* is used as a flavoring, solvent, anti-inflammatory, anti-spasmodic, and antibacterial agent which acts as a bactericidal against pathogens such as *Escherichia coli* and *Staphylococcus aureus*.¹⁸ Sorbitol functions as a sugar substitute sweetener which has caloric value, low glycemic index, and carcinogenic effects free. In addition, sorbitol prevents the formation of plaque and tooth decay owing to resistance to the metabolism of oral bacteria.¹⁹ The preservatives are sodium benzoate and benzoic acid.^{8,9} Sodium benzoate and benzoic acid has an activity as ⁸ antifungal and antibacterial. Benzoic acid and sodium benzoate act as buffer in mouthwash.²⁰ Polyoxyl 40 (PEG-40) hydrogenated castor oil is used as a detergent which lowers the surface tension in the solution, so the substances become more soluble and are able to kill bacteria in the oral cavity by destroying the bacterial cell walls.¹² The therapeutic agents are calcium lactate and potassium thiocyanate. The functions of calcium lactate are strengthening tooth structure, antibacterial agents, antitartar agents, and bone substitute materials.¹³ Potassium thiocyanate acts as an antifungal and anti-caries agent in teeth.²¹

Zingiber officinale var. *officinale* is natural ingredient which carries antimicrobial and antifungal effects to prevent caries and oral candidiasis, so that ginger influences to maintain oral health.²² Phytochemical test of *Zingiber officinale* var. *officinale* proves that it contains alkaloids, flavonoids, quinones, saponins, monoterpenoids, and sesquiterpenoids.²³ Based on previous studies, mouthwashes containing natural ingredients are safe for the body and have fewer side effects.²⁴

Stability tests are performed to evaluate changes of the mouthwash preparation that may ⁶ determine the mouthwash suitability. The pH test shows the value is in the range of 4.90-5.83. The quality standard of herbal mouthwash ⁶ is 5-7 pH value.²⁵ The mouthwash formulations in 0%, 3.125% and 6.25% concentration meet the quality standards of herbal mouthwash with a pH range of 5.46-5.83. The 12.5% mouthwash formulation reaches 4.90 to 4.97 pH value, which is the lowest, indicates it does not meet the quality standards of herbal mouthwashes in consideration of being too acidic. When mouthwash solution proven too acidic, it makes the bacteria grow faster and irritate to the oral cavity.^{3,26} Surprisingly, when the mouthwash formulation 12.5% is compared to commercial mouthwash which is a positive control in this study, it shows that the pH value of the mouthwash is higher than the mouthwash used by the public. The pH value of a mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 0% as a negative control, 6.25%, and 12.5% concentration ¹ have been stabled for 56 days. However, the pH value of the 3.125% concentration mouthwash formulation has found to be unstable for 56 days. In addition, there are the differences in pH values between mouthwash formulations in 3.125%, 6.25% and 12.5% concentration, negative and positive control at one-time measurement. This strongly indicates that the difference of the mouthwash ingredients will affects the pH value of the mouthwash.

The viscosity test shows that the value is in the range of 3.03-3.50 cP. The best mouthwash viscosity is one that closest to the viscosity of water for convenient reason when patients rinse their mouths. The water viscosity value is 0.89 cP.²⁷ The viscosity values of a mouthwash formulations containing *Zingiber officinale* var. *officinale* juice are found higher than the standard value of mouthwash. This can be caused by the content of *Zingiber officinale* var. *officinale* and sorbitol which is quite high. In fact, when these mouthwash formulations are compared with commercial mouthwashes which are the positive controls in this study, it prove ⁶ that the viscosity values of the mouthwash formulations 3.125%, 6.25% and 12.5% are closer to the viscosity of

water than the commercial ones. The mouthwash formulation in this test shows a stable viscosity for 56 days. In addition, there are differences in the viscosity values between the positive controls (enkasari and chlorhexidine), negative controls, and mouthwash formulations 3.125%, 6.25% and 12.5% at one-time measurement which is tested on day 0, 14, 28, 42 and 56. Based on these results, it can be concluded that the difference of mouthwash ingredients will affect the viscosity value of the mouthwash. The higher the concentration of *Zingiber officinale* var. *officinale*, the higher the viscosity of the mouthwash will be.

9 CONCLUSION

Based on the research that has been done, it can be concluded that the pH of *Zingiber officinale* var. *officinale* juice mouthwash in 3.125% concentration has been unstable for 56 days with 5.50-5.61 of pH value. The viscosity value has been stabled for 56 days. It has the lowest viscosity value compared to concentrations of 6.25% and 12.5%, that is 3.03-3.09 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 6.25% concentration has a stable pH and viscosity value for 56 days. It has the highest pH value compared to concentrations of 3.125% and 12.5%, that is 5.78-5.83, while the viscosity value ranges from 3.12-3.13 cP. Mouthwash formulation containing *Zingiber officinale* var. *officinale* juice in 12.5% concentration has a stable pH value for 56 days with the lowest pH value compared to concentrations of 3.125% and 6.25%, that is 4.90-4.97. The viscosity value has been stabled with the highest viscosity value compared to concentrations of 3.125% and 6.25%, that is 3.43-3.50 cP.

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