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Streptococcus Mutans Antibacterial Study: Mouthwash Preparations Formulation Using Cinnamon and Betel Leaf Essential Oils (*Cinnamomum burmannii*) (*Piper betle* L)

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Mouthwash on the market contains active ingredients that are not natural and often cause side effects to the mouthwash user. Therefore, mouthwash has been developed that uses natural active ingredients such as cinnamon and betel leaf essential oils. This research aims to determine the physical and antibacterial properties of mouthwash preparations. The results obtained by the mouthwash that contains essential oils have a more pungent smell and are more turbid than the mouthwash without essential oils. Mouthwash viscosity shows the value of F1 to F6, respectively for 1.0445; 0.9464; 1.0031; 0.9905; 0.9586; and 0.9010. The density of mouthwash has a value close to 1, which is the density of water. The test results showed the entire formula has a pH of acidic nature ranging from 3.7. The antibacterial power test showed that the diameter of the biggest obstacle is the mouthwash, which contains single essential oils of cinnamon.

Keywords: *Mouthwash, Essential Oils of Cinnamon, Betel Leaf Essential Oil*.

Introduction

Oral health in Indonesia requires serious attention (Anitasari and Rahayu, 2005). As many as 89% of Indonesian children under 12 years old suffer from oral disease (Besford and Yuwono, 1996). In Jakarta, 90% of children have problems with cavities (caries), and 80% suffer from gum disease (Zatnika, 2009). Children are very susceptible to tooth decay because most

children like sweets (Besford and Yuwono, 1996). Although that is the case, oral health problems not only occur in children but can occur in adolescents and adults. Research conducted by Boy (1991) and Boy (2019) showed that as many as 72% of teenage respondents at high school in the city of Jambi have the status of dental caries in the unfavourable category, and the prevalence of dental caries in adolescents was 98.3%.

Dental cavities (caries) is the most common disease and often interferes with human activities. Dental caries can occur in one part of the tooth and then extend to the deepest part of the tooth (Tarigan, 1989). Dental caries begins with the formation of plaque on the teeth (Kuntari, 1996). Dental plaque can be prevented by brushing teeth properly to obtain maximum results (Nunung and Zubaidah, 1993). Proper dental care is an appropriate effort to avoid complications of diseases caused by a bacterial infection that causes toothache (Muis, 2010).

Cleaning teeth insufficiently causes infection: *streptococcus mutans* is one of the bacteria causing the infection, creating dental caries events (Kidd, 1991). These bacteria are found in large amounts in patients with dental plaque caries (Roeslan, 1996).

Streptococcus mutans can convert sugar from sweets to acid (Besford and Yuwono, 1996). When the degree of acidity (pH) is low it will damage tooth enamel organic material to form cavities (Hastuti and Indriyani, 2010). By reducing the production of acid by the bacteria, then the excess acidity of saliva can be prevented so as not to exceed the critical limits. The use of mouthwash (mouthwash) stimulates the major salivary glands, and salivary pH returns to normal to prevent the formation of dental caries (Juwita, 2013).

Mouthwash is a solution to rinse the mouth in the fashion of breath fresheners, which contain astringent, demulcent or surfactant and antibacterial ingredients, to refresh and cleanse the respiratory tract (Akarina, 2011). Mouthwash contains an antibacterial substance, thereby reducing the number of microorganisms in the mouth (Wardani, 2012). Toothpaste in circulation today includes the kinds of active elements that have proved useful for oral health, the active substances, among others, are chlorhexidine and triclosan. Chlorhexidine is often used as a positive control for comparison of other products. However, chlorhexidine has side effects, some of which are tooth discoloration, unpleasant taste, dry and burning sensation in the mouth (Bishara, 1991). At this time, most mouthwashes on the market using active ingredients that are not natural and often cause side effects to the mouthwash user.

In addition to using synthesic ingredients, mouthwash can use natural ingredients as nutritious substances (Wardani, 2012). Natural materials are already known to be useful for health. Utilisation of natural materials, especially plants, animals, and marine organisms, cannot be separated from the chemical content contained therein, e.g., betel leaf and cinnamon essential oils.

Several researchers have analysed the chemical components and known materials making up the essential oil of betel leaf (*Piper betle*) – the main component constituents of this volatile oil include others amongst others: kariofilena (30%), isoeugenol (22%), and α-kubobena (9%) (Agusta, 2000; Sulianti & Chairul, 2002; Hertiani & Purwantini, 2002). Narlina and Rahim (2002) reported that the water decoction of betel leaf extract has antibacterial activity against mutant *streptococcus*.

Cinnamomum burmannii (Cinnamon) is one type of the Lauraceae family that was selected for this study. This plant is widely available in sub-tropical and tropical regions. A review of the essential oil of *Cinnamomum burmannii* originating from Guangzhou, China conducted by Wang et al. (2009), reported that the significant components contained in this essential oil are trans cinnamaldehyde (60.72%), eugenol (17.62%) and coumarin (13.39%).

Various formulas can be used to make preparations for mouthwash. According to Yosephine et al. (2013), in his research, basil leaf essential oil mouthwash formula is the basis of the mouthwash formula tween 80, and a glycerin ratio of 3.75 ml and 1.25 ml with inhibition of bacterial growth amounted to 87.50 ± 3.33%.

Based on the description above, we then tested the physical properties and antibacterial activity of essential oil of cinnamon bark and betel leaf essential oil in a variety of dosage formulations of mouthwash.

Methods

The design of this study is experimental. The dependent variable found in this study is the preparation of mouthwash. While the independent variable in this study is a mixture of essential oil of cinnamon (Cinnamomum oil) and betel leaf (Piper oil). Subjects in this study are a mixture of essential oil of cinnamon (Cinnamomum oil) and betel leaf (Piper oil) formulated in mouthwash dosage forms with a ratio of F1 (0: 0), F2 (1: 0), F3 (0: 1), F4 (1: 1), F5 (1: 3) and F6 (3: 1).

Tool

The tools used in this research are laboratory glassware, hose, tweezers, light spirits, hot plate, balance, pH meter, Pycnometer, Ostwald viscometer, Autoclave, and incubators.

Material

Materials used include essential oils of cinnamon, betel leaf essential oil, tween 80, glycerin, peppermint oil, a solution of Na-Benzoate, Na-saccharin solution, NaCl, dye, Blood agar plate (BAP), disk medicine, *streptococcus mutans,* and distilled water.

Procedure

1. Preparation of mouthwash with a mixture of essential oils of cinnamon and betel leaf

Development of a toothpaste follows the formula of Yosephine, et al. (2013). Comparison mix of the active ingredients in the form of essential oils of cinnamon and betel leaf can be seen in Table 1.

Table 1. Essential Oil Blend or active ingredient

Essential Oils / Active Ingredients	Formula components					
	F10: 0	F2 1: 0	F3 0: 1	F4 1: 1	F5 1: 3	F6 3: 1
Cinnamon	0 ml	1 ml	0 ml	0.5 ml	0.25 ml	0.75 ml
Betel leaf	0 ml	0 ml	1 ml	0.5 ml	0.75 ml	0.25 ml

2. Testing mouthwash preparations with a mixture of essential oils of cinnamon and betel leaf

Examination preparations undertaken in this study include organoleptic test, viscosity test, a test of density and pH test; Tests performed on six mouthwash preparations as produced. The organoleptic test was conducted in mouthwash preparations to observe the change in the form of, among others, odour and clarity of mouthwash. Viscosity test conducted by Ostwald viscometer covering sees fluid viscosity by entering the mouthwash solution viscometer dosage up to the upper limit. Used rubber reckoner to drain the mouthwash solution preparation and allowed it to flow to the lower limit. Prepared stopwatch to record the time , then calculated viscosity. The density of the test is done using a pycnometer by weighing an empty pycnometer, considering the pycnometer containing mouthwash solution preparation to be measured for its density. I have then calculated the thickness of the obtained preparation solution.

3. Testing antibacterial mouthwash preparations with a mixture of essential oils of cinnamon and betel leaf

In this study, the antibacterial test was done using a diffusion test using the media blood agar plate (BAP).

Results and Discussion

Tests conducted on six types of mouthwash formula preparations have been made. Formula mouthwash made with a blend of essential oils of cinnamon (Cinnamomum oil) and essential

oil of betel leaf (piper oil) in comparison F1 (0: 0), F2 (1: 0), F3 (0: 1), F4 (1: 1), F5 (1: 3), F6 (3: 1).

Organoleptic testing results (Table 2), the viscosity test (Table 3), the density of the analysis (Table 4), pH test (Table 5), are as follows:

Table 2.Mouthwash Preparations Organoleptic Test

No.	formula	Smell	Clarity
1	F1 (0: 0)	weak odour	Clear
2	F2 (1: 0)	tang	somewhat Cloudy
3	F3 (0: 1)	tang	cloudy
4	F4 (1: 1)	weak odour	cloudy
5	F5 (1: 3)	tang	slightly turbid
6	F6 (3: 1)	tang	cloudy

Table 3. Viscosity Test Mouthwash Preparations

No.	formula	viscosity
1	F1 (0: 0)	1.04
2	F2 (1: 0)	0.95
3	F3 (0: 1)	1.00
4	F4 (1: 1)	0.99
5	F5 (1: 3)	0.96
6	F6 (3: 1)	0.90

Table 4. Test preparations Massa type Mouthwash

No.	formula	Density
1	F1 (0: 0)	1.02
2	F2 (1: 0)	1.04
3	F3 (0: 1)	1.02
4	F4 (1: 1)	1.02
5	F5 (1: 3)	1.02
6	F6 (3: 1)	1.03

Table 5. Test preparations pH Mouthwash

No.	formula	pH
1	F1 (0: 0)	4.09
2	F2 (1: 0)	3.75
3	F3 (0: 1)	3.70
4	F4 (1: 1)	3.74
5	F5 (1: 3)	3.80
6	F6 (3: 1)	3.78

The test results showed that the antibacterial activity of the essential oil of cinnamon bark is inhibitory to the bacteria s*treptococcus mutans* . A mixture of essential oils has lower antibacterial activity against *Streptococcus mutans* than preparations with single essential oil of cinnamon bark. The diameter of the positive control barriers originating from mouthwash "X" containing the essential oil of betel leaf has a diameter of 16.2 mm barrier. The test results for antibacterial mouthwash preparations can be seen in Table 6.

Table 6. Power Test preparations Antibacterial Mouthwash

No.	formula	Barriers Diameter (mm)
1	F1 (0: 0)	6.00
2	F2 (1: 0)	13.80
3	F3 (0: 1)	7.08
4	F4 (1: 1)	8.75
5	F5 (1: 3)	9.05
6	F6 (3: 1)	8.73

The organoleptic test is one of the physical parameters to determine the quality of the mouthwash. From the results of the organoleptic test of formula 1, 2, 3, 4, 5 and 6 show, the mouthwash preparations were visually observed as cloudy to very cloudy. This caused the reaction between tween 80 essential oils that are not mixing with eugenol (phenol) contained in the essential oil extracts. In the cinnamon, the extract showed more turbidity than betel leaf extract mouthwash. Phenol content in the betel leaf showed smaller polarity than the polarity of phenol volatile oil on cinnamon.

Viscosity test is one of the parameters to assess the cast of mouthwash (Martin A, 1993). Observations on the entire formula preparations have viscosity values ranging from 0.9 to 1.0 pm. This mouthwash preparation's viscosity value is within the dosage value range according to the standard mouthwash preparations (0.8 - 1). The closer the mouthwash preparation approaches a viscosity grade level of 0.89, it then becomes a more comfortable and convenient product for gargling (Rowe, 2009; Tranggono, 2007). The thickness of the preparation is due to the materials such as the Tween and span contained therein. Viscosity dosage formulations can also be changed with change in temperature. Generally, fluid viscosity decreases with increasing temperature (Ansel, 2005).

The density of the entire formula preparation (1 - 6) is in the range of 1.02 to 1.04 and on the average the mouthwash preparations are fit for circulation (1.03). Mass type is very influenced by glycerin as a wetting agent to decrease the water surface. With voltage (contact angle) and increases of insoluble dispersion material based on the evaluation of the value of the density of formula 1, 2, 3, 4, 5, 6, there is no significant difference.

In preparation pH probe showed the entire formula has acidic properties ranged between approximately 3.7. The degree of acidity of 5.0 to 9.5 is a safe pH; the optimum pH is from 6.5 to 7.0 for liquid oral use (Martin, 1971). Mouthwash preparations that have met with success by POM preparation showed pH range 4 - 7. So, the entire formula shows the formula 2 to method 6 has a pH less than the pH range. This is because the content of essential oil extracts has the acidic properties of both materials. To overcome these problems, it is necessary to increase the buffer in the formulation.

Testing antibacterial mouthwash preparation of betel leaf oil and cinnamon oil using a diffusion method against streptococcus mutants showed the presence of inhibition ranged from 7.08 to 13.80. Mouthwash qualifications inhibition without extract (6.00) is smaller than the second using essential oil extracts in the range (7.08 to 13.80). The repression against s*treptococcus mutans* was highest in formula two (13.80) containing extract of cinnamon. In the three methods containing betel leaf extract (7.08), all have inhibitory capacity lower than compared to another dosage formula.

According to Akiyama and Iwatsuki (2001), polyphenols have hydrophobic properties, so it possibly will damage the cell membrane of s*treptococcus mutans* bacteria. Polyphenols contained in the betel leaf oil and cinnamon oil are compounds that tend to be polar. The polarity of these compounds results in synthesis that may more easily penetrate the bacterial cell wall. Polyphenols appear to function as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes (Cowan, 1999).

Conclusion

Conclusions from this research is as follows: organoleptic mouthwash dosage makes all formulas acrid and slightly cloudy, the viscosity of the entire method ranges from 0.9 to 1.0, the density of the whole formula range is 1.02 to 1.04, the average pH of the preparation weakly acidic (3-4) and mouthwash formula preparations using good essential oil of betel leaf and cinnamon have a direct inhibitory effect on the growth of s*treptococcus mutans*. In subsequent studies it will be necessary to add a pH buffer to the stocks of mouthwash preparations; proper development and research must be done using the form of extracts for the addition of essential oils in mouthwash preparations to obtain a more precise result.

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