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RESEARCH **Open Access**

Antibacterial Mouthwash of Kesum Leaf (*Polygonum Minus Huds*) Essential Oil on *Streptococcus Mutans* and Sanguinis

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Abstract
Polygonum Minus Huds methanol extract from several previous studies has antibacterial properties in bacteria in the mouth such as *Streptococcus Mutans*, Staphylococcus Aureus, Salmonella Typhi, Escherichia Coli, etc. The cause of gingivitis is *Streptococcus Sanguinis* and anaerobic bacteria in the subgingival, which is porphyromonas gingivalis. *Polygonum Minus Huds* has the potential to be an antibacterial mouthwash for bacteria that causes caries and gingivitis. This research aims to make a mouthwash based on the essential oil of *Polygonum Minus Huds* and determine its antibacterial properties in mouthwashes of 0.025% and 0.05% concentrations to find antibacterial activity on *Streptococcus Mutans* and Sanguinis. The research was conducted in June 2019 at the Chemistry Laboratory of Faculty of Mathematics and Natural Sciences, the University of Tanjungpura in Pontianak Lab and Health Polytechnique Pontianak Integrated Lab. Design Quasi-experimental research used post-test only control group design, mouthwash formulation with essential oil concentrations of 0.025% and 0.05%, positive control using non-alcoholic mouthwash, and negative control (Aquades). Statistical analysis was performed univariately. The results showed that the best mouthwash was 2.5 ml of Tween 80 and 2.5 ml of glycerin. The results showed the essential oil gargle of Kesum leaves (*Polygonum Minus Huds*) at concentrations of 0.05%, and 0.02%, which is the bacteria of *Streptococcus Mutans* and *Streptococcus Sanguinis* were not able to inhibit the growth of the two bacteria. Increasing the amount of concentration or making mouthwash in other forms is expected to increase its antibacterial effect.

Keywords: Polygonum Minus Huds, Mouthwash, Streptococcus Mutans, Streptococcus Sanguinis

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1. INTRODUCTION

The results of Basic Health Research 2018 stated that Indonesians brush their teeth every day (94.7%) but still suffer from dental disease 57.6%, and only 10.2% receive health services from dental, medical assistance. It is because only 2.8% brush their teeth properly (Kemenkes, R.I., 2018). If you do not brush your teeth properly, the diseases often occur caries and periodontal tissue disease. The source of the disease that supports tooth tissue and dental caries is a neglect of dental and oral hygiene, resulting in plaque accumulation (Juwita, L., 2013).

Periodontal disease attacks the gingiva and tooth-supporting tissue through an inflammatory process, which can result in the detachment of the tooth from the tissue (Newman, et al., 2012). Periodontal disease affects the supporting tissues of the teeth. Gingivitis, the mildest form of periodontal disease, is generally caused by inadequate oral hygiene (Aspalli, et al., 2014).

Plaque, apart from causing caries, also causes gingivitis. *Streptococcus Mutans* bacteria are the main causes of caries. Betel fruit mouthwash with a level of 5% has an antibacterial effect against *Streptococcus Mutans* (Ririn, et al., 2013). *Streptococcus Sanguinis*, *S. Milleri*, *Fusobacterium Nucleatum*, *Actinomyces Israeli*, and *Bacteroides Intermedius* are the most bacteria that cause chronic gingivitis. To maintain trouble-free oral health, plaque control is a mainstay in preventing oral disease. Various plaque control methods include mechanical methods (toothbrush, floss) and chemical/therapeutic methods (Bagchi, et al., 2015).

Polygonum Minus Huds plants are found in many West Kalimantan areas, especially areas with peatlands, which in the local language is called Kesum. This plant is used to cure stomachaches and is often made into boiled drinks for herbal medicine after childbirth (Lumbantoruan, et al., 2013). This plant's leaves are used as a seasoning for various types of special dishes because they provide a delicious aroma and delicious taste to food (Syaiful, et al., 2015). Research (Wibowo, et al., 2009) regarding *Polygonum Minus Huds* leaves' chemical content shows that there are phenolic compounds, flavonoids, alkaloids, steroids, and terpenoids, tannins, saponins, and essential oils. *Polygonum Minus Huds* has antimicrobial activity against Escherichia Coli and Bacillus Subtilis and is bacteriostatic (Purwaningsih, et al., 2018). The content of phenolic and flavonoid leaves of kesum was higher in ethanol extract than water extract (Othman, et al., 2014). Previous research also said that *Polygonum Minus Huds*simplicia contains alkaloids, polyphenols, tannins, flavonoids, steroids-triterpenoids, and saponins (Tommy, 2013). Diethyl ether and leaf methanol fraction, petroleum ether extract, methanol, and chloroform of *Polygonum Minus Huds* leaves showed an inhibition zone against the growth of Helicobacter Pylori (Qader, et al., 2011).

One way to inhibit plaque formation is done chemically. Chemical inhibitors such as antiseptics play an essential role in plaque control. Products on the market to inhibit plaque are toothpaste, mouthwash, sprays, irrigation, gum, and varnish. Fluid/mouthwash is a simple and widely accepted method as an antimicrobial agent (after toothpaste), which patients can use as an oral hygiene aid (Parwani, et al., 2013).

Mouthwash or mouthwash containing oil in water media (emulsion form) requires an emulator and stabilizer. This addition is to improve the physical properties of the mouthwash so that the mouthwash formula becomes stable. The mouth rinse needs to be tested for its antibacterial properties to know its potential (Yosephine, et al., 2013).

A part from the active ingredients, another ingredient that is no less important in the composition of mouthwash is humectants. Humectants keep the active substances in

the mouthwash formula from evaporating, thereby prolonging the contact with the active substances on the teeth and improving the stability of an ingredient in the long term. Furthermore, humectants also keep mouthwash ingredients from evaporating into the air. Humectants often used are glycerin, which can also act as a solvent and viscosity regulator.

This follow-up study was to test the effectiveness of *Polygonum Minus Huds* essential oil on *Streptococcus Mutans* (the cause of caries) and *Streptococcus Sanguinis* (the cause of gingivitis) when using variations in glycerin levels and tween 80 as a humectant in mouthwash formulas.

2. RESEARCH METHOD

This study used a Quasi-laboratory experimental research design with posttest only control group design. This research was conducted in two different places, the manufacture of essential oils and tests of viscosity, density, and physical properties were performed at the Chemistry Laboratory of Faculty of Mathematics and Natural Sciences, the University of Tanjungpura in Pontianak. Antibacterial activity test was administered at the Microbiology Laboratory of Health Polytechnique Pontianak. This study's sample population was the leaves of *Polygonum Minus Huds*, which were obtained in Sambas Regency, West Kalimantan Province. The sample selection used purposive sampling. This study's inclusion criteria were fresh *Polygonum Minus Huds* leaves, no defects, and dark green color.

The leaves of *Polygonum Minus Huds* were distilled in water vapor for 8 hours. The oil at the top was then taken and then added with an amount of anhydrous sodium sulfate (Na2SO4) to remove the remaining water contained in the oil during the extraction process. The results obtained were 0.258 g of *Polygonum Minus Huds* leaf essential oil.

Figure 1. *Polygonum Minus Huds* leaf essential oil

Preparation of mouth rinses using additives tween 80 and glycerin to achieve the emulsion. Peppermint oil, sodium benzoate, sodium saccharin for taste and aroma, and distilled water. The cultures of *Streptococcus Mutans* and *Streptococcus Sanguinis* were obtained from the Faculty of Mathematics and Natural Sciences, University of Tanjungpura.

Table 1. *Polygonum Minus Huds* leaf mouthwash formula

Materials	Formula 1	Formula 2	Formula 3
Essential oil	0.5	0.5	0.5
*Tween 80 (*mL*)*	3.75	2.5	1.25
*Gliserin (*mL*)*	1.25	2.5	3.75
*Peppermint oil (*mL*)*	0.5	0.5	0.5
*Na-benzoat (*mL*)*	0.2	0.2	0.2
*Na-sakarin (*mL*)*	3	3	3
Green dye	q.s	q.s	q.s
Aquades ad (mL)	50	50	50

Polygonum Minus Huds essential oil was made a mouthwash formulation into a concentration of 0.05% and 0.025%. Each concentration was made in 3 mouthwash formulas to see the viscosity and physical properties of the liquid. The first group was mouthwash with 0.05% content of *Polygonum Minus Huds* leaf essential oil, and the second group with 0.025% content of *Polygonum Minus Huds* leaf essential oil.

Furthermore, the six mouth rinses were tested physically which included: organoleptic test (taste, smell, and color). The viscosity was tested using the Ostwald viscosimeter. The density used a pycnometer. The test results are to see the physical properties that may change, such as creaming, appearance, smell, and color. Then, look at the antibacterial activity. The way to determine the bacterial inhibition by diffusion method is through preparing a sterile microplate. A total of 50 µl of media was added with 50 µl of the sample at a certain concentration in the microplate holes. It was also made a positive control (media + bacteria) and negative control (media). In each hole, 5 µl of bacterial suspension was dropped and incubated for 24 hours at 37˚C. This research has received approval from the health research commission of the Health Polytechnic of Pontianak Health Ministry with No.007/KEPK-PK.PKP/V/2019.

3. RESULTS AND DISCUSSION

The essential oil of kesum leaves taken from the Kesum plant in Sambas Regency, West Kalimantan with water vapor distillation was dominated by dodecanal (34.11%), decanal (10.32%), caryophyllene (9.13%), eugenol (7.71%) and precocene 1 (6.37%). Caryophyllene is an anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, and local anesthetic. It also has antimicrobial properties against E. coli, S. aureus, K. pneumonia, P. aeruginosa and C. Albicans. The phenolic compounds identified in the essential oil of kesum leaves are eugenol compounds with a content of 7.71%, which are antioxidants and antimicrobials and anti-inflammatory and chemoprevention (Rusiardy, et. al., 2014). Phenolic compounds from flavonoids work by denaturing protein and inhibiting bacterial cell wall synthesis and resulting in cell death.

Table 2. Physical Properties of Mouthwash

Mouthwash Samples	Clarity	Stable	Smell	Taste	Viscositys (µs/ml)	Density (ρ)
0.05% formula 1	✓	✓	Mint smell	Sweet Fresh	1.6097 X 10^{-3}	1.0121
0.05% formula 2	✓	✓	Mint smell	Sweet Fresh	1.5425 X 10^{-3}	1.0115
0.05% formula 3	Cloudy	✓	Mint smell	Sweet Fresh	1.4251 X 10^{-3}	1.014

0.025% formula 1	✓	✓	Mint smell	Sweet Fresh	1.5042 X 10^{-3}	1.002
0,025% formula 2	✓	✓	Mint smell	Sweet Fresh	1.4390 X 10^{-3}	1.008
0.025% formula 3	Cloudy	The smell of mint on the 5th day became clear	Mint smell	Sweet Fresh	1.3434 X 10^{-3}	1.0102

The higher the concentration of tween 80, the higher the viscosity value. Compared with water's absolute viscosity, it is 1 X 10-3, and the resulting mouth rinse is thicker than water. The lowest density and the lower the viscosity value will be selected as a mouthwash, which will be tested for antibacterial, which is the second formula with 2.5 ml of Tween 80 and 2.5 ml of glycerin. The mouthwash's physical properties are transparent in color, stable in nature, sweet, smelly, and fresh due to a mixture of glycerin and mint.

The smell of *Polygonum Minus Huds* leaf essential oil is like the original smell of the leaves. The resulting mouth rinse smells minty due to the addition of mint leaf essential oil. The volatile components provide aroma sensation, provide top notes, and evaporate quickly. The non-volatile components give the sensation of taste, namely sweet, bitter, sour, and salty, do not offer aroma sensation but become a medium for volatile components, and help resist volatile components' evaporation. (Rumayati, et. al., 2014).

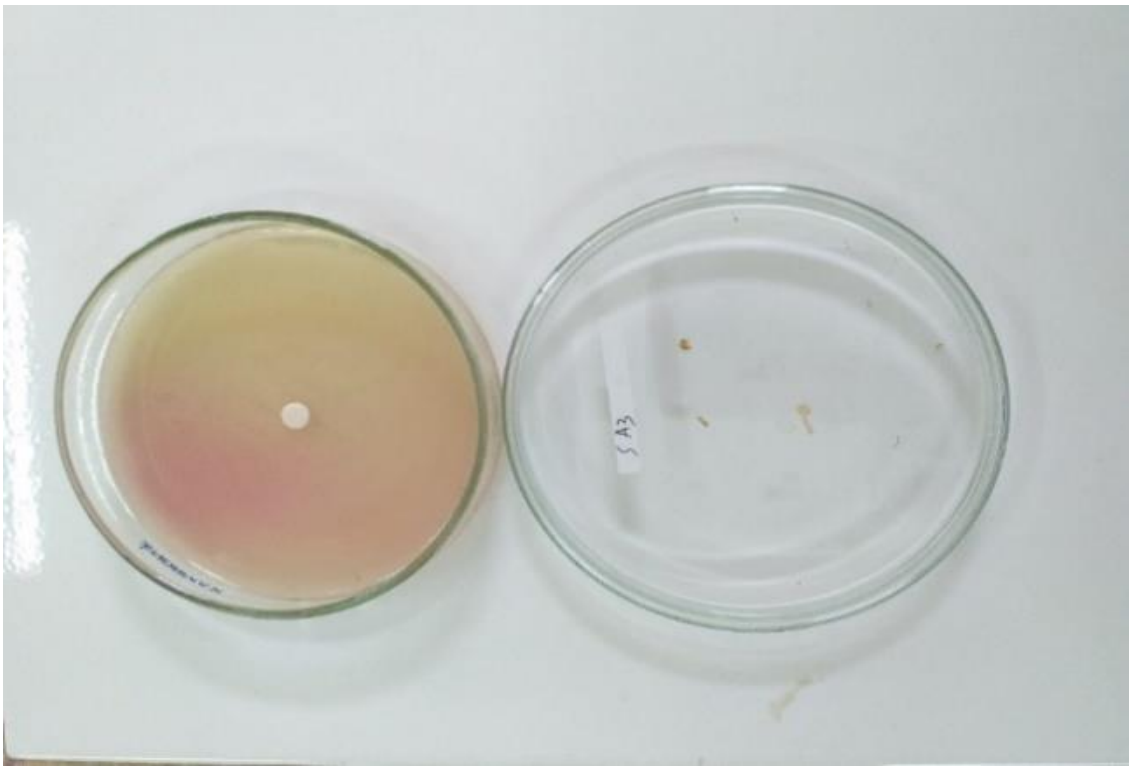

Figure 2. Inhibition of Sanguinis Bacteria in Mouthwash

On day 2, there was no clear or germ-free zone outside the diameter of the paper disc that was dyed with active or controlled substances, so that multivariate statistical analysis could not be performed, and only univariate or descriptive analysis was performed.

Table 3. Antibacterial Inhibition Properties of *Streptococcus Sanguinis*

	Average of Replication 1	Average of Replication 2	Average of Replication 3	Average
Concentration 5 %	0	0	0	0
Concentration 2.5%	0	0	0	0
Control (-)	0	0	0	0
Control (+)	0	0	0	0

The study (Figures 1, 2, and table 3) shows no antibacterial properties against *Streptococcus Sanguinis* in the essential oil gargle of *Polygonum Minus Huds* leaves and in control, which is a mouth rinse that is sold in the market.

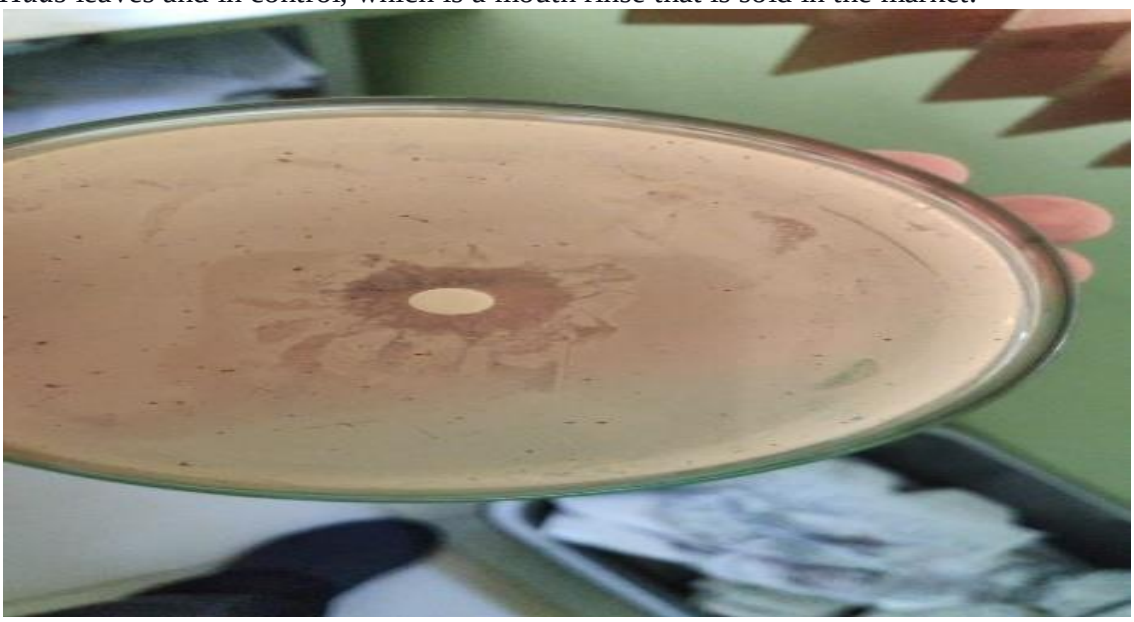

Figure 3. Antibacterial Inhibition of *Streptococcus Mutans* Bacteria in Positive Control

The results showed no clear germ-free zone outside the paper discs in the mouthwash containing *Polygonum Minus Huds* essential oil, which means that there was no inhibition of 0.025 and 0.05% mouthwash against *Streptococcus Mutans* bacteria. In the positive control, which is a non-alcoholic mouth rinse which is sold freely in the market. There is a 6 mm inhibition zone from the edge of the paper disc soaked with the mouthwash.

Table 4. Inhibitory Properties of *Streptococcus Mutans* Antibacterial

	Average of Replication 1	Average of Replication 2	Average of Replication 3	Average
Concentration 5 %	0	0	0	0
Concentration 2.5%	0	0	0	0
Control (-)	0	0	0	0
Control (+)	6	6	6	6

The research results showed that there was no inhibition of *Streptococcus Mutans* bacteria in the essential oil gargle of *Polygonum Minus Huds* leaves, and there was little resistance to *Streptococcus Mutans* bacteria in positive controls (mouthwash on the market).

The results showed no antibacterial activity in the mouthwash of *Polygonum Minus Huds* leaves at a concentration of 5% and 2%, on both *Streptococcus Mutans* and *Streptococcus Sanguinis* bacteria. If the concentration is increased, the

viscosity tends to increase, which means that the viscosity will increase. A good mouthwash should have the same viscosity as plain water so that it is easy to flow over the entire tooth surface and through the narrow gaps of the teeth. It can be concluded that the essential oil of *Polygonum Minus Huds* leaves has no potential to be used as an ingredient in mouthwash as an antibacterial agent for *Streptococcus Mutans* and *Streptococcus Sanguinis*. *Streptococcus Mutans* and *Streptococcus Sanguinis*are common bacteria present in many oral cavities, Aeron, and facultative anaerobes. Sanguinis Streptococcus is a bacterium that has a characteristic chain shape. Based on the structure of the cell wall, these bacteria are classified as Gram-positive bacteria. Based on the need for oxygen, *Streptococcus Sanguinis*is ranked as a facultative anaerobic bacterium because it can utilize oxygen to produce energy by respiration. However, when oxygen is not sufficient, bacteria can perform fermentation for ATP synthesis (Xu, et. al., 2007).

In the positive control, which is the mouth rinses on the market, there were antibacterial properties in *Streptococcus Mutans* but not in Sanguinis. Mouthwash, which has been shown to have antibacterial properties against *Streptococcus Mutans*, contains triclosan and sodium fluoride. Triclosan works by inhibiting the activity of the enzyme protein enoyl-acyl reductase (ENR), which is an essential enzyme in the formation of fatty acids in bacteria so that it can inhibit bacterial growth. Sodium fluoride effectively reduces caries by inhibiting the use of carbohydrates with oral microorganisms by inhibiting enzymes in the glycolytic pathway. Triclosan is a chemical with antibacterial, antifungal, and antiviral properties (Febriany, 2013).

Based on the results of GC-MS analysis, the *Polygonum Minus Huds* extract contained aldehyde and aliphatic alcohol compounds. Aldehyde affects the distinctive aroma of *Polygonum Minus Huds*, and aliphatic alcohol affects the taste. Another component in *Polygonum Minus Huds* leaf essential oil is caryophyllene, known for its anti-inflammatory, local anesthetic, and antifungal properties (Rumayati, et. al., 2014). The essential oil of *Polygonum Minus Huds* leaves in small concentrations (0.05% and 0.025%) is not able to inhibit the growth of *Streptococcus Mutans* and *Streptococcus Sanguinis*, while high concentrations are not economical because Kesum leaves or *Polygonum Minus Huds* do not contain much oil, so no potential used as an essential oil, and more likely to be made in the form of an infusion. *Polygonum Minus, Huds* in ethanol and liquid (infusion), has high antioxidant and phenolic properties compared to *Andrographis paniculata, Curcuma xanthorrhiza, Momordica charantia,* and *Strobilanthes Crispus* (Qader, 2011). For further research, more research is suggested in the form of infusion and the anti-inflammatory and antifungal properties.

4. CONCLUSION

Mouthwash with a level of Tween 80 as much as 2.5 ml and 2.5 ml of glycerin content is the mouthwash choice. The results showed no antibacterial activity in the essential oil gargle of *Polygonum Minus Huds* leaves at concentrations of 0.05% and 0.025%, both for *Streptococcus Mutans* and *Streptococcus Sanguinis* bacteria. There was no difference between the antibacterial activity of the essential oil rinse of *Polygonum Minus Huds* leaves at a concentration of 5% and 2% for *Streptococcus Sanguinis* the study samples with positive and negative controls.

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