

Formulation of Mouthwash for Gingivitis from Combination Infusion of Salam leaves (*Eugenia Polyantha Wight*) and Betel leaf (*Piper betle. L*)

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ABSTRACT

Introduction: Gingivitis is inflammation of the gingiva associated with bacterial activity in dental plaque. The combination of bay leaves and betel leaves infusion has been used as a toothbrush disinfectant. The purpose of this study was to obtain a stable and effective mouthwash formulation from the combination of both infusions to prevent and relieve gingivitis. **Methods:** Five mouthwash formulas were made by mixing the active ingredients which is a combination of bay leaves and betel leaves infusion, with additives. The combination namely F-I (100% : 0%); F-II (75% : 25%); F-III (50% : 50%); F-IV (25% : 75%) and F-V (0% : 100%). Antibacterial activity tests against *Streptococcus mutans*, *Streptococcus sanguis*, and *Porphyromonas gingivalis* and physical stability tests were carried out. Stability test was carried out by storing the formula at room temperature (28°C), hot temperature (40°C) and cold temperature (4°C) for 3 storage cycles (6 weeks) and observing changes in the physical indicators of the solution, namely pH, specific gravity, viscosity and organoleptic conditions, namely homogeneity, clarity, color, aroma and taste at the end of every 2nd week. **Results:** F-I to F-V had no inhibitory activity against *Streptococcus mutans* and *Streptococcus sanguis*, but had inhibitory activity against *Porphyromonas gingivalis* in the range (7.19±0.48) to (8.29 ±0.82) mm (ANOVA with a significance value of 0.237 > 0.05). The 5 formulas were more stable at cold storage, with organoleptic observations at the end of the 3rd cycle showing a slight precipitate (+), clear solution, light brown color, mint aroma and sweet taste. F-II formulas showed better physical indicators values because they were closer to the standard values. **Conclusion:** The F-II formula is recommended as a mouthwash for gingivitis because significant for antibacterial effect and showed better physical indicators values as the standard values for herbal medicines.

Keywords: antibacterial activity; bay leaves - betel leaves infusion; gingivitis; mouthwash formulation.

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INTRODUCTION

Gingivitis, an inflammatory condition of the gingiva due to plaque, is indicated by clinical

symptoms in the form of swelling at the gingival margin, the redder color of the gingiva, excess gingival fluid from the gum pocket, and bleeding when brushing teeth. This inflammation of the

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gingiva is associated with bacterial activity in plaque in conditions where the composition of dental plaque which was previously dominated by Gram-positive bacteria turns into dental plaque which is starting to be dominated by anaerobic Gram-negative bacteria.¹ Mouthwashes from herbal plants can help prevent or relieve gingivitis because of their metabolite compounds that are antibacterial, anti-inflammatory and palliative, or pain relievers.² Bay leaf (*Eugenia polyantha* Wight) as an active herbal ingredients for mouthwash has chemical contents, namely tannins, flavonoids, and 0.05% essential oil consisting of citral and eugenol function as anesthetics and antiseptics. Tannins cause protein denaturation by forming protein complexes.

The formation of protein complexes through nonspecific forces such as hydrogen bonding and hydrophobic effects as well as the formation of covalent bonds, inactivates bacterial adhesion (molecules to adhere to host cells) and stimulates phagocytic cells that play a role in cellular immune responses.² Flavonoids denature protein and nucleic acid molecules causing protein coagulation which will eventually occur metabolism and physiological function of bacteria.^{2,3,4,25}

Mechanism of phenol toxicity in essential oils causes denaturation of proteins on the germ cell wall by forming a tertiary protein structure with nonspecific bonds or disulfide bonds.² A combination of the active compounds will accelerate the death of bacteria. The betel plant (*P. betle* L) comes from the *Piperaceae* family. *Piperaceae* family has 13 genus that are commonly known to the primary as medicinal plants.⁵ In dental health, betel leaf extract has long been known as a mouthwash to remove dental plaque.

There are four main chemical compounds contained in essential oils and plant extracts/betel leaf, namely phenolic compounds, terpenoids, flavonoids, and alkaloids, and of the four main compounds, 56 active compounds have been found. With a strong antiseptic power from its essential oil content, for example, the presence of catechins, which are one of the phenolic contents of betel leaf, the betel leaf extract is bactericidal, antiprotozoal and antifungal. Mouth rinsing with betel leaf extract can relieve aphthous stomatitis and other oral lesions caused by fungi, viruses, and bacteria.^{6,7,8,9} Research conducted by Nurjanah et

al¹⁰, regarding the use of a combination of bay leaf-betel leaf infusion as a toothbrush disinfectant concluded that the combined infusion of bay leaf (65%) and betel leaf (35%) has bactericidal properties against toothbrush contaminants, which are mixed bacteria from the oral cavity originating from plaque, saliva, and water carried by toothbrushes after use.

This infusion combination is better than single-use. Meanwhile, this combination has never been used in mouthwash formula. Utilization of bay leaf combined with betel leaf expects the synergism of the antibacterial power of the active compounds of the two ingredients so that the antibacterial effect of the combination of bay leaf and betel leaf decoction is greater than the single effect. In addition, the purpose of mixing several herbs is to make mouthwash more effective to prevent microorganisms resistance.¹¹

Several studies have shown that natural extracts mixed in proper proportion affect pathogenic oral bacteria thus not have impact on nonpathogenic oral bacteria. It is assumed that naturally derived extracts that have been properly formulated can preserve a healthy oral environment and even replace commercial mouthwash made of chemical compound mixtures.^{22,23,24} In this study, we will develop a mouthwash based on a combination of bay leaf and betel leaf infusion by proposing 5 combination formulas, and further testing the antibacterial activity against 3 test bacteria that cause gingivitis, namely *Streptococcus mutans*, *Streptococcus sanguis*, and *Porphyromonas gingivalis*, followed by stability physical testing of the five mouthwash formulas. The purpose of this study was to obtain a stable and effective mouthwash formulation from a combination of bay leaves and betel leaves infusion to prevent and relieve gingivitis.

METHODS

The method used in this study started with making a mouthwash formulation with an active combination of bay leaf and betel leaf infusion, followed by carrying out anti-bacterial activity tests on *Streptococcus mutans*, *Streptococcus sanguis*, and *Porphyromonas gingivalis*. After that, the physical stability test was carried out. The design of this study was an experimental study

with a laboratory scale. Variations of mouthwash formulas based on active substances from an

infusion of bay leaves and betel leaf are proposed as shown in table 1 below:

Table 1. Variations of mouthwash formulas based on active substances from infusion of bay leaves and betel leaf

Ingredients	Formula				
	I	II	III	IV	V
Bay leaves (%)	100	75	50	25	0
Betel leaf (%)	0	25	50	75	100
Tween 80 (%)	10	10	10	10	10
Peppermint (%)	1	1	1	1	1
Na-benzoate (%)	0.4	0.4	0.4	0.4	0.4
Na-saccharin (%)	6	6	6	6	6
Dyes (%)	0.2	0.2	0.2	0.2	0.2
Aquadest add up to 100 mL	100	100	100	100	100

The five formulas of the mouthwash formula were then tested for their antibacterial activity against three bacteria that cause gingivitis, namely *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguis* (ATCC 10556), and *Porphyromonas gingivalis* (ATCC BAA-308). *S. mutans* and *S. sanguis* are Gram-positive bacteria, while *P. gingivalis* are Gram-negative anaerobic bacteria.

Bacterial Culture Method and Antibacterial Activity of Mouthwash Preparations Test

The bacteria test *S. mutans* and *S. sanguis* were cultured by taking one oase of each bacterium from stock culture, inoculated on blood agar media, and incubated for 24-48 hours at 37°C under aerobe conditions. The bacteria test *S. mutans* and *P. gingivalis* were cultured by suctioning 0.5-1.0 ml from the test tube (5-6 ml) with a 1.0-mL Pasteur pipette, then moistening the cotton pellet. Aseptically, transfer this bacterial liquid to a tube containing Brain Heart Infusion Broth (BHIB) medium supplemented with 2% glucose for testing *S. mutans* and *S. sanguis*. Meanwhile, for *P. gingivalis*, BHIB supplemented with vitamin K and hemin. Additional broth tubes can be inoculated with 0.5 ml each suspension. Furthermore, from the broth medium that has been inoculated with bacteria, 0.2 mL was taken and inoculated on slanted. Then instill in several bred blood to purify the bred and observe colony morphology. Furthermore, the tubes and culture plates were incubated anaerobe, at 37°C for 48 hours. The broth culture will look cloudy after incubation in the incubator for 48 hours. In order

to recommended for culture, *P. gingivalis* is the PRAS Brucella Blood Agar Plate from the anaerobic system (AS-111)^{12,14,15} To test the antibacterial activity of mouthwash preparations, there are 9 paper disks were prepared, each 3-paper disk was dipped in a) each mouthwash formula, b) standard solution infusion to the combination of betel leaf, bay leaf, and c) negative control (that was additives without the active of infusion were bay leaf and betel leaf) d) positive control (chlorhexidine). Solutions a), b), c) and d) were provided as much as 20 mL each. All paper disks were dyed for 1 hour, and then each paper disk was implanted in a petri dish containing blood agar medium, and the bacterial culture of *P. gingivalis* was incubated for 1 x 24 hours at 37°C, then the inhibition zone of bacterial growth was measured.

Stability test of mouthwash preparations

This test was intended to assess the stability of mouthwash by measuring several parameters before and after storage. The mouthwash formulas were stored at 4°C, room temperature, and 40°C for 3 cycles each, the range between cycles was 2 weeks. The parameters measured were organoleptic observations, which were assessed through physical observation by expert panelists. The second parameter is viscosity measurements (cSt). This parameter is measured with an Ostwald viscometer at Chemistry Lab. Third parameter was pH measurements. This parameter is measured with a pH meter at Chemistry Lab. The last parameter is specific gravity measurements (g/cm³), this parameter is measured with a pycnometer at the Chemistry lab.

RESULTS

The antibacterial activity results

The antibacterial tests were carried out for the 5 combination solutions (marked by L-I - L-V) of bay leaf and betel leaf infusion (without additives) and for the 5 mouthwash formulas (marked by F-I - F-V). The results of the antibacterial activity test are shown in the table 2. Table 2 showed that the L-I and L-II solutions have antibacterial activity against *Streptococcus mutans*, but in combination with the same composition (L-III) or composition

containing more infusion of betel leaf (L-IV) and (L-V), the formula could not inhibit the growth of *S. mutans*. The results of the antibacterial activity test of the combination solution of bay leaf and betel leaf infusion against *Streptococcus sanguis* showed slightly different results, namely the compositions L-I, L-II, L-IV, and L-V had inhibitory power against *S. sanguis* with an average inhibition zone varying between 6.95 - 11.72 mm, and the composition with the largest inhibition zone

Table 2. The average diameter of zone of inhibition of the mixture of bay leaf and betel leaf infusion without additives*

Sample	Diameter of zone inhibition the mixture of bay leaf and betel leaf infusion towards		
	<i>S. mutans</i> Mean \pm SD (cm)	<i>S. sanguinis</i> Mean \pm SD (cm)	<i>P. gingivalis</i> Mean \pm SD (cm)
L-I	7.55 \pm 0.16	8.07 \pm 0.13	NA
L-II	6.96 \pm 0.35	6.95 \pm 0.11	7.96 \pm 0.77
L-III	NG	NG	8.25 \pm 0.45
L-IV	NG	8.44 \pm 1.16	8.35 \pm 0.66
L-V	NG	11.72 \pm 0.49	11.92 \pm 0.86
Control (+) Chlorhexidine	18.82 \pm 0.27	18.51 \pm 0.72	24.14

*NG = no bacterial growth

Additives* are ingredients that are added so that the mouthwash formula is stable, the active substance is well dissolved so that it is not easy to precipitate (Tween-80), has a good taste, aroma and color (Na-Saccharin, Peppermint and dyes) and the formula can inhibit the growth of bacteria and fungi (Na-Benzate).

was the L-V formula (0% bay leaf infusion: 100% betel leaf infusion). *P. gingivalis* is sensitive to the combination formula, the sensitivity increases with the increase in the concentration of betel leaf infusion, but in this study, betel leaf infusion alone did not have antibacterial activity against these bacteria.

The results of the antibacterial test of the 5 mouthwash formulas, namely the combination of betel leaf bay leaf infusion with the addition of additives can be seen in Table 3

Table 3 shows that the additive (negative Control) did not have antibacterial activity against the three bacteria test. The formula from F-I - F-V did not have antibacterial activity against *Streptococcus mutans* and *Streptococcus sanguis* (NA) but had antibacterial activity against *Porphyromonas gingivalis*.

The results of the homogeneity of variance test followed by the ANOVA Test on Inhibitory Zones between groups and within groups are written in table 4 and 5.

Table 3. The average diameter of zone of inhibition of bay leaf infusion-based mouthwash and betel leaf

Samples	<i>S. mutans</i> (cm)	<i>S. sanguinis</i> (cm)	<i>P. gingivalis</i> (cm)
F-I	NG	NG	7.19 \pm 0.48
F-II	NG	NG	7.25 \pm 0.22
F-III	NG	NG	8.06 \pm 0.84
F-IV	NG	NG	8.09 \pm 0.81
F-V	NG	NG	8.29 \pm 0.82
Control (+): Chlorhexidine			23.55
Control (-): (additive without infusion)	NG	NG	NG

*NG = no bacterial growth

Table 4 regarding the *Test of Homogeneity of Variances* from the *Based on Mean* results obtained a significant result of $0.189 > 0.05$ which can be concluded that Inhibition zone data variance in samples 1-5 is homogeneous. Table 5, ANOVA with a significance value of 0.237

> 0.05 show no significant variation in the zone of inhibition based on the five samples. In the sense that each mouthwash formula has the same opportunity to be determined as the selected formula, for the implementation of further tests in the development of mouthwash manufacture.

Table 4. The variance homogeneity test for Mouthwash Formula (F)

		Levene Statistics	dFI	dFII	Sig.
Inhibition zone	Based on Mean	1.887	4	10	.189
	Based on Median	.330	4	10	.852
	Based on Median and with adjusted df	.330	4	7.191.850	.
	Based on trimmed mean	1.674	4	10	.232

Source: SPSS Output

Table 5. ANOVA test for Mouthwash Formula (F)

Inhibition zone					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.205	4	.801	1.652	.237
Within Groups	4.850	10	.485		
Total	8.056	14			

Source: SPSS Output

Determination of the Formula's Physical Properties

The mouthwash's physical properties such as pH, specific gravity, and viscosity were determined because these parameters could affect the mouthwash's quality. The results of measuring the physical properties of the mouthwash formula are listed in Figure 1. The pH of Formula solution I-V ranged from 4.47-4.59 as shown in figure 1,

while the infusion of bay leaf and betel leaf was 4.48 and 4.33, respectively. The graph of specific gravity of the mouthwash formula (FI-FV) ranged from 1.0126-1.0155 g/cm³, while the infusion of the bay leaf and betel leaf were 1.0085 and 1.0103 g/cm³. The viscosity values for the infusion of bay leaf and betel leaf are 1.0988 and 1.0155 cSt, respectively. This value is close to the value of water viscosity, which is 1 cSt. However, when

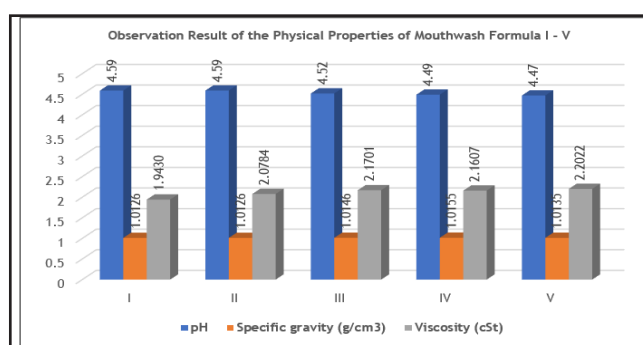


Figure 1. Results of Observation of Physical Properties of Mouthwash Formula I - V

additives are added, the value of each viscosity increases. The viscosity value of bay leaf from 1.0988 cSt increased to 1.9430 cSt (F-I), while that of betel leaf from 1.0155 cSt increased to 2.2022 cSt (FV), this indicates each infusion's viscosity was increased by the addition of additives. The increase in viscosity was further increased when the two infusions were mixed

Results of Stability Testing of Mouthwash Preparations Based on Organoleptic Observations

Organoleptic tests included pH, homogeneity, clarity, color, aroma, and taste of the mouthwash formula before the storage cycle and after storage cycles 1, 2, and 3 in a storage room at room temperature (28°C), hot (40°C) and cold

Table 6. The results of the stability test for the combination mouthwash formula infusion of Salam and Betel leaf for Formula II

Stability at room temperature						
Period of time	pH	Homogeneity	Clarity	Color	Aroma	Taste
Before	4.52	V	V	Light Brown	Mint	Sweet
	4.57	V	V	Light Brown	Mint	Sweet
	4.59	V	V	Light Brown	Mint	Sweet
Average	4.56±0.03					
Cycle 1	4.52	Precipitation +	V	Light Brown	Mint	Sweet
	4.50	Precipitation +	V	Light Brown	Mint	Sweet
	4.49	Precipitation +	V	Light Brown	Mint	Sweet
Average						
Cycle 2	3.88	Precipitation ++	V	Dark Brown	Mint	Sweet
	3.87	Precipitation ++	V	Dark Brown	Mint	Sweet
	3.86	Precipitation ++	V	Dark Brown	Mint	Sweet
Average						
Cycle 3	5.25	Precipitation ++	V	Dark Brown +	Mint	Sweet
	5.23	Precipitation ++	V	Dark Brown +	Mint	Sweet
	5.20	Precipitation ++	V	Dark Brown +	Mint	Sweet
Average	5.22±0.03					
Stability at 40°C						
Cycle 1	4.47	Precipitation +	V	Light Brown	Mint	Sweet
	4.46	Precipitation +	V	Light Brown	Mint	Sweet
	4.46	Precipitation +	V	Light Brown	Mint	Sweet
Average						
Cycle 2	3.77	Precipitation ++	V	Dark Brown	Mint	Sweet
	3.76	Precipitation ++	V	Dark Brown	Mint	Sweet
	3.78	Precipitation ++	V	Dark Brown	Mint	Sweet
Average						
Cycle 3	3.72	Precipitation ++	V	Dark Brown +	Mint	Sweet
	3.71	Precipitation ++	V	Dark Brown +	Mint	Sweet
	3.70	Precipitation ++	V	Dark Brown +	Mint	Sweet
Average	3.71 ±0.01					
Stability at 4°C						
Cycle 1	4.52	Precipitation +	V	Light Brown	Mint	Sweet
	4.52	Precipitation +	V	Light Brown	Mint	Sweet
	4.51	Precipitation +	V	Light Brown	Mint	Sweet
Average						
Cycle 2	3.94	Precipitation +	V	Light Brown	Mint	Sweet
	3.94	Precipitation +	V	Light Brown	Mint	Sweet
	3.92	Precipitation +	V	Light Brown	Mint	Sweet
Average						
Cycle 3	4.05	Precipitation +	V	Light Brown	Mint	Sweet
	4.08	Precipitation +	V	Light Brown	Mint	Sweet
	3.98	Precipitation +	V	Light Brown	Mint	Sweet
Average	4.03±0.03					

temperature (4°C). Duration per cycle was 2 weeks. Before entering the cycle, organoleptic test was carried out in the chemistry lab by expert panelists. The results of the organoleptic test of the mouthwash regarding homogeneity,

clarity, color, aroma, and taste, for all formulas and controls gave the same results, namely, the mouthwash solution was homogeneous, clear, and did not cause deposits or cloudiness. This light brown color came from bay leaf and betel leaf

infusion which is a dark brown color so being a light brown because of the addition of additive substance.¹³ The dominant aroma of mint comes from the mint flavor and sweet taste from the added saccharin.

Compared to the 5 proposed mouthwash formulas, we chose Formula II, which is a formula with a combination of 75% Salam leaf infusion and 25% Betel leaf infusion to be developed as a mouthwash. This is based on the consideration of a more favorable pH value when compared to other combinations. The results of the stability test for the combination mouthwash formula infusion of Salam and Betel leaf for Formula II can be seen in Table 6.

In the table, it can be seen that before stability testing the average pH of the Formula II mouthwash was 4.56, the solution was homogenous, clear, light brown in color, with a mint aroma, and sweet taste. At room temperature storage after completion of the third cycle, the clarity, aroma, and taste did not change, but the pH rose to 5.22 and was within the standard pH range, the solution became non homogenous, precipitated ++, and the color changed to dark brown +, and a precipitate increased with increasing storage time. This indicates that Formula II is unstable at room temperature storage.

Storage of Formula II at 40°C, after the third cycle ended the clarity, aroma, and taste did not change, but the pH dropped to 3.71, the solution became non homogenous, precipitated ++, and the color became dark brown+. In cold storage at 4°C, the clarity, color, aroma, and taste did not change but the pH increased to 4.03, and the solution became non homogeneous with a slight + precipitate. Storage conditions at cold temperatures were better than storage at room temperature and 40°C.

DISCUSSION

Antibacterial Test

Regarding the antibacterial test of the combination of bay leaf and betel leaf infusion without additives listed in table 2, it can be seen that the solution with a higher composition of bay infusion has antibacterial activity against *S. mutans* and *S. sanguis*. Aldhafer's research (2017) stated that the aqueous extract of bay

leaves had a good antibacterial effect against *S. mutans*, and the best inhibition was obtained at an infusion concentration of 60% (20.4 mm) even when compared to 0.2% Chlorhexidine gluconate as a mouthwash golden standard (16 mm)^{16,17}. However, if both infusions equally combined or betel leaf infusion proportion higher than bay leaf, the antibacterial effect is inactive.

The sensitivity of *S. sanguis* and *S. mutans* to the infusion of the bay leaves and betel leaves mixture can occur due to the content of tannins, flavonoids, and essential oils. These results are in line with research that concluded that gargling with bay leaf infusion (*Eugenia polyantha* Wight) with concentrations of 100%, 75%, and 50% can reduce the number of colonies *Streptococcus spp.*^{16,18} Infusion and bay leaf extract contains chemical compounds, namely tannins, flavonoids, and essential oils (0.05%) consisting of citric acid and eugenol. Tannins are glycoside solutions derived from polypeptides and ester polymers which can be hydrolyzed by bile (3, 4, 5 trihydroxide benzoic acids) and glucose. Flavonoid is a term for oxygen heterocyclic aromatic compounds derived from 2 phenyl benzopyran. Flavonoids are one of the naturally occurring phenolic compounds found in many plants.

Tannins, one of the compounds contained in bay leaves, are included in the phenol group, which can inhibit the growth of various bacteria through precipitation and denaturation of bacterial proteins. Flavonoids also have antibacterial properties because these compounds are able to interact directly with bacterial DNA. The structure of DNA plays an important role in the process of transcription and replication of bacteria. Flavonoids are considered capable of disrupting the stability of the DNA double helix structure, which results in disruption of the growth process and bacterial metabolism.

Flavonoid interaction with bacterial DNA Flavonoids can also produce energy transduction which also affects the cytoplasm of bacteria and weakens its movement. The hydroxyl ions resulting from the energy transduction can inhibit bacterial growth through the mechanism of precipitation and denaturation of bacterial proteins.^{17,21} Presumably, this mechanism also causes the combination to have antibacterial activity against *P. gingivalis*. Phenolic compounds,

terpenoids, flavonoids, and alkaloids are also found in betel leaf plants, so it is hoped that the combination of the two infusions can increase the antibacterial effect against the three bacteria tests. But the results of this study provide data that L-III (50% bay leaf and 50% betel leaf), L -IV (25% bay leaf and 75% betel leaf), and L-V (100% bay leaf and 0% betel leaf) have no antibacterial activity against *S. mutans*. The explanation for this is the possibility of an antagonistic effect between the active compounds present in both infusion solutions.^{22,24}

Table 3 shows the antibacterial power of 5 different mouthwash formula variants. The results showed that F-I to F-V did not have antibacterial activity against *S. mutans* and *S. sanguis*, but had antibacterial activity against *P. gingivalis*, although relatively weak compared to standard Chlorhexidine mouthwash. In comparison, *Streptococcus sp* is a Gram-positive bacterium with the characteristic of having a thick peptidoglycan layer on its cell wall, while *P. gingivalis* is a Gram-negative bacterium with a thin peptidoglycan.¹² Tannins and flavonoids are compounds contained in bay leaves and betel leaves. The mechanism of action of tannins as an antibacterial is to interfere with the synthesis of peptidoglycan so that the formation of cell walls becomes less than perfect. This situation will cause bacterial cells to become lysed due to osmotic and physical pressure so that bacterial cells die.

The mechanism action of flavonoids as an antibacterial is to form complex compounds with extracellular and soluble proteins which result in phospholipids not being able to maintain the shape of the bacterial cell membrane, as a result, the cell membrane will leak and the bacteria will be inhibited in growth and even death.^{20,21} It is possible that in this study, it is necessary to carry out phytochemical tests on tannins and flavonoids as the most active ingredients in the formula. The tannins and flavonoids contained in the infusion of deep leaves and betel leaf were affected by the heating process in the manufacture of the infusion, so that their antibacterial activity, especially against the test bacteria *S. mutans* and *S. sanguis* which were Gram positive, was reduced. However, *P. gingivalis*, which has a thinner peptidoglycan layer, is still sensitive to the tannins and flavonoids contained in the combined infusion.

Physical Properties of Mouthwash Formula

The physical properties of the mouthwash formula are determined by the pH value, specific gravity and viscosity of the solution. The pH of Formula solution I-V ranged from 4.47- 4.59 as shown in figure 1, while the infusion of bay leaf and betel leaf was 4.48 and 4.33, respectively. This pH value is still below the standard range of oral pH which is between 5.5-7.9¹⁹, while based on the Herbal Medicine Standard Quality, the pH of mouthwash is required to be between 5-6. For this matter, there needs to be an effort to increase the pH value, by adding an alkaline material, so that when the preparation is consumed it does not cause irritation to the oral mucosa. The pH of the formula close to the oral pH can also prevent the growth of bacteria and fungi. Bacteria will grow easily in an acidic solution, while fungi will grow easily in an alkaline solution.

Betel leaf infusion has a slightly lower pH value than bay leaf infusion. In the graph, it can be seen that as the ratio of betel leaf concentration increases in the formula, the pH of the formula decreases. Meanwhile, the addition of additives seems to raise the pH value. This is indicated by the previous pH value of the bay leaf infusion of 4.48, after being in the form of formula (F-I) pH value change to 4.59. In addition, it is shown by the changing of pH of the betel leaf infusion which was originally 4.33 becomes 4.47 when it is in the form of Formula (F-V).

The specific gravity values of all samples met the standard of 1 g/cm³, approaching the water density value of 1 g/cm³. The viscosity of liquid indicates the level of resistance to flow. The greater the viscosity, the slower the flow. The results in the diagram Figure 1 show that the addition of additives increases the viscosity of each infusion. The increase in viscosity was further increased when the two infusions were mixed. It becomes necessary to think about rearranging the formula's composition.

The specific gravity and viscosity of the formula with the composition of the higher bay leaf infusion content (F-I and F-II) were relatively more advantageous when compared to F-IV and F-V. Since the results of this study showed that there were no significant differences in antibacterial power, further research is needed to make more effective mouthwash formulations.

CONCLUSION

The F-II formula is recommended as a mouthwash for gingivitis because it has antibacterial effect and showed better pH, specific gravity, and viscosity values as the standard values for herbal medicines.

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REFERENCES

1. Sunarto, H. Plaque as the Main Cause of Periodontal Tissue Inflammation. Dept Periodonsia FKG UI: Jakarta. 2014. p.1-15
2. Safiaghdam H, Oveissi V, Bahramsoltani R, Farzaei MH, Rahimi R. Medicinal plants for gingivitis: a review of clinical trials. Iran J Basic Med Sci. 2018;21(10):978-91. DOI: [10.22038/IJBMS.2018.31997.7690](https://doi.org/10.22038/IJBMS.2018.31997.7690).
3. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020;25(22):5243. DOI: [10.3390/molecules25225243](https://doi.org/10.3390/molecules25225243).
4. Gundala SR, Aneja R. Piper betel leaf: a reservoir of potential xenohormetic nutraceuticals with cancer-fighting properties. Cancer Prev Res (Phila). 2014;7(5):477-86. DOI: [10.1158/1940-6207](https://doi.org/10.1158/1940-6207).
5. Shukla R, Sachan S, Mishra A, Kumar S. A Scientific Review on Common Chewing Plant of Asians: Piper Betle Linn, Jour. Harmo. Res. Pharm. 2015;4(1):01-10
6. Das S, Parida R, Sriram Sandeep I, Nayak S, Mohanty S. Biotechnological intervention in betelvine (Piper betle L.): A review on recent advances and future prospects. Asian Pac J Trop Med. 2016; 9(10): 938-46. DOI: [10.1016/j.apjtm.2016.07.029](https://doi.org/10.1016/j.apjtm.2016.07.029).
7. Purba RAP, Paengkoum P. Bioanalytical HPLC method of Piper betle L. for quantifying phenolic compound, water-soluble vitamin, and essential oil in five different solvent extracts. JAPS. 2019;9(5):33-39. DOI: [10.7324/JAPS.2019.90504](https://doi.org/10.7324/JAPS.2019.90504)
8. Syahidah A, Saad CR, Hassan MD, Rukayadi Y, Norazian MH, Kamarudin MS. Phytochemical analysis, identification and quantification of antibacterial active compounds in betel leaves, piper betle methanolic extract. PJBS, 2017;20(2):70-81. DOI: [10.3923/pjbs.2017.70.81](https://doi.org/10.3923/pjbs.2017.70.81)
9. Atiya A, Sinha BN, Lal UR. Bioactive phenylpropanoid analogues from Piper betle L. var. birkoli leaves. Nat Prod Res. 2017;31(22):2604-11. DOI: [10.1080/14786419.2017.1285297](https://doi.org/10.1080/14786419.2017.1285297).
10. Nurjanah N, Herijulianti E, Putri MH, Sukmasari S. Eugenia polyantha (Wight) infusion against oral microorganisms on toothbrushes. Sci Dent J. 2020;4(3):105-9. DOI: [10.4103/SDJ.SDJ_38_20](https://doi.org/10.4103/SDJ.SDJ_38_20)
11. Carmona F; Pereira AMS. Herbal medicine: Old and new concepts, truths and misunderstandings. Rev Bras Farmacogn. 2013;23(2):379-85. DOI: [10.1590/S0102-695X2013005000018](https://doi.org/10.1590/S0102-695X2013005000018)
12. Samaranayake L. Essential Microbiology for Dentistri, 5th ed. Elsevier. 2018.
13. Simatupang DP, Susanti N, Purba J. Stability of styrax benzoin extract and fraction with the addition og glycerol and tween 80. J Unimed. 2021;13(2):143-50. DOI: [10.24114/jpkim.v13i2.26986](https://doi.org/10.24114/jpkim.v13i2.26986)
14. Wunsch CM, Lewis JP. Porphyromonas gingivalis as a model organism for assessing interaction of anaerobic bacteria with host cells. J visualized experiments: JoVE. 2015;(106):e53408. DOI: [10.3791/53408](https://doi.org/10.3791/53408)
15. Rajaram A, Kotrashetti VS, Somannavar PD, Ingalagi P, Bhat K. Culture-based identification of pigmented porphyromonas and prevotella species in primary endodontic infections. J Dent Res Dent Clin Dent Prospects. 2016;10(3):136-41. DOI: [10.15171/joddd.2016.022](https://doi.org/10.15171/joddd.2016.022).
16. Aldhafer ZA, Merza WM, Amelan MF, Shaker RM, Yas LS. Effectiveness of bay leaves aqueous extract on streptococcus mutans in comparision to chlorhexidine gluconate. IOSR-J Pharm Bio Sci. 2017;12(4):12-16. DOI: [10.9790/3008-1204081216](https://doi.org/10.9790/3008-1204081216)
17. Avrilliyanti F, Suparwitri S, Alhasyimi AA. insing effect of 60% bay leaf (Syzygium polyanthum wight) aqueous decoction on the accumulation

- of dental plaque during fixed orthodontic treatment, *Dent J-MKG*. 2017;50(1):1-5. DOI: [10.20473/j.djmkg.v50.i1.p1-5](https://doi.org/10.20473/j.djmkg.v50.i1.p1-5)
18. Ayu Nala El Muna Haerussana, Wulan Putri Dwiastuti, Cindi Arwan Sukowati, Widyastiwi. Inhibitory Test of Bay Leaf (*Eugenia polyantha*) Extract Against *Pseudomonas aeruginosa* and *Escherichia coli*. 4(2) DOI: [10.35877/454RI.asci772](https://doi.org/10.35877/454RI.asci772)
19. Patil SS, Yadav AR, Chopade AR, Mohite SK. Design, development and evaluation of herbal mouthwash for antibacterial potency against oral bacteria. *J Univ Shanghai Sci Tech*. 2020;22(11):1137-48.
20. Meigy NM, Mahendradatta A, Laga A, Natsir D. Antimicrobial activities of tannins extract from guava leaves (*psidium guajava* l) on pathogens microbial. *Int J Sci Techno Res*. (2014):236-241.
21. Alghazeer R, Elmansori A, Sidati M, Gammoudi F, Azwai S, Naas H, Garbaj A, Eldaghayes I. In vitro antibacterial activity of flavonoid extracts of two selected libyan algae against multi-drug resistant bacteria isolated from food products. *J Biosci Med*. 2017;5(1):26-48. DOI: [10.4236/jbm.2017.51003](https://doi.org/10.4236/jbm.2017.51003).
22. Lee, S-H.; Kim, W-H.; Ju, KW.; Lee, M-S.; Kim, HS; Lee, J-H.; Jung, YJ.; Kim, B. Anti-bacterial and anti-inflammatory potential of mouthwash composition based on natural extracts. *Appl. Sci*. 2021, 11, 4227. <https://doi.org/10.3390/app11094227>
23. Aspinall, S. R., Parker, J. K., & Khutoryanskiy, V. V. Oral care product formulations, properties and challenges. *Colloids and Surfaces B: Biointerfaces*, 200, 111567. 2021. <https://doi.org/10.1016/j.colsurfb.2021.111567>
24. Chandrashekar, B., Nagarajappa, R., Mruthunjaya, K., Shekar, S., Girish, M., & Thakur, R. Antiplaque and antimicrobial efficacy of polyherbal mouth rinse among adult human volunteers - A short term randomized controlled trial. *Journal of Herbal Medicine*, 17-18, 100273. 2019. <https://doi.org/10.1016/j.hermed.2019.100273>
25. Serafini, M., Peluso, I., & Raguzzini, A. Flavonoids as anti-inflammatory agents. *Proceedings of the Nutrition Society*, 69(3), 273-278. 2010. <https://doi.org/10.1017/S002966511000162x>