

FORMULATION AND PHYSICAL STABILITY TESTING OF MOUTHWASH PREPARATIONS CONTAINING A COMBINATION OF ETHANOL EXTRACTS OF BETEL LEAF (*Piper betle* L.) AND BASIL LEAF (*Ocimum basilicum* L.)

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A B S T R A C T

Background: Betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) are two traditional plants that are often used as herbal medicines. Betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) were collected from Waulangi Hamlet in Wolowa Baru Village, Wolowa Subdistrict, Buton Regency. **The purpose** of this study was to create a mouthwash preparation and test the physical stability of the preparation consisting of a combination of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) extracts under different temperature conditions. **The method** used in the testing was the cycling test method by stimulating extreme temperature changes where the mouthwash preparation was stored for one day at cold and hot temperatures, respectively 4 degrees Celsius and 40 degrees Celsius. During six testing cycles, physical changes in the preparation were observed, including organoleptic properties, pH, homogeneity, and viscosity. **The results** showed that ethanol extracts of betel leaf and basil leaf could be made into mouthwash preparations with various concentrations of FI=2:1, FII=1:2, and FIII=2:2. The results indicated that as the concentration of the preparation increased and the temperature change became higher, changes would occur in the mouthwash preparation. **The conclusion** is that the combination of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) extracts can be used to make mouthwash preparations, and differences in their concentrations can affect the pH and viscosity properties of the mouthwash preparations tested using the cycling test method.

INTRODUCTION

Oral health is very important for the community, and its cleanliness should not be neglected. Poor oral hygiene can not only affect the oral cavity but also other parts of the body, such as increasing the risk of heart attack and stroke, worsening diabetes, disrupting digestion, contributing to respiratory diseases, and reducing the body's resistance to infection. (Fmipa *et al.*, 2024). Mouthwash is a medicinal solution used to rinse and clean the mouth. Mouthwash is a pharmaceutical product that can be used to prevent cavities and bad breath caused by bacteria and to treat secondary infections.

Mouthwash is a diluted solution that can be used to relieve topical pain, topical antibacterial, topical anti-inflammatory, or to prevent cavities. Mouthwash is used to clean the mouth, eliminate bad breath, and give the mouth a fresh feeling. One of the many mouthwashes currently on the market is produced with natural ingredients. One of the natural ingredients used as a mouthwash is betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.), which contain various compounds (Suryani *et al.*, 2019 dalam Banu & Gayathri, 2016).

Using mouthwash is the easiest way to maintain oral health. Mouthwash can be used chemically to remove plaque from teeth and supporting tissues, or mechanically using a toothbrush, dental floss, or interdental brush. To help prevent bacterial growth and reduce the number of bacteria in dental plaque, mouthwash should be used as an antiseptic (Khamilatusy Sholekhah, 2020).

The use of basil leaves in the manufacture of mouthwash preparations is reliable because basil leaves can prevent bad breath. In addition, basil leaves contain essential oils that act as antibacterials, while flavonoids and eugenol act as antioxidants that can stop the growth of fungi, viruses, and bacteria. Research results from (Yuniarti, 2022) show that basil leaves contain alkaloids, flavonoids, saponins, and tannins, which have the physical qualities of mouthwash preparations that meet testing requirements. In addition to inhibiting bacterial growth, basil leaves can also be used to freshen breath due to their unique aroma. The chemical composition of basil includes essential oils such as cineole and eugenol, saponins, flavonoids, polyphenols, and tannins (Harmely *et al.*, 2015). Many current scientific studies are investigating the benefits of antioxidants and seeking new sources of antioxidants. Previously, the leaves of this plant were examined for antioxidant compounds (Hamzah *et al.*, 2022).

In addition to basil leaves, which can be used in the manufacture of mouthwash, there are also betel leaves, which are very popular in social life. Betel leaves are needed to maintain dental and oral health. In addition, betel leaves are also often used to eliminate body odor and treat thrush or small wounds in the mouth as well as bad breath caused by bacteria (Almasyhuri & Pusat, 2019).

Phenolic compounds, tannins, and essential oils, such as betelfenol, kavicol, estragol, augenol, and carvacrol, are found in green betel leaves. In addition to having strong antibacterial and antifungal properties, these compounds have the ability to stop the growth of various bacteria. This essential oil with its unique aroma is present in 4.2% of betel leaves. Aromatic phenolic compounds, particularly betelphenol and cavicol, as well as their derivatives, including cavicol, carvacrol, eugenol, allylpyrocatechol, and catechin (Sayekti *et al.*, 2022).

Therefore, it is necessary to conduct research on the formulation and physical stability testing of mouthwash preparations containing a combination of ethanol extracts of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) to utilize the synergy or cooperation of active compounds such as flavonoids, essential oils, and phenols in observing physical changes in a mouthwash preparation that will be used by passing various preparation tests using the cycling test method.

METHOD

Research Design

This research is an experimental laboratory study, namely betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) formulated into mouthwash preparations with formulations I, II, and III. The physical stability of each preparation was evaluated in terms of organoleptic properties, pH, viscosity, and homogeneity.

Research Time and Place

This study was conducted from June to August 2025 at the Pharmacy Laboratory of Baubau Polytechnic and at Mandala Waluya in Kendari City.

Equipment and Materials

The equipment used in this study included plastic sieves, stirring rods, 100 mL plastic bottles, wooden stirring rods, glass funnels, glass jars, horn spoons, blenders, filter paper, gauze, glass containers, mortars and pestles, porcelain dishes, 100 mm funnels, Erlenmeyer flasks, 10 mL measuring cups, 100 mL measuring cups, 250 mL measuring cups, 1,000 mL measuring cups, viscometers, aluminum foil, and cotton.

The materials used in this study included betel leaf extract (*Piper betle* L.) and basil leaf extract (*Ocimum basilicum* L.), distilled water, 96% ethanol, glycerin, sorbitol, peppermint oil, and sodium lauryl sulfate.

Sample Collection

The betel leaves (*Piper betle* L.) and basil leaves (*Ocimum basilicum* L.) used as samples in this study were obtained from Waulangi Hamlet, Wolowa Baru Village.

Working Procedure

Sample Preparation.

Betel leaves (*Piper betle* L.) and basil leaves (*Ocimum basilicum* L.) were picked and sorted to remove stems and debris from the leaf samples. The samples used were obtained from Waulangi Hamlet, Wolowa Baru Village. The samples were then cleaned and washed under running water and then drained. After draining, the leaves were divided into several parts separated by their stems, then dried in the sun until dry, with the drying process taking up to 7-10 days. The dried samples were then taken and ground into powder.

Preparation of Betel Leaf (*Piper Betle* L) and Basil Leaf (*Ocimum Basilicum* L) Extracts.

500 grams of betel leaf (*Piper betle* L.) samples and 500 grams of basil leaf (*Ocimum basilicum* L.) samples are weighed and then soaked in 5,000 mL of 96% ethanol solvent, with a ratio of 1:10 (100 grams of simplisia = 1,000 mL of solvent). The mixture was then covered and left to stand for 3 to 5 days, with occasional stirring. After 3 to 5 days, the filtrate was collected using filter paper and filtration to separate the filtrate from the residue. A rotary evaporator was used to evaporate the filtrate. In general, rotary evaporators generate electrical energy as kinetic energy and heat energy. Kinetic energy is used to rotate the round-bottom flask, and heat is generated from a hot water bath (Prastyo, 2023).

Formulation of Betel Leaf and Basil Leaf Extracts

Table 1. Ethanol Extract Mouthwash Formula of Basil Leaves and Betel Leaves

No.	Ingredient	Concentration (% b/v)			Function
		Formulation I	Formulation II	Formulation III	
1.	Betel leaf extract	2	1	2	Active ingredient
2.	Basil leaf extract	1	2	2	Active ingredient
3.	Glycerin	10	10	10	Humectant
4.	Sorbitol	8	8	8	Sweetener
5.	<i>Sodium lauryl sulfate</i> (SLS)	1	1	1	Surfactant
6.	Peppermint oil	<i>q.s</i>	<i>q.s</i>	<i>q.s</i>	Refresher
7.	Distilled	ad. 100 mL	ad. 100 mL	ad. 100 mL	Solvent

Source: Primary Data, 2025

Notes:

F1 : Mouthwash formulation containing 2% betel leaf extract and 1% basil leaf extract

F2 : Mouthwash formulation containing 1% betel leaf extract and 2% basil leaf extract

F3 : Mouthwash formulation containing 2% betel leaf extract and 2% basil leaf extract

Preparation of Mouthwash

The ingredients are weighed according to Table 1. Betel leaf and basil leaf extracts as active ingredients are put into a mortar and ground until homogeneous. Then, 10 mL of glycerin is added as a humectant and ground until mixed. Next, 8 mL of sorbitol is added as a sweetener and ground again until homogeneous. After homogenization, sodium lauryl sulfate (SLS) as a surfactant or foaming agent, then ground again until mixed. Finally, 6 drops of peppermint oil are added as a freshener into the mortar and ground. After all ingredients are mixed, the mixture from the mortar is poured into a 100 mL chemical glass, then distilled water is added up to the marked level. It is then stirred until homogeneous using a stirring rod. The mixed ingredients are then filtered using gauze twice, and the final filtration is performed using filter paper twice. After filtration, the preparation is left to stand for 24 hours to observe any sediment. If sediment is present, filtration is performed again.

Physical Stability Test of Mouthwash Preparations

Before further testing, the initial drug preparation was evaluated after it was made. After that, the mouthwash preparation was stored at a cold temperature of 40C for 24 hours and then placed again at a hot temperature of 40oC for approximately 24 hours. The storage time at these two temperatures was considered one cycle. During six cycles, or twelve days, testing was conducted every two days, with each cycle observing physical changes in the mouthwash formulation, including organoleptic observations, homogeneity, pH, and viscosity (Mudhofar *et al.*, 2024).

Organoleptic Test

The organoleptic test is an examination of the form, color, and odor of the mouthwash preparations made from ethanol extracts of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) (Mudhofar *et al.*, 2024).

Homogeneity Test

The mouthwash preparation that has been placed in a clear bottle with a white background is used to perform the homogeneity test. A good preparation is not contaminated and is not damaged (Djafar *et al.*, 2021).

Viscosity Test

The viscosity of the mouthwash is measured using a viscometer. Rotor No. 1 is set at 60 rpm on the viscometer. After the sample is prepared, it is dipped to the rotor limit mark. After ten seconds of operation, the viscometer is turned off after the results are obtained. The mouthwash has a viscosity range of 3.64 to 7.25, with 7.25 cP as the standard viscosity available on the market (Rachmawati *et al.*, 2022).

pH Test

Using a Universal pH meter, the pH test of the mouthwash formulation was conducted by dipping the pH stick into the formulation. Wait a few seconds to see the results. The ideal pH of mouthwash is 5-7. Saliva pH ranges from 6.2-7.6, with an average pH of 6.7 (Sawitri, 2021).

RESULTS & DISCUSSION

The results of the maceration process were carried out for 3 days. The filtrate was separated using a plastic funnel lined with filter paper, then the filtrate was collected and evaporated using a rotary evaporator to obtain ethanol extracts of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.). After the solvent evaporation process using a rotary evaporator, the extract was weighed and placed in a glass container.

Mouthwash is a diluted solution that is usually used to rinse the mouth. In this study, the process of making mouthwash preparations used ethanol extracts of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.), after which their stability was tested using the cycling test method, where the mouthwash preparations were stored at a temperature of 40°C for 24 hours and then placed again at 40°C for approximately 24 hours. The storage time at these two temperatures is considered one cycle (Ilyas) *et al.*, 2023).

Physical stability is an evaluation of changes in the physical properties of a product over time (storage period). Examples of physical changes include color migration (change), taste change, odor change, and texture or appearance change. The evaluation of physical stability testing includes organoleptic examination, homogeneity, pH, and specific gravity. However, chemical stability is defined as the amount of time required for a pharmaceutical preparation to maintain its properties and stability, as stated on the label, during the specified packaging period (Mudhofar *et al.*, 2024).

Table 2. Organoleptic Observation Results After *Cycling Test*

Cycle To	Organoleptis	Formula I	Formula II	Formula III	Controls+
I	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	Red
Cycle 1 is eligible					
II	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	Red
Cycle 2 is eligible					
III	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	Red
Cycle 3 is eligible					
IV	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	Red
Cycle 4 is eligible					
V	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	Red
Cycle 5 is eligible					
VI	Shape	Liquid	Liquid	Liquid	Liquid
	Smell	Betel leaves and peppermint oil	Basil leaves and peppermint oil	Peppermint oil	Mint
	Color	Deep green	Easy green	Deep green	red
Cycle 6 qualified					

Source: Primary Data, 2025

In Table 2, Formulations 1, 2, 3, and K+ in cycles 1-2 are liquid, with a distinctive scent of betel leaf and basil in F1, peppermint oil in F3, and mint in K+, dark green and red in color without coarse particles. Formulations 1, 2, 3, and K+ in cycles 3-6 are liquid, with a characteristic scent of betel leaf and basil and a mint scent in K+, dark green and red in color without coarse particles. Each formula tested in each cycle met the requirements. Organoleptic testing of the betel leaf and basil leaf extract mouthwash preparations showed that the preparations did not change during six cycles in formulas 1, 2, 3, and 4. The mouthwash preparations produced were liquid, similar to a solution, and had the same

properties as general mouthwashes. The color of formulation 1 is dark green, formulation 2 is light green, formulation 3 is dark green, which is consistent with the color of betel leaf and basil leaf extracts, and formulation 4, as a positive control, is red. The aroma of the preparation has a distinctive peppermint oil scent and a distinctive scent due to the presence of peppermint oil and betel leaf and basil leaf extracts in the formulation. The results of the post-cycling test are shown in Table 2. Therefore, the organoleptic observations of formulations 1, 2, 3, and the positive control have been deemed satisfactory.

Table 3. Homogeneity Observation Results After *Cycling Test*

Formula	Homogeneity Test Results on the Cycle to					
	I	II	III	IV	V	VI
F1	Homogeneous	Not homogeneous	Not homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Not homogeneous	Not homogeneous	Not homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Control +	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Source: Primary Data, 2025

In Table 3, formulations 1, 2, 3, and K+ mouthwash showed that F2 was not homogeneous in the first cycle, F1 and F2 were not homogeneous in cycles 2-3, while in cycles 4-6 they were homogeneous. F3 and K+ were homogeneous in the first to sixth cycles. The results of the homogeneity test on the mouthwash preparations after the cycling test can be seen in Table 3, where in the first cycle there was a change in formula 2, which was not homogeneous, and the other 3 formulas were homogeneous. In the second and third cycles, formulas 1 and 2 were not homogeneous, while formulas 3 and 4 were homogeneous. Then, in cycles 4, 5, and 6, there were no changes and they remained homogeneous. Therefore, the results of the observation of formulations 1, 2, 3, and the positive control of the mouthwash have been declared that F3 and the positive control meet the testing requirements.

Table 4. pH Observation Results After *Cycling Test*

pH Test Results							Standard pH Value of Mouthwash
Formula	Cycle to						
	I	II	III	IV	V	YOU	
F1	5	5	5	4	4	4	5-7
F2	6	5	5	4	4	4	
F3	5	5	5	6	6	6	
Control +	5	6	6	6	6	6	

Source: Primary Data, 2025

Table 4 shows that the mouthwash in F1 and F2 experienced a change in pH in cycles 4-6, which did not meet the pH requirements for mouthwash. In F3 and K+, there was an increase in pH, but it still met the pH requirements for mouthwash and oral pH standards. The pH test results for the mouthwash preparations after testing for six cycles showed different changes for each formula. The pH test results for formula F1 decreased in cycles 4 and 6, falling from 5 to 4, and increased in cycles 2 and 4. The pH test results for formula F2 decreased from 6 to 5 and increased to 4 in cycles 2 and 4. The pH test results for formula P increased from 5 to 6 in cycles 1 and 4. The pH values of F3 and the positive control were outside the pH range intended for oral health, thus meeting the requirements of (Pradewa *et al.*, 2008). However, the pH values of F1 and F2 obtained in cycles 3 to 6 were quite acidic, influenced by the active ingredient and extreme temperature changes, especially as an increase in temperature can cause the mouthwash to become acidic. Therefore, the pH test results for formulations 1, 2, 3, and the positive control mouthwash have been declared to meet the requirements for formulation 3 and the positive control.

Table 5. Viscosity Observation Results After *Cycling Test*

Formula	Viscosity test results in the Cycle to						Viscosity Range of Mouthwash (mPa.s)
	I	II	III	IV	V	YO U	
F1	4,86	3,28	3,00	1,36	1,29	1,24	3,64-7,25
F2	3,33	3,26	1,84	1,70	1,63	1,60	
F3	4,76	4,00	3,93	1,60	1,58	1,37	
Control +	6,84	6,48	13,3	1,62	1,49	1,42	

Source: Primary Data, 2025

Table 5 shows that all mouthwash formulations made using formulas 1, 2, 3, and K+ are below the standard viscosity range for mouthwash of 3.64-7.25 cps. Results of viscosity testing of mouthwash formulations containing betel leaf and basil leaf extracts. The testing was conducted using an Nd J-8s Rotor No. 1 at a speed of 60 rpm. Testing over 6 cycles showed changes, and the results can be seen in Table 4.10. In F1, the viscosity obtained was cycle 1=4.86, cycle 2=3.28, cycle 3=3.00, cycle 4=1.36, cycle 5=1.29, and cycle 6=1.24. For F2, the viscosity obtained was cycle 1 = 3.33, cycle 2 = 3.26, cycle 3 = 1.84, cycle 4 = 1.70, cycle 5 = 1.63, and cycle 6 = 1.60. In F3, the viscosity obtained was cycle 1 = 4.76, cycle 2 = 4.00, cycle 3 = 3.93, cycle 4 = 1.60, cycle 5 = 1.58, and cycle 6 = 1.37. Meanwhile, the positive control (F4) viscosity obtained was cycle 1=6.84, cycle 2=6.48, cycle 3=13.3, cycle 4=1.62, cycle 5=1.49, and cycle 6=1.42. The formulation underwent changes after the cycling test and could not meet the pH range requirement of 3.64-7.25 cP. Extreme temperature changes from 4 degrees Celsius to 40 degrees Celsius may have influenced these viscosity changes over six cycles.

CONCLUSION

The results showed that a mixture of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) extracts could be used to make a mouthwash solution. In organoleptic testing, the concentration and combination of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) extracts did not change the shape, color, or smell of all formulation preparations. In the homogeneity test, changes occurred in cycles 1, 2, and 3 for F1 and F2, while no changes occurred in F3 and K+. In the pH test, F1 and F2 showed a change in pH to acidic from cycles 4-6. In the viscosity test, changes occurred in F1, F2, F3, and K+ from cycles 4-6, so the preparations did not meet the viscosity standards for mouthwash. The stability test of the mouthwash preparation using the cycling test method during a storage period of 12 days or 6 cycles was declared stable in the organoleptic and homogeneity tests because there were no changes in the form of the preparation during the storage period. In the pH test, the preparation was declared stable in F3 and K+ during the storage period and met the mouthwash testing standards, while in the viscosity test, the preparation did not meet the viscosity range of 3.64-7.25 mPa.s during storage and did not meet the viscosity standard.

Researchers are advised to conduct further research using various tests to determine the effectiveness of mouthwash preparations that combine ethanol extracts of betel leaf (*Piper betle* L.) and basil leaf (*Ocimum basilicum* L.) as antibacterial agents using different concentrations of ingredients and the agar diffusion (Kirby-Bauer) test method. In this study, the limitations of the researchers in conducting the research often focused on one aspect of testing the preparation, where the physical stability testing equipment had constraints.

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