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FORMULATION AND ANTIBACTERIAL ACTIVITY OF MOUTHWASH COMBINATION OF GUAVA LEAF (*Psidium guajava L.*) AND BASIL LEAF (*Ocinum basilicum L.*) EXTRACT AGAINST *Streptococcus mutans*

FORMULASI *SEDIAAN MOUTHWASH KOMBINASI EKSTRAK DAUN JAMBU BIJI (Psidium guajava L.) DAN DAUN KEMANGI (Ocinum basilicum L.) TERHADAP AKTIVITAS BAKTERI Streptococcus mutans*

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Abstract

Dental caries is basically caused by cariogenic bacteria, including Streptococcus mutans, which are found in the oral cavity, especially dental plaque. To treat dental caries, antiseptics packaged in the form of mouthwash use unnatural active ingredients which often cause side effects. Therefore, it is necessary to carry out preventive measures to reduce side effects by using waste from guava leaves and basil leaves. This research purpose were to making mouthwash formulation with the active ingredients guava leaf extract and basil leaf extract and determine the antibacterial activity of mouthwash formulation. The method used is the diffusion method, a combination of concentrations of guava leaf extract and basil leaves, namely F1(0%+20%), F2(5%+15%), F3(10%+10%), F4(15%+ 5.0%), F5(20%+0%), F6(15%+15%). In this study, the variable measured in this study was the diameter of the inhibition zone formed by the combination of extracts. Data analysis was tested using One Way Anova. The significant value obtained was 0.00 ≤ 0.05, indicating a significant difference from the formulation containing a combination of extracts. The results of this research showed that the largest inhibitory zone was at F5 with the largest average value of 15.07mm which had a strong inhibitory response. This research can be concluded that the mouthwash formulation of extracts meets the physical requirements and can inhibit S. mutans.

Keywords **:** *Guava, basil, mouthwash, S. mutans*

Abstrak

Karies gigi merupakan infeksi yang disebabkan oleh bakteri kariogenik, termasuk *Streptococcus mutans*, yang ditemukan di dalam rongga mulut, terutama pada plak gigi. Untuk mengobati karies gigi, antiseptik yang dikemas dalam bentuk obat kumur biasanya menggunakan bahan aktif yang tidak alami, yang sering menimbulkan efek samping. Oleh karena itu, perlu dilakukan upaya pencegahan untuk mengurangi efek samping tersebut dengan memanfaatkan limbah dari daun jambu biji dan daun kemangi. Tujuan dari penelitian ini adalah untuk membuat formulasi obat kumur dengan bahan aktif berupa ekstrak daun jambu biji dan ekstrak daun kemangi, serta mengetahui aktivitas antibakteri dari formulasi obat kumur tersebut. Metode yang digunakan adalah metode difusi, dengan kombinasi konsentrasi ekstrak daun jambu biji dan daun kemangi, yaitu F1 (0% + 20%), F2 (5% + 15%), F3 (10% + 10%), F4 (15% + 5%), F5 (20% + 0%), dan F6 (15% + 15%).

Variabel yang diukur dalam penelitian ini adalah diameter zona hambat yang terbentuk dari kombinasi ekstrak tersebut. Analisis data diuji menggunakan One Way Anova. Nilai signifikansi yang diperoleh adalah 0,00 ≤ 0,05, yang menunjukkan adanya perbedaan yang signifikan dari formulasi yang mengandung kombinasi ekstrak. Hasil penelitian menunjukkan bahwa zona hambat terbesar terdapat pada F5 dengan nilai rata-rata terbesar sebesar 15,07 mm, yang menunjukkan respons hambatan yang kuat. Dapat disimpulkan bahwa formulasi obat kumur dari ekstrak tersebut memenuhi persyaratan fisik dan dapat menghambat pertumbuhan *S. mutans*.

Kata Kunci **:** Basil, Jambu Biji, Pasta gigi, *S. mutans*

INTRODUCTIONS

The mouth and teeth often experience infections caused by bacterial and fungal infections, one of the infectious diseases that arises is dental caries. Caries is a disease caused by food residue left on the teeth, which then forms acid on the surface of the teeth by bacteria in the mouth, which can destroy the tooth structure [1].

Dental caries is basically caused by cariogenic bacteria found in the oral cavity, especially dental plaque. These bacteria include Actinomyces, Lactobacillus, *Streptococcus mutans* (*S. mutans*) and *Streptococcus Sanguis* [2]. S. mutans is a bacteria that is cariogenic because it is able to stick to the surface of teeth. Ecological changes are characterized by an increase in the proportion of S. mutans and other species that are aciduric and acidogenic [3].

The increase in the prevalence of dental caries in the Indonesian population in 2018 increased by 88.8% with the prevalence of root caries amounting to 56.6%. The prevalence of caries tends to be high above 70% in all age groups. The highest prevalence of caries was in the 55-64 year age group, 96.8%, while the highest prevalence of root caries was in the 35-44 year age group [4].

Treatment of dental caries has currently used antiseptics packaged in the form of mouthwash, which on the market use unnatural active ingredients, namely synthetics containing alcohol, which often causes side effects such as taste, changes in color of teeth and tongue, desquamation of the oral mucosa and burning of the tongue [5]. In general, commercial mouthwash preparations on the market contain quite high alcohol levels, namely 25% or more. Using mouthwash with high alcohol content can increase the risk of mouth, throat and pharynx cancer by around 50% [6].

Synthetic mouthwash that causes side effects requires finding alternative medicines made from herbal ingredients that have active substances that are safer for the body. In an effort to reduce this risk, mouthwash can use natural ingredients. Empirically, guava leaves have been proven to treat various diseases of swollen gums, canker sores, and have activity against S. mutans bacteria [2]. Basil leaves have been proven to have antibacterial properties against *S. Mutans* and *S. Aureus* [7]. Basil leaf extract also has antibacterial activity against S. Mutans [8]. In previous research, guava leaf extract had antibacterial activity [9]. Guava leaf extract can be formulated as a mouthwash preparation and has antibacterial activity

against *S. Mutans* [10]. Basil leaf extract can be formulated as a mouth freshener [11-13].

The purpose of the research were to making mouthwash formulation with the active ingredients guava leaf extract and basil leaf extract and determine the antibacterial activity of mouthwash formulation.

MATERIALS AND METHODS

Materials

The tools used for this research were glass, mortar and stamper, pH Meter, Ostwald Viscometer (Pyrex), Autoclave, Petri Dish, Hot Plate, Incubator, Test Tube, Ose, Tweezers, Micro Pipette, Rotary Evaporator (SCI TEK RE-100s), Oven, Bunsen Burner, Laminar Air Flow (Faithful CJ -600P), caliper, filter paper, analytical balance.

The materials used in this research were distilled water, guava leaves, basil leaves, ethanol (technical 70%), Streptococcus mutans bacteria, Sorbitol (merck), Peppermint Oil (Apercap), Glycerin (oneMed), Mueller Hinton Agar (himedia, paper discs.

Methods

Preparation of Simplicia

Guava leaves and basil leaves are sorted wet, washed with running water and drained for 5 days. Next, the guava leaves and basil leaves were chopped and then each leaf was weighed at 2 kg. Guava leaves and basil leaves are dried separately by drying them in the sun and covered with a black cloth so that the heat from the sun is evenly distributed and prevents the entry of impurities [14].

Preparation of Guava Leaf and Basil Leaf Extracts

Making guava leaf extract is done by maceration. The dried and powdered simplicia is then extracted with 70% ethanol. Simplicia is soaked in 70% ethanol until completely submerged (1:250), then let stand for 24 hours. The maserate obtained was filtered with filter paper (filtrate 1) and the remainder was extracted again with 70% ethanol for 24 hours (filtrate 2). The treatment was repeated until the maceration process was considered complete. The resulting filtrate is collected and then evaporated using a rotary evaporator to obtain a liquid extract[10-11]. The basil leaf extract is done in the same way as guava leaves.

Phytochemical Test

Saponin Identification

Each extract of guava leaves and basil leaves which had been diluted to the amount of 0.5 mL was put into a test tube separately. Add 10 ml of hot water to both test tubes and shake for 1 minute. If the foam formed remains stable for ±15 minutes, then the sample is positive for containing saponin [15-16].

Alkaloid Identification

Each extract of guava leaves and basil leaves which had been diluted to the amount of 1.0 mL was put into a test tube separately then added with 1 mL of 2N HCl and 9 ml of distilled water, heated over a water heater for 2 minutes, cooled and filtered. , The filtrate obtained is used for testing. Take 10 drops of the extract and put it in a test tube plus 2 drops of Wagner's reagent (a mixture of iodine and potassium iodide) so that a brown to black precipitate forms. If the reactant produces a precipitate, it is positive for containing alkaloids [15-16].

Flavonoid Identification

Each extract of guava leaves and basil leaves which had been diluted to the amount of 1.0 mL was put into a test tube separately. Add 0.05 mg of Mg powder and 1 ml of concentrated HCl to each test tube, then shake vigorously. A positive test is indicated by the formation of red, yellow or orange [15-16].

Tannins Identification

Each extract of guava leaves and basil leaves which had been diluted to the amount of 1.0 mL was put into a test tube separately and 2 - 3 drops of 10% $FeCl_3$ were added . A positive result contains phenol if it produces a green, red, purple, blue or dark black color [15-16].

Mouthwash Formulation

The combination of extract used in this research were the concentrations of guava leaf and basil leaf extracts shown in Table 1.

The basis of each mouthwash formulation consisting of 0.1 mg sodium benzoate dissolved in distilled water, 20 ml Sorbitol, 10 ml glycerin added little by little, then stirred homogeneously and added 0.5 ml aqua menthae piperitae. Wet solution was added to each formula F1 – F6 and filled with 100 ml of distilled water [17].

Tabel 1. the concentrations of guava leaf and basil leaf extracts in Formulation

Treatment	Concentrations of extract (%)	
	guava leaf	basil leaf
F1	0.0	20.0
F2	5.0	15.0
F3	10.0	10.0
F4	15.0	5.0
F5	20.0	0.0
F6	15.0	15.0
K+	Chlorhexidine	
K-	Basis of the formula	

Evaluation of Mouthwash Formulation

Organoleptic Observations

Observations of color, aroma and taste have been carried out by looking at the color, smelling the aroma and tasting the taste of each mouthwash formulation.

pH measurement

Measuring and checking pH can be done with a pH meter. How it works: Calibrate the pH meter at pH 4 and pH 7. Dip the pH meter into the formulation. Read the pH listed. The pH of mouthwash formulation is pH 5 – 7 [10].

Viscosity Test

Test the viscosity of the mouthwash formulation using an Ostwald viscometer. 50 ml of the formulation is inserted through tube B and then sucked until the liquid passes through part A and passes the "a" level. The liquid is then allowed to flow from the "a" limit to the "b" limit. The time required for the formulation to flow is calculated using a stopwatch. The viscosity measurement was repeated 3 times for each formulation. The time required for the formulation to flow then the viscosity was calculated [10].

Test the Antibacterial Activity of Mouthwash formulation

Sterilization

In the antibacterial test, the treatment must be sterile, for this reason all tools and materials used must be sterile. The purpose of sterilization is to kill microorganisms on tools and materials, because it is feared that this will disrupt the course of research. Sterilization was carried out in an autoclave at 120 °C for 15 minutes.

Preparation of Media Agar

NA media is made by dissolving 2 grams of NA powder in 100 ml of distilled water

and then heating it while stirring until it dissolves and boils for approximately 10-15 minutes. The medium was sterilized by autoclaving at 121°C, 1 atm pressure for 15 minutes. After that, put the still warm NA into a test tube and let it sit in a petri dish until it solidifies.

Preparation of Bacterial Cultures

S. mutants bacteria are cultured in slanted agar media by pure culture of *S. mutans* bacteria obtained at the Microbiology Laboratory of Tulang Bawang University, Lampung, the culture is taken in 1 eye of the loop then streaked into slanted NA media which has solidified in a test tube, close the top test tube with sterile gauze filled with cotton, incubate for 24 hours at 37°C .

Antibacterial Power Test

Test antibacterial power using the hole method (cup plate). Two petri dishes containing solidified NA were made into four wells in each. The holes in the first petri dish were sequentially filled with F1, F2, F3 and F4 using a micropipette (50 µL). The holes in the second petri dish are filled with F5, F6, K(-) and K(+). All petri dishes were incubated for 24 hours at 37 ^{o}C in an incubator. Measurement of the inhibition zone formed using a caliper. Each treatment was carried out three times [10].

Data Analysis

From the results of the inhibition zone measurements, the inhibition zone was then analyzed using a one-way Anova test with a confidence level of 95%, by comparing F count and F table, if F count > F table then there is an influence of the treatment given then proceed with a further test of the smallest real difference , analysis assisted with SPSS (Statistical Product and Service Solutions) software. Evaluation of mouthwash preparations was analyzed descriptively. Depicting data in descriptive analysis in the form of pictures, tables and bar graphs.

RESULTS AND DISCUSSION

The results of the determination at the Botany Laboratory of the Biology Department, FMIPA, University of Lampung, showed that the samples used were actually guava leaves (*Psidium guajava* L.) and basil leaves (*Ocinum basilicum* L.). The correctness of the plant species resulting from this determination is the basis for the use of plants in making mouthwash formulations in this research.

The results of making basil leaf simplicia and guava leaves are ± 300 grams each (15%), brown in color. The dried simplicia is ground using a blender to obtain fine simplicia powder from guava leaves and basil leaves so that the process of making the extract using the maceration method with 70 % ethanol solvent will take place more quickly. The maceration process was chosen because it effectively attracts secondary metabolites and compounds in plants. When simplicia is soaked in a solvent, the cell membrane and walls break down due to the difference in pressure inside and outside the simplicia cell [15].

The results of determining the water content of guava leaf simplicia and basil leaves were 2.80% and 5.32%, this is in accordance with the required water content, namely less than 10% [18]. The results of determining the simplicia ash content of guava leaves and basil leaves were 10.2% and 12.1% . The ash content value of both is less than 16% , this shows that the ash content of guava leaf simplicia and basil leaves meets the ash content requirements [18]. The ash content aims to provide an overview of the internal and

external mineral content originating from the initial process until the formation of the extract and to control the amount of determination of inorganic objects and contamination [19-20]. The result of phytochemical secondary metabolite extracts leaf basil and guava leaves shown in Table 2.

Table 2. The result of phytochemical secondary metabolite extracts.

Compound	Results	Information	
		Basil leaf	Guava leaf
Saponin	Forms stable foam	+	+
Alkoloid	Brownish yellow and there is a brown precipitate	+	+
Flavonoids	Black	+	+
Tannin	Blackish green	+	+

Based on Table 2 above, the results of testing the two leaf extracts for saponin with the addition of distilled water and shaken vigorously produce stable froth or foam. This shows that basil leaf extract contains saponin compounds. The glycoside content in saponin will be hydrolyzed into glucose and other compounds, causing foam or foam in the liquid [10,15].

The results of alkaloid analysis in the extract using Wagner's reagent produced I 3- ions which were brownish in color. This brownish precipitate is produced by a complex bond of potassium-alkaloid which is formed from the K + metal ion in potassium which forms a coordinate covalent bond with nitrogen in the alkaloid [10,15].

In guava leaf and leaf extracts basil shows yellow, this shows that the two leaf extracts contain flavonoid compounds. The yellow color is formed because flavonoids are compounds in the phenol group which have many OH groups to form complex compounds of flavilium salts with Cl – ions [10,15].

Guava leaves and basil leaves that have been added with distilled water and heated produce a blackish green color after being dripped with a few drops of 0.1% $FeCl_3$. This proves that basil leaf extract contains tannin compounds. The phenol group found in tannin compounds will form a complex compound with Fe 3+ ions , resulting in a color like inky blue or blackish green [10,15].

Organoleptic observations of the colors of the six different mouthwash formulations are as shown in Figure 1, namely brown, light brown, yellowish brown, brownish yellow and yellow .

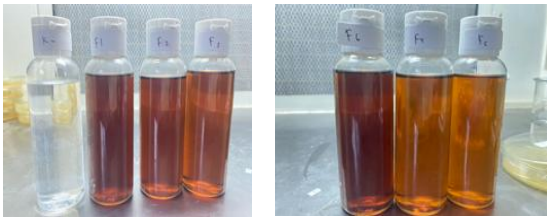


Figure 1. Color of each mouthwash formulation

These differences differ based on the concentration of the extract mixed. The brown color is caused by the presence of basil leaf extract which is dark brown, while the yellow color is obtained from guava leaf extract which is yellow. The mint aroma in formula 1 is mint aroma from the additional ingredients used (peppermint oil), while the aroma in formulas 2 and 3 is mint and is followed by the distinctive aroma of guava leaves. There is a difference in taste between

formulas 1, 2, 3 and formulas 4, 5 and 6. The sweet taste in the six formulas is due to the addition of sorbitol which is used as a sweetener , while the chelating taste in formulas 4, 5 and 6 is obtained from the chelating taste of leaf extract. Guava is more dominant than tasteless basil leaf extract10 [10-11].

The test results show that the average results obtained from calculating the pH limit values for mouthwash formulas in all formulas are outside the optimum pH for bacterial growth, shown in Table 3.

Table 3. The results of pH mouthwash formulas

Mouthwash Formulation	pH value		
	Week 1	Week 2	Week 3
F1	6.2	6.2	6.0
F2	6.1	6.1	6.0
F3	5.0	5.2	5.2
F4	5.2	5.1	5.2
F5	5.5	5.4	5.4
F6	5.1	5.1	5.4

In Table 3. pH 5.0 - 6.2 is a safe pH for oral fluids. Most bacteria have an optimum pH, namely the pH at which bacterial growth is maximum, which is around pH 6.5-7.5. Therefore, the pH value of the mouthwash preparation formulation of guava leaf and basil leaf extract meets the requirements for the formulation of antibacterial mouthwash preparations [10]. The results of measuring the viscosity of the six formulas at temperature show that the mouthwash preparation combining guava leaf extract with basil leaves has a low viscosity of 1.53 – 1.65 Ns/m^2 as shown in Table 4. The combination of mouthwash that combines of extracts has a reasonable viscosity value. The specifications for mouthwash preparations maximum viscosity value. Since it is neither too thick nor too runny, the viscosity values are consistent with mouthwash. The viscosity value of mouthwash formulas is determined by the concentration of the ingredients it contains, such as glycerol, sorbitol and others [21].

Table 4. Results of the viscosity of mouthwash formulas

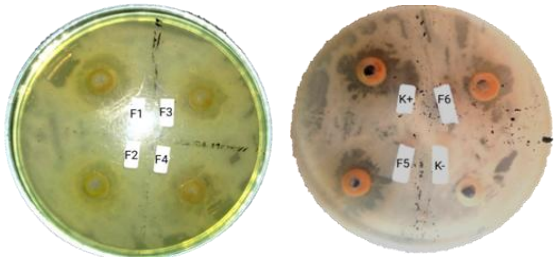
Dosage formulation	Viscosity (Ns/m^2)
F1	1.654
F2	1.616
F3	1.602
F4	1.608
F5	1.609
F6	1.635

The results of the antibacterial power test carried out on each S bacterium preparation . Mutants using the hole or well method that have been cultured in NA media are shown in Table 5 and Figure 2.

Table 5. The results of antibacterial of mouthwash formulas

Treatment	Mean zone of inhibition (mm)	Inhibited response
F1	14.92 ± 0.56 ^{d}	strong
F2	11.94 ± 0.62 ^{c}	strong
F3	13.28 ± 0.60 ^{c}	strong
F4	9.74 ± 0.98 ^{b}	moderate
F5	15.07 ± 0.95 ^{e}	strong
F6	12.79 ± 0.47 ^{c}	strong
K+	20.76 ± 0.73 ^{f}	Very strong
K-	0 ^{a}	Nothing

Note: the numbers in the same column followed by the same uppercase letter indicate that they are not significantly different at the 5% test level



A B

Figure 2. The results of the antibacterial power test of the combination of extract concentrations against *S. mutants*. (A) inhibition zones F1, F2, F3, F4 and (B) inhibition zones F5, F6, K (-), K(+)

Based on Table 5, the results of the *One Way Anova test* obtained a sig value. 0.000 ≤ 0.05 which shows that the content of guava leaf and basil leaf extracts in the mouthwash formulation has a significant effect on the growth of S. mutans colonies. The Tukey test was carried out to see differences in samples that showed real differences between each mouthwash formulation. The average value of the combination of extracts against *S. mutant bacteria* at K- was significantly different from F4 and also significantly different from F2. F2 is not significantly different from F3 and F6, F3 and F6 is not significantly different from F1 and is not significantly different from F5 and is very significantly different from K+. The results of research on *mutant Streptococcus* bacteria show that the inhibition zone for K+ is very strong because the inhibition zone is more than 20 mm, while for F1, F2, F3, F5 and F6 it is categorized as strong because the diameter of the inhibition zone is between 11-20 mm. K- does not have an inhibition zone, this indicates that the negative control used has no effect on the antibacterial test [12].

The antibacterial activity of the mouthwash formulas combined with guava leaf extract and basil leaves is supported by the results of phytochemical tests which show that the mouthwash formulation still contains saponins, alkaloids, flavonoids and tannins. The chemical compounds contained in each formulation are antibacterial against *S. mutans* [22].

Saponin as an antibacterial works by reducing the surface tension of cell membranes which results in increased cell permeability or hemolysis so that intracellular compounds will come out [23-24]. Alkaloids have antibacterial capabilities because they have quaternary aromatic groups that are able to interact with DNA, apart from that, alkaloids are also able to disrupt the integrity of the peptidoglycan components in bacterial cells. Peptidoglycan is a component that makes up bacterial cell walls, so this disruption will cause the cell wall layer to not form completely and cause cell death [22].

Flavonoids as antibacterials work through complex bonds with soluble extracellular proteins so that they can disrupt the integrity of the bacterial cell membrane, as a result the cell membrane will leak and the bacteria will experience growth inhibition and even death. Flavonoids also inhibit bacterial energy metabolism by blocking the bacterial respiration process so that the inhibited energy will affect the metabolite absorption activity and biosynthesis of bacterial macromolecules [25].

Tannin compounds can interfere with peptidoglycan synthesis in the formation of bacterial cell walls, resulting in cell walls no longer forming. This situation will cause bacterial cells to lyse due to osmotic pressure so that the bacterial cells die. Plants' ability to fight germs is influenced

by the quantity and kind of secondary metabolites they contain.

The highest amount of guava leaf extract in the F5 formulation provides the most potent antibacterial properties. This may occur because guava plants have a higher concentration and kind of antibacterial metabolites than basil leaves. [21, 26].

CONCLUSION

The combination of guava leaf extract with basil leaves can be formulated into a mouthwash formulation that meets physical requirements including pH test, viscosity test and organoleptic test. The mouthwash formulation with a combination of guava leaf extract and basil leaves has antibacterial power against *S. mutans bacteria* . The largest average zone of inhibition is found in F5, namely 15.07 ± 0.95 mm, which is classified as inhibiting strong growth .

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