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Formulation and effectiveness testing of mouthwash (*Gargarisma*) combination of guava leaves (*Psidium guajava L. folium*) and bay leaves (*Syzygium polyanthum folium*) extracts as an antibacterial agent against *Streptococcus mutans*

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ABSTRACT

The dental caries disease is the result of the bacterial infection by Streptococcus mutans bacteria which cause the decay of the teeth. The extraction of guava leaves (*Psidium guajava L. folium*) and bay leaves (*Syzygium polyanthum* (Wight) Walp.) has secondary metabolites with antibacterial action. This research is intended to investigate the effect of combination of both plant extracts as the inhibitors of *Streptococcus mutans* by making the mouthwash in the three concentrations of F1, F2, and F3. Plant identification, phytochemical testing, physical testing, and disc diffusion test are done in order to identify the identity of the plants, metabolite content of plant, physical quality of the formulation, and antibacterial activity of the formulation. Statistical analysis used for the inhibition zone diameter (IZD) value is one-way ANOVA. From the phytochemical test result, both of plant extracts have secondary metabolites of alkaloid, flavonoid, tannin, and phenol. Physical quality of the F1, F2, and F3 formulations remained stable until the four weeks of storage period. Antibacterial assays revealed that F3 exhibited the highest inhibitory activity against *Streptococcus mutans* with an IZD of 8.67 ± 0.764 mm, followed by F2 at 7.67 ± 0.577 mm and F1 at 4.33 ± 3.786 mm. One-way ANOVA demonstrated significant differences in IZD between groups ($F = 17.135$; $p < 0.001$), following the order of F3 > F2 > F1. Post hoc analysis using the Games-Howell test showed that F2 and F3 had significantly higher antibacterial activity than the negative control. However, the differences in effectiveness among F1, F2, and F3 were not statistically significant.

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1. INTRODUCTION

Dental caries continues to be a common problem affecting oral health in the community. One of the main bacteria responsible for causing tooth rot is *Streptococcus mutans*. This type of Gram-positive bacteria can stick to the tooth surface and generate acid by breaking down carbohydrates, which lowers the pH level and leads to damage of the dental tissues [1]. Controlling the growth of

Streptococcus mutans is an important step in keeping the mouth healthy and preventing the development of tooth decay [2]. The use of mouthwash products is a common way to help keep the mouth clean and lower the amount of bacteria present. Commercially available mouthwashes usually include certain antibacterial components; however, the prolonged use of artificial ingredients may lead to various drawbacks [3]. Therefore, using medicinal plants as a source of natural active ingredients offers an alternative approach that can be explored for developing mouthwash formulations [4].

The leaves of guava are known scientifically as *Psidium guajava L.folium*, while the leaves of bay can be called *Syzygium polyanthum* (Wight) Walp. Guava and bay leaves can be considered as two plants that contain secondary metabolites that might exhibit the antibacterial effect. According to the results of previous phytochemical analysis, the secondary metabolites like alkaloids, flavonoids, and tannins/phenols were found in the extracts of guava and bay leaves. Such secondary metabolites are very important for showing antibacterial effect because they prevent the development of bacteria by preserving the integrity of the bacterial cell wall [5,6]. Guava and bay leaves' extracts in the mouthwash solution are expected to inhibit the activity of bacteria *Streptococcus mutans*. There is not much scientific information concerning the antibacterial effect of both guava and bay leaves in mouthwash solution. With the addition of guava leaves and bay leaves to the mouthwash, there will be a development of synergy that will have antibacterial properties towards the *Streptococcus mutans*. This will be attributed to the complementary functions of the secondary metabolites, especially flavonoids and tannins, which will break down the bacterial cell wall and inhibit the functioning of the bacteria's enzymes.

Thus far, many of the research works have concentrated on the individual extracts of the plants for their antibacterial properties and very few studies have looked at these properties when applied collectively. Thus, the experiment was carried out to acquire the scientific data about the mouthwash formulations, containing the extract of these two plants as the natural antibacterial component against *Streptococcus mutans*. The findings of this investigation will provide the insights about the possible benefits of natural components and promote the use of Indonesian medicinal plants in the production of pharmaceutical products. Furthermore, the goal of this investigation is to set a basis for the future research on the development of the natural mouthwash formulations that could have some benefits compared to the artificial ones. Different quantities of the combined extracts were used to produce the mouthwashes, which were then tested for the preparation and antibacterial properties by the method of disc diffusion [7].

2. RESEARCH METHODS

2.1. Materials

The ingredients which comprise guava leaves and bay leaves were taken from Kampung Durian, Lubuk Alung District, Padang Pariaman Regency, West Sumatra. Ingredients that were used include 96% ethanol, peppermint oil, sorbitol, sodium benzoate, glycerin, distilled water, 2% HCl, Mayer's reagent, Dragendorff's reagent, magnesium powder, concentrated HCl, and 1% $FeCl_3$ solution. In terms of the antibacterial test, ingredients that were used include Mueller-Hinton Agar (MHA), Nutrient Agar (NA) media, *Streptococcus mutans* culture, 70% alcohol, Whatman No. 40 filter paper, aluminum foil, sterile cotton swab, sterile cotton, and Listerine.

2.2. Sample Preparation and Extraction Procedure

Sorting was done manually to remove any impurities from the collected guava and bay leaves. After that, the plant materials were cleaned by washing them under tap water, chopped, and air-dried. After air-drying, the leaves were grinded to get the powder form of simplicia using a laboratory blender.

The two types of extracts were prepared by soaking 250 g of each guava leaf and bay leaf powder separately in 96% ethanol with the solvent-to-solid ratio of 1:10. Maceration for 72 hours was done with intermittent stirring. Lastly, the extracts obtained were combined and concentrated through evaporation using rotary evaporator at 40°C [8].

2.3. Phytochemical Screening

Qualitative phytochemical analysis was conducted using tests in tubes for the presence of alkaloids, flavonoids, tannins, and phenolics.

2.3.1. Alkaloid Analysis

The presence of alkaloids was found using Mayer's and Dragendorff's test. The precipitation of the substances showed the presence of the compound [9].

2.3.2. Flavonoid Analysis

Flavonoids were confirmed by the use of Mg powder and concentrated HCl on the extract, resulting in a change in color [10].

2.3.3. Tannin/Phenolic Analysis

For the identification of tannins and phenols, the extract was subjected to treatment with 1% ferric chloride solution ($FeCl_3$), and a change in color to green-black indicated a positive reaction [11].

2.4. Formulation Design

Table 1 presents the mouthwash formulations containing guava and bay leaf extracts [12].

Table 1. Design of formulation for mouthwash with extracts from guava and bay leaves [12].

Ingredients	Control		Formulation (%)			Function
	(+)	(-)	F1	F2	F3	
Guava leaf extract	-	-	4	4.5	5	Active ingredient
Bay leaf extract	-	-	4	4.5	5	Active ingredient
Peppermint oil	-	0.2	0.2	0.2	0.2	Flavoring agent
Sorbitol	-	0.25	0.25	0.25	0.25	Sweetener
Sodium benzoate	-	0.1	0.1	0.1	0.1	Preservative
Glycerin	-	2.0	2.0	2.0	2.0	Humectant
Aquadest add	-	100	100	100	100	Solvent
Listerin	0.1	-	-	-	-	Positive control

2.5. Physical Quality Evaluation of the Mouthwash Formulation

2.5.1. Organoleptic Evaluation

The organoleptic properties which included color, smell, and taste were tested through direct sensory assessment. Every formula underwent continuous observation during the storage time, and all changes were recorded [13].

2.5.2. pH Determination

A digital pH meter, calibrated using buffer solutions, was used to measure the pH value. An aliquot of 10 ml of the mouthwash solution was placed in a beaker, and the electrode was immersed till the reading was steady. The pH values were subsequently validated against quality standards [14].

2.5.3. Clarity Test

The preparations clarity was tested visually under proper lighting using contrasting backgrounds of white and black. This test was done to confirm that there were no particles or precipitates in the preparation [15].

2.5.4. Viscosity Measurement

To find the viscosity of the formulation, an Ostwald viscometer was employed. The process involved making the readings after the formulations were placed inside the viscometer. Every formulation was repeated three times to get the average value [16].

2.5.5. Homogeneity Test

Homogeneity of formulation was tested by spreading out a minute amount of sample on a glass slide. The mouthwash would be termed homogeneous if it did not contain any coarser particles, agglomerates, or phase separation [17].

2.5.6. Hedonic Test

The sensory analysis experiment was conducted with a consumer panel consisting of 25 people (5 male and 20 female), whose age ranged from 17 to 55 years. Inclusion criteria required excellent oral health, with no dental caries, mouth ulcers, or allergies to mouthwash components. Participants were instructed not to eat, drink coffee, or smoke for one hour before testing. Color, smell, and taste of the mouthwashes F0, F1, F2, and F3 were determined using the five-point hedonic scale (where 1 means strongly dislike and 5 means strongly like). In-between testing periods, participants were required to rinse their mouths with mineral water [18].

2.6. Antibacterial Activity Test

2.6.1. Equipment Sterilization

The laboratory equipment was cleaned and dried before sterilization. It was sterilized using the autoclave at 121°C for 15 minutes, while the inoculation loops were sterilized by holding them over the Bunsen burner flame until they became red hot. The sterilized lab equipment was then left to cool down to room temperature.

2.6.2. Mueller Hinton Agar (MHA) Medium Preparation

The preparation of the medium included mixing 5.70 grams of MHA in 150 ml of distilled water using a 500 ml Erlenmeyer flask. This was done by stirring the solution until boiling. The neck of the flask was covered with sterile cotton wool and sterilized by autoclaving at 121°C for 15 minutes. The dissolved medium was subsequently poured into sterile Petri dishes [19].

2.6.3. Subculturing of *Streptococcus mutans*

Strain subculture of *Streptococcus mutans* was done using the method of inoculating bacteria on agar slants. Aseptic technique was used whereby a loopful of the bacteria was streaked on NA slants, which were then incubated at 37°C for 24 hours. The culture obtained was stored in a refrigerator as a stock culture. However, in the case of working culture, bacteria biomass was suspended in 0.9% NaCl to give 0.5 McFarland turbidity [20].

2.6.4. Peparation of *Streptococcus mutans*

Preparation of bacterial suspension was done by transferring *Streptococcus mutans* bacteria obtained from patient to test tube that contained 5 ml of 0.9% NaCl solution by using sterile inoculating loop. This suspension was used directly in the experiment for antibacterial test [21].

2.6.5. Preparation of Disc Papers

Paper disks (6 mm in diameter) made from Whatman No. 40 filter paper were placed in a Petri dish and sterilized through autoclaving at 121°C for about 15 minutes. The disks were then placed completely in 10 ml of their corresponding test solutions consisting of Listerine (positive control), F0 (negative control), and F1, F2, and F3. Each set was run in triplicates [22].

2.6.6. Inhibition Zone Test *Streptococcus mutans*

A total of 20 ml of MHA media was dispensed into sterile Petri dishes and was allowed to settle for approximately 30 minutes. Once the MHA had solidified, the *Streptococcus mutans* culture was swabbed onto the medium using the lawn streak method. The discs that had been immersed in different mouthwashes (F1, F2, and F3) and those from positive and negative controls were carefully placed on the surface of the MHA media. The Petri dishes were inverted and incubated at 37°C for 24 hours. After incubation, clearance zones around the discs were measured using a vernier caliper [23].

3. RESULTS AND DISCUSSIONS

3.1. Plant Determination Results

Plant determination confirmed that the samples were *Psidium guajava L.folium* (leaves of guava) and *Syzygium polyanthum* (Wight) Walp. (leaves of bay) from *Myrtaceae* family. This process was

authenticated in Certificate Number 344/K-ID/ANDA/VI/2026 for leaves of guava and Number 371/K-ID/ANDA/VII/2026 for leaves of bay.

3.2. Yield and Processing Result of Guava Leaf and Bay Leaf Simplicia

From the drying of the two raw materials, it was found that 2 kg of guava leaves gave 736 grams of dried leaves, indicating a yield of 36.8%. On the other hand, 2 kg of bay leaves gave 776 grams of dried leaves, giving a yield of 38.8%. After drying, both of the dried leaves exhibited a unique brown color.

3.3. Extraction Yields of Guava Leaf and Bay Leaf Simplicia Powder

Extraction of 250 g of guava leaf powder with 96% ethanol produced 42.5 g of thick extract, which is an equal to 17% yield. Some of the physical characteristics of the extract obtained were: greenish black color, very viscous, and possessing the strong smell of guava leaf powder. On the other hand, the extraction of 250 g of bay leaf powder gave a total of 27.5 g of thick extract, equaling to 11% yield. The bay leaf extract had similar physical properties of being thick, greenish-black, and having an aromatic smell. The difference in the extraction yields can be explained by the nature of the plant matrix.

3.4. Phytochemical Screening

Phytochemical testing is an important process of discovering the types of secondary metabolites found in plants that have biological activities. In this experiment, qualitative phytochemical testing was done on the ethanolic extract of *Psidium guajava L. folium* and *Syzygium polyanthum* (Wight) Walp. for the presence of alkaloids, flavonoids, tannins, and phenolics. The results of the phytochemical testing of the two extracts are shown in Table 2.

Table 2. Screening results of phytochemicals present in guava leaves and bay leaves.

Metabolite group	Reagent	Guava leaves	Bay leaves	Results
Flavonoids	Mg + concentrated HCl	Positive, demonstrated a yellow color shift	Demonstrated a yellow color shift	(+)
Tannins and Phenolic	1% $FeCl_3$	Formation of a distinct greenish-black coloration	Formation of a distinct greenish-black coloration	(+)
Alkaloid	Mayer and dragendroff	Positive, giving orange and yellowish-white precipitates	Produced orange and yellowish-white precipitates	(+)

It can be observed from the above test results that both the guava and bay leaf extracts provided positive results for all types of the screened secondary metabolites. In the case of the flavonoid test conducted with Mg powder and concentrated HCl, an obvious shift to orange red was seen in both extracts. In the tannin / phenolic test with 1% $FeCl_3$ solution, there was greenish black coloration. On the other hand, in the case of the alkaloid test with Mayer's and Dragendorff's reagent, orange and yellowish white precipitate was observed. This shows that 96% ethanol has succeeded in extracting various types of secondary metabolites. The presence of all these metabolites together is thought to be responsible for the antibacterial activity of the extracts against *Streptococcus mutans*. But since this test was only qualitative, these results only show the presence of the different metabolites.

3.4.1. Alkaloid Analysis

Presence of the alkaloid compounds in guava and bay leaves was confirmed by the use of Mayer's and Dragendorff's reagents. Orange and yellowish white precipitate was obtained in both samples confirming the presence of alkaloids. The precipitated compounds arise from reactions that take place between the nitrogen- containing alkaloids and the metal precipitating reagents to form complexes or salts which are not soluble. It is known that alkaloids have different biological effects, especially their antibacterial effect. The reason behind this property is that the alkaloids affect the metabolic processes

in bacteria. Therefore, the presence of the alkaloids in the tested samples makes them have antibacterial activity against *Streptococcus mutans* [24,25].

3.4.2. Flavonoid Analysis

The presence of flavonoids in both extracts was confirmed using Mg powder and concentrated HCl, where the color changed to orange-yellow. It is the process where magnesium reduces the flavonoids through an acid catalyzed process. Flavonoids prevent the growth of bacteria by interaction with cell proteins, alteration of membrane permeability and enzyme inhibition. This shows that the flavonoids present are responsible for the antibacterial effects on *Streptococcus mutans* [25,26].

3.4.3. Tannin/Phenolic Analysis

For the presence of tannins and phenolics in the extract, the test was carried out using 1% $FeCl_3$, producing a greenish-black color. This reaction is due to the chelating effect of Fe^{3+} ions by the phenolic compound to produce highly colored complexes. These chemicals have antimicrobial properties because of their ability to cross-link the cell proteins and deactivate the bacterials enzymes [27,28].

3.5. Organoleptic Evaluation

Tests of organoleptic properties were carried out to determine the physical characteristics of the mouthwash formula in terms of color, taste, and smell. There was also variation in the concentration of the active herbal extract in the three formulations (F1, F2 and F3), all other factors remaining the same particularly the concentration of the inactive ingredients. In formulation F1, the concentration of the total herbal extracts was 8%, comprising of 4% of guava leaf extract and 4% of bay leaf extract. In F2, the concentration of total extracts was 9% while using 4.5% of each extract, whereas in F3, the concentration was the highest that is 10%, consisting of 5% of each extract. The ratio of the two extracts was consistently maintained in all the formulations to make sure of the consistent use of herbals in terms of concentration. The other ingredients, such as peppermint oil, sorbitol, sodium benzoate and glycerin, were also consistent to see the effect of changing concentrations of herbals on the physical and antibacterial properties of the mouthwash. The tests for the formulas of mouthwash F0, F1, F2, and F3 were conducted within 4 weeks of storage. The results of the organoleptic test are presented in Table 3.

Table 3. Organoleptic evaluation.

Formula	Parameter	Week 0	Week 1	Week 2	Week 3	Week 4
F0	Color	Clear	Clear	Clear	Clear	Clear
	Taste	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint
	Aroma	Fresh mint	Fresh mint	Fresh mint	Fresh mint	Fresh mint
F1	Color	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Reddish brown
	Taste	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint	Slightly sweet, mint
	Aroma	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint
F2	Color	Brownish red	Brownish red	Brownish red	Brownish red	Brownish red
	Taste	Astringent, mint	Astringent, mint	Astringent, mint	Astringent, mint	Astringent, mint
	Aroma	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint
F3	Color	Darker reddish brwon	Darker reddish brwon	Darker reddish brwon	Darker reddish brwon	Darker reddish brwon
	Taste	Stronger astringent, mint	Stronger astringent, mint	Stronger astringent, mint	Stronger astringent, mint	Stronger astringent, mint
	Aroma	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint	Distinct extract, mint

As seen from the data presented in Table 3, no changes in color, odor, and flavor were noted for the formulas F0, F1, and F2 during the period of 28 days. On the other hand, the formula F3 showed a slight decrease in flavor intensity after the first week.The rinsing agent had diverse organoleptic properties due to the presence of different percentage of the extract and it had a strong smell and taste. As the quantity of the extract increased, it had a stronger taste and smell of the herb. The obtained results are consistent with the research conducted by Kartikasari and Masykuroh (2024) on the mouthwash kesum, according to which different quantities of the extract resulted in varied organoleptic properties like the distinct smell of herb and menthol and the taste of layers [29].

3.6. pH Determination

The pH of the prepared mouthwashes was checked through a well-defined pH profiling test. The pH stability tests were performed at different intervals during the process of stability testing. The pH stability results of the guava and bay leaf extract mouthwashes are described in Table 4.

Table 4. pH measurement.

Formula	pH formula				
	Week 0	Week 1	Week 2	Week 3	Week 4
F0	6.6	5.8	6.5	6.7	6.8
F1	5.4	4.8	4.9	5.0	5.1
F2	5.4	4.8	4.6	5.1	5.2
F3	5.5	4.8	4.9	5.2	5.2

From the pH readings in Table 4, it can be seen that there were changes in pH among the tested formulations during four weeks of storage period. There was an increment in pH reading of the negative control (F0) with pH reading from 6.6 at week 0 to 6.8 at week 4. On the other hand, there was a significant drop in pH of formulations which used the mixture of the two extracts (F1, F2, and F3), especially during the second week of storage when the lowest reading of pH occurred, which is 4.6 in formulation of F2. pH values were increased subsequently and were stabilized at around 5.1-5.2 until the end of the experiment. According to the Indonesian National Standard (SNI 12-3524-1995), the pH range for mouthwash is 4.5-10.5. The pH change during storage could be caused by the process of hydrolysis or chemical reaction which occurs between phenol and flavonoid compounds found in the extract. Although there was a change in pH, it still was in the required range according to the standard [30].

3.7. Clarity Test

Evaluation for clarity using visual analysis included analysis of the formulations present in clean and clear containers in well-lit conditions against black and white backgrounds. A formulation would be considered clear if it did not contain any foreign particles, since such foreign particles could compromise both clarity and quality structurally. Longitudinal results of clarity for formulations F_0 to F_3 in the stability test are shown in Table 5.

Table 5. Clarity test.

Formula	Clarity test				
	Week 0	Week 1	Week 2	Week 3	Week 4
F0	Clear	Clear	Clear	Clear	Clear
F1	Clear	Clear	Clear	Clear	Clear
F2	Clear	Clear	Clear	Clear	Clear
F3	Clear	Clear	Clear	Clear	Clear

As depicted from Table 5 below, formulas F_0 to F_3 remained completely transparent from weeks 0 through 4. No foreign particle or insoluble precipitate was found in any of the formulas during the whole evaluation period. This is in agreement with the observations made by Litaay et al. (2023),

who noted that properly formulated mouthwashes contain no light and dark particles in clarity assays [31].

3.8. Viscosity Measurement

The rheological behavior of the developed mouthwashes was measured in order to assess the resistance of the mouthwashes against flow. This was done using an Ostwald viscometer that is well suited for such fluids. The viscosities of the formulations F0 to F3 over time are shown in Table 6.

Table 6. Measurement of viscosity.

Formula	Viscosity test (cP)				
	Week 0	Week 1	Week 2	Week 3	Week 4
F0	1.01	1.19	1.12	1.83	1.47
F1	1.03	1.57	0.98	1.01	1.35
F2	1.25	1.66	1.16	1.04	1.67
F3	1.19	1.69	1.40	1.09	1.54

Dynamic changes in viscosity profiles were noted in all formulations during the period of storage, as shown in Table 6. The viscosity profile of formula F0 was seen to be 1.01 cP at week 0, increased to 1.19 cP at week 1, reduced to From 1.12 cP at week 2, it was elevated to 1.83 cP at week 3 and subsequently fell to 1.47 cP at week 4. As regards formula F1, the chronologically recorded viscosity levels were 1.03, 1.57, 0.98, 1.01, and 1.35 cP, respectively. In contrast, the formula F2 produced a viscosity level of 1.25 cP (week 0) and 1.67 cP (week 4) with the intermediate levels of 1.66, 1.16, and 1.04 cP. There were differences in the viscosity during the period of storage and no linear pattern was detected among the formulations. These variations might have been due to the complexity of herbal preparation consisting of many plants extracts according to Anjalikha et al. (2026). The mouthwash formulations should be prepared with low viscosity in order to facilitate its flow and comfortable rinsing inside the mouth cavity [32]. According to Fadhillah (2026), the appropriate viscosity range of liquid mouthwash formulation is about 1.0-5.0 cP which is close to the viscosity of water. Low viscosity ensures easy flow of mouthwash to reach interdental spaces while rinsing [30]. In this research, the viscosity of F1, F2 and F3 formulations varied between 0.98 to 1.69 cP throughout four weeks storage period. This means that the viscosity of the formulations was relatively low. Therefore, these formulations can be used as mouthwash but they still need further evaluations regarding their adherence to certain criteria concerning the viscosity standard since some values were lower than 1.0 cP.

3.9. Homogenity Test

The visual assessment of clarity involved inspecting the formulations in clean and clear containers in well-lit conditions using a black or white background. If the formulation was clear, it should not contain any suspended particles as their presence could have an adverse effect on its clarity as well as structure. The results of the longitudinal clarity test of formulas F0 to F3 in the stability test are presented in Table 7.

Table 7. Homogenity test.

Formula	Homogenity test				
	Week 0	Week 1	Week 2	Week 3	Week 4
F0	No floating particles	No floating particles	No floating particles	No floating particles	No floating particles
F1	No floating particles	No floating particles	No floating particles	No floating particles	No floating particles
F2	No floating particles	No floating particles	No floating particles	No floating particles	No floating particles
F3	No floating particles	No floating particles	No floating particles	No floating particles	No floating particles

From weeks 0 to 4, the lack of any suspended particles in mouthwash samples F0 through F3 was evident using longitudinal screening (Table 7). Such a situation means that the samples had physical stability since no phase separation occurred in the process of storing them. The stability is in agreement with the results presented by Bila et al. (2024), which says that the mouthwash formulations are homogeneous in such a way that they have an even distribution of all the ingredients without any precipitate [33].

3.10. Hedonic Test

The hedonic analysis was done in order to find out the level of acceptability of the sensory properties of the mouthwashes that were formulated, taking into consideration the properties of their color, smell, and taste (see Table 8). The analysis was done by analyzing the preferences of the individual towards the different mouthwashes that were tested. The test subjects that were used in the analysis were 25 people whose ages were between 17 and 55 years, 5 males and 20 females.

Table 8. Hedonis test [33].

Formula	Parameter	Level of preference				
		STS	TS	N	S	SS
		(1)	(2)	(3)	(4)	(5)
F0	Aroma	-	-	3	19	3
	Color	-	-	7	12	6
	Flavour	-	-	9	11	5
F1	Aroma	-	-	2	20	3
	Color	-	-	8	11	6
	Flavour	-	-	10	12	3
F2	Aroma	-	-	-	16	9
	Color	-	-	1	17	7
	Flavour	-	2	8	14	1
F3	Aroma	-	-	1	4	20
	Color	-	-	1	13	11
	Flavour	1	5	7	9	3

There were clear differences in the responses from participants concerning the evaluated formulas for the mouthwashes through the process of hedonic assessment. F3 mouthwash managed to get a considerable number of positive responses especially when it came to the aspect of smell and taste. F0 mouthwash on the other hand had an acceptable tolerance to all the properties evaluated. In relation to F1 mouthwash there was a clear cluster of responses that fell under the category of 'like' and 'strongly like'. In contrast to this, F2 mouthwash evaluation had a variety of responses concerning smell, color and taste of the product [34].

3.11. Antibacterial Activity Analysis

The antibacterial effectiveness of the combinations of the ethanolic extract mouthwashes of the leaves of *Psidium guajava L.* (guava) and *Syzygium polyanthum* (Wight) Walp. (bay leaves) against the *Streptococcus mutans* are listed in Figure 1.

As shown in Figure 1, all experimental groups were performed in triplicate for reproducibility purposes. The positive control group had a mean ZOI diameter of 11.00 ± 0.87 mm while the negative control showed no antibacterial action at all, with a mean of 0.00 ± 0.00 mm. As for the extracts, formula F1 had a mean inhibition diameter of 4.33 ± 3.79 mm, while formula F2 increased the inhibition up to 7.67 ± 0.58 mm and formula F3 had a maximum mean inhibition diameter of 8.67 ± 0.76 mm. Finally, formula F3 was the most efficient against bacteria among all formulas tested, while F1 had the lowest antibacterial effect. The reduced antibacterial properties of the formulations F1, F2, and F3 as compared to the commercial positive control might be attributed to the properties of plant crude extracts. The ethanolic extracts of *Psidium guajava L.* and *Syzygium polyanthum* include a wide variety of secondary metabolites besides other components of the plant that might not necessarily play an antibacterial role.

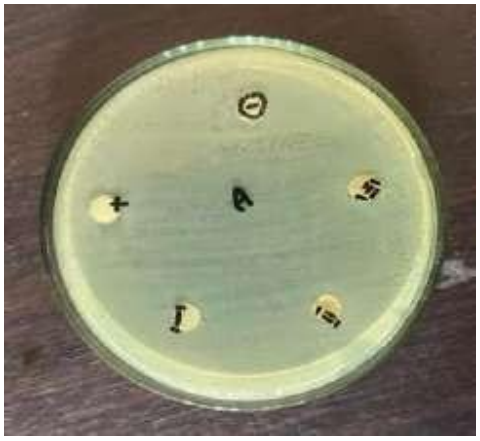
Thus, the antibacterial compounds such as flavonoids and tannins that might affect the bacteria by damaging their cell walls and interfering with their activities occur alongside other compounds in the extract. On the other hand, the commercial positive control includes antibacterial agents with known properties. Hence, the differences in composition, solubility, and diffusion through the Mueller-Hinton agar can explain the larger zone of inhibition of the positive control as compared to the formulations after 24 hours of incubation (see Figure 2).



Replicate 1



Replicate 2



Replicate 3

Figure 1. Antibacterial activity assay results.

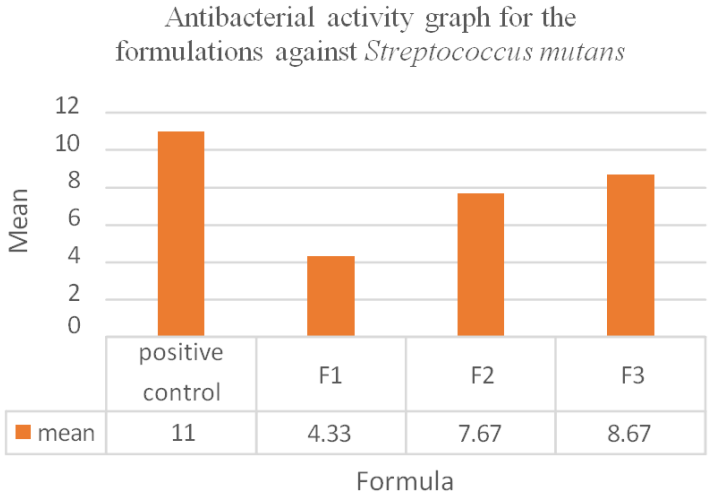


Figure 2. Inhibition zone of antibacterial activity of each formulation.

3.12. Statistic Processing

Antibacterial activities were statistically analyzed using One-Way ANOVA in terms of the changes in inhibition zones of *Streptococcus mutans* due to treatments.

3.12.1. Homogeneity Test

The homogeneity test was performed to establish whether there is homogeneity of variance in the inhibition zone for all the groups under study. The Levene test on variance homogeneity was conducted on DIZ values. The results of the time series analysis on homogeneity of variance are provided in Table 9.

Table 9. The output from Levene's test for assessing variance equality.

Method	Statistic	df1	df2	Sig.
Based on Mean	9.387	4	10	0.002
Based on Median	1.082	4	10	0.416
Based on Median and with adjusted df	1.082	4	2.532	0.509
Based on trimmed mean	7.976	4	10	0.004

As can be seen from Table 9, Levene's test was conducted to check the homogeneity of variance across the experimental groups. The Levene test based on the mean produced a significance value of 0.002 ($p < 0.05$), implying that there was no homogeneity in terms of variance in the diameter of the inhibition zone. However, the Levene test based on the median produced significance values of 0.107, 0.416, and 0.509 ($p > 0.05$), respectively. Since there were heterogeneous variances according to the Levene test based on the mean, it was necessary to consider the presence of heterogeneous variances in the study. Thus, the Games-Howell post-hoc test was used to conduct further analysis of pair differences.

3.12.2. Games-Howell Post-Hoc Test

Games-Howell test was used to detect localized differences in the diameters of the inhibition zones between the different groups in the experiments after detecting the statistical differences using One-Way ANOVA. The selected method acted as a post hoc analysis based on the initial heteroscedasticity check by Levene's test. Post hoc results of the Games-Howell test are presented in Table 10.

As shown in Table 10, the application of the One-Way ANOVA test resulted in F-value equal to 17.135 at $p < 0.001$. It means that there was a statistically significant difference in antibacterial activity among five experimental groups in relation to *Streptococcus mutans*. In particular, the highest mean value of inhibition zone diameter (IZD) was observed in the positive control (11.00 ± 0.87 mm), while the negative control had no inhibitory effects (0.00 ± 0.00 mm). As regards the herbal formulations, their mean values of IZD increased gradually, i.e. 4.33 ± 3.79 mm for F1, 7.67 ± 0.58 mm for F2, and 8.67 ± 0.76 mm for F3.

Despite the presence of the positive tendency in the increase of mean values of IZD from F1 to F3, according to the results of the Games-Howell post-hoc test (see Table 11), there were no statistically significant differences between F1 and F2 ($p = 0.638$), F1 and F3 ($p = 0.484$), and F2 and F3 ($p = 0.479$), as all p-values exceeded 0.05. Hence, the current results do not prove a statistically significant dose-dependent effect among three herbal formulations.

Moreover, the total antibacterial properties of F1, F2, and F3 were still less compared to those of the commercial positive control whose mean inhibition zone was recorded to be 11.00 mm. This may be due to the complexity of crude ethanol extract whose active secondary metabolites include flavonoids, tannins, and alkaloids among others. On the other hand, the commercial positive control is made up of standardized antibacterial components which may be different in solubility and diffusion into the agar medium. However, the significant differences observed between the negative control and F2 ($p = 0.006$) and F3 ($p = 0.008$) show that there was antibacterial property of these formulations against *Streptococcus mutans*. The results obtained suggest that the mixture of guava leaves and bay leaves may have the possibility of being used as a mouthwash formulation.

Table 10. Post hoc results of the games-Howell test for antibacterial inhibition zones.

Group comparison	Mean difference (I-J)	Sig.
Positive control – Negatice control	11.000	0.006
Positive control – F1	6.667	0.256
Positive control – F2	3.333	0.032
Positive control – F3	2.333	0.107
Negative control – F1	-4.333	0.481
Negative control – F2	-7.667	0.006
Negative control – F3	-8.667	0.008
F1 – F2	-3.333	0.638
F1 – F3	-4.333	0.484
F2 – F3	-1.000	0.479

4. CONCLUSION

The phytochemical screening conducted in this study identified the presence of key secondary metabolites like flavonoids, tannins/phenolics, and alkaloids in the ethanol extracts of the leaves of *Psidium guajava L. (guava)* and *Syzygium polyanthum (Wight) Walp. (bay).* The two plant sources have been effectively incorporated as active constituents of polyherbal mouthwashes at different concentrations (F1, F2, and F3). Stability tests showed that the F0 to F3 mouthwashes maintained their physical stability throughout the 4-week storage period. No significant degradation was recorded in the evaluation of organoleptic, homogeneity, clarity, and viscosity characteristics. While pH variations were experienced in the early stages of the storage period, stability was eventually achieved after the first week. F3 received maximum consumer acceptability in color and aroma while F0 was favored on the basis of taste.

ZOI for Antibacterial Screening on *Streptococcus mutans* was done to prove that there were clear ZOIs for all the extract-containing formulas. From the results, the ZOI diameters were 4.33 ± 3.79 mm for F1, 7.67 ± 0.58 mm for F2 and 8.67 ± 0.76 mm for F3. For the positive control, the maximum diameter was 11.00 ± 0.87 mm, whereas the negative control showed no activity whatsoever. Results indicated a positive descriptive correlation of antibacterial activity against Streptococcus mutans as the concentration of the herbal mixture was increased, with F3 demonstrating the highest inhibition zone. Nevertheless, no significant differences in the antibacterial activity of the three herbal formulations (F1, F2, and F3) were observed.

Levene's criteria parametric pre-test revealed that the data were heteroscedastic with a significance value of 0.002 ($p < 0.05$). However, globally, the One-Way ANOVA revealed very significant differences in growth inhibition within the samples ($F = 17.135$; $p < 0.001$). The local post-hoc Games-Howell tests revealed the significant differences in particular samples to be those of the positive control against the negative control and F2, the negative control against F2 and F3. On the other hand, the local significant variations of F1-F3 were statistically insignificant. Therefore, the combination extract of guava and bay leaves has immense potential as a natural pharmaceutical ingredient in the preparation of mouthwashes to reduce the infection of *Streptococcus mutans* since F3 has proven to be the most active (8.67 ± 0.76mm) although it is locally different from the other herbal extracts.

AUTHOR CONTRIBUTIONS

Anggun Rizca Gustiny: Investigation, sample preparation, mouthwash formulation, experimental testing, data curation, formal analysis, visualization, and writing—original draft. Azuxetullatif Azuxetullatif: Conceptualization, methodology, research planning, supervision, validation, interpretation of findings, and writing—review and editing. Reren Oktivana: Investigation, preparation of plant extracts, physical quality testing, antibacterial assays, and verification of experimental records. M Saka Abeiasa: Data processing, statistical analysis, visualization, interpretation of results, and manuscript review. Eldya Mossfika: Methodological evaluation, validation, interpretation of findings, and writing—review and editing. All authors contributed to manuscript revision, approved the final version, and agreed to be accountable for the accuracy and integrity of the work.

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DECLARATION OF AI USE

The authors declare that no AI tools were used in preparing this manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting this study are included in the article. No separate datasets are available.

ETHICAL APPROVAL

Ethical approval was unnecessary, as no humans or animals were involved.

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