Guava and Bilimbi Leaves Extract as an Active Ingredient for Antibacterial Liquid Hand Soap

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Abstract- This research study aimed to evaluate the antibacterial activity of Averrhoa bilimbi L. and Psidium guajava extracts individually and in combination for formulating antibacterial liquid soap. Three sets of liquid soap formulations were tested, including 50% bilimbi extract and 50% guava extract, 100% bilimbi extract, and 100% guava extract. The assessment focused on pH values, foam retention capacity, color, and odor of the formulated liquid soaps. All formulations met the required pH level for liquid soap and demonstrated superior foam retention capacity compared to commercially available alternatives. The liquid soap prototypes exhibited an appealing planter green color and a pleasant odor characterized by a fresh and musky fragrance, further enriched by the addition of lavender essential oil. Significantly, the liquid soap containing the combined guava and bilimbi extract displayed remarkable antibacterial properties against both E. coli and S. aureus, surpassing the effectiveness of commercial liquid soaps. While bilimbi extract alone did not exhibit antibacterial activity against the tested bacteria, its combination with guava extract exhibited potential improvements in efficacy against E.coli and S.aureus The statistical analysis using the Kruskal-Wallis test revealed statistically significant differences between the tested concentrations. For E. coli, the p-value was 0.0310, and for S. aureus, the p-value was 0.0200, leading to the rejection of the null hypothesis for both bacteria.

Indexed Terms- Antibacterial activity, Averrhoa bilimbi, disk diffusion, extract, foam retention, pH, Psidium guajava, zone of inhibition

I. INTRODUCTION

Skin is the largest portal of entry for several microorganisms. A hair follicle, sweat gland duct, puncture, or cut can provide infection access. Up until the turn of the century, biomedical researchers disputed the existence of sensitive skin. Despite the use of ambiguous vocabulary to characterize sensitive skin and the acknowledgment of its diversity, few studies have focused on objective methods of identifying kinds.In the past, iodine was widely used as an antiseptic for the purpose of disinfecting the skin, as stated by Jing et al. (2020). It can enter microbial cells and form complexes with amino acids or unsaturated fatty acids, which hinder the synthesis of cellular components. Several of the qualities that make for effective disinfection or antiseptics are present in alcohols, as stated by Morley (2021). Their bactericidal, bacteriostatic, virucidal, and fungicidal efficacies are all top-notch, and they even have some virucidal activity (particularly against enveloped viruses). Additionally, as stated by Burton et al. (2011), antiseptic soaps contain bactericidal active substances that remove both harmful and helpful germs.

Psidium guajava is a fast-growing small tree that normally reaches heights of 1 to 6 m but can occasionally reach 10 meters. The leaves are positioned in pairs and opposite from one another along the stems. The bases of the leaf blades are rounded, while the tips are either round or pointy. They have the shape of an elliptic and are 7 to 15 cm in length by 3 to 7 cm broad. (Prabhudesai et al., 2019)

According to Wirahmi (2021), a combination of 75% guava leaf infusion +25% betle leaf with an average inhibitory diameter of 10.2 mm, is the best formula natural disinfectant solution of combined guava leaf infusion (Psidium guajava L.) and betle leaf infusion (Piper Betle L.)

Another study of Delorino et al. (2021) reported that guava leaf extract demonstrated much less persistent edema and elevation of wound compared to povidone-iodine and ordinary normal saline solution. In addition, guava leaf extract outperforms povidone iodine, whereas Plain Normal Saline Solution (PNSS) fares the worst.

Averrhoa bilimbi L., known locally by the Malay name 'Belimbing buluh,' is a member of the Oxalidaceae family. Particularly in Malaysia, A. bilimbi fruits were renowned for their use as pickles or flavor enhancers in traditional Malays meals. A. bilimbi leaves are used medicinally to cure venereal disease.

In addition, the infusion or decoction of the leaves is used as a cure for coughs and used as a tonic after childbirth and alleviates rectal inflammation. In addition, fruits of A. bilimbi are used to cure acne, diabetes, and hypertension. A. bilimbi fruits, leaves, and flowers are additionally used to remedy coughs. Meanwhile, in the Philippines, the leaves are used as a paste to cure rheumatism-related itching and swelling, while in Java, Indonesia, the fruits are used to treat internal hemorrhoids, fever, coughs, rectal bleeding, and inflammation.

In the study of Sabaani et al. (2019), many herbal plants possess bioactive components that are highly effective in killing and preventing the growth of microorganisms. Additionally, these herbal plants are considered as less toxic and free from side effects rather than using synthetic compounds present in commercial liquid soaps.

This study aims to formulate an antibacterial liquid hand soap using combined extract of Averrhoa bilimbi and Psidium guajava plants to investigate its antibacterial activity. Furthermore, this study will be evaluating the physicochemical characteristics of each antibacterial liquid hand soap created.

II. METHODOLOGY

Research Design

The researchers used the quantitative experimental research design to conduct the study since it aims to determine the antibacterial activity of Psidium guajava and Averrhoa bilimbi leaf extract as an antibacterial liquid hand soap. By utilizing this research design, the study aims to provide empirical evidence regarding the effectiveness of these plant extracts as active ingredients in combating bacterial pathogens commonly found on hands. This choice of methodology allows for the precise manipulation of variables under controlled laboratory conditions, ensuring the reliability of the results (Sabaani et al., 2019). Moreover, the quantitative nature of the design enables the researchers to quantitatively measure the extent of antibacterial activity exhibited by the plant extracts, thus, facilitating objective comparisons.

Locale of the study

The study was conducted at World Citi Colleges in Quezon City, Philippines. To initiate the study, leaves of Averrhoa bilimbi and Psidium guajava were collected from Cogeo, Antipolo City. These leaves were then validated at the Jose Vera Santos Memorial Herbarium, located at the University of the Philippines Institute of Biology in Diliman, Quezon City.

To create the liquid soap base, the researchers obtained the required ingredients from Alysons' Chemical Enterprises, Inc., a reputable chemical wholesaler located on Araneta Avenue in Quezon City. To complete the purchase, the researchers obtained a request letter from their research adviser. This letter permitted them to acquire the essential materials necessary for producing the liquid soap base.

Figure 1. Tree (A) *P. guajava* (B) *A. bilimbi*

Samples of the Study

Commercial Soap

The researchers utilized online store-bought, commercial antibacterial liquid hand soap to determine which of the two liquids is effective - the utilized commercial soap brand or the combined guava and bilimbi extract. The researchers used a liquid soap brand named A La Maison, Lavender Aloe Liquid Soap which contains Filtered Water, Coconut Oil, Olive Oil, Vegetable Glycerin, Argan Oil, Tocopherol (Vitamin E), Aloe Leaf Extract, Fragrance Blend with Plant Extracts and/or Essential Oils. The researchers have selected this product because it has natural ingredients and has the same composition as the combined extract of guava and bilimbi liquid hand soap.

The commercialized sample served as the positive control to test the validity of accessing the antibacterial activity of combined guava and bilimbi extracts as an active ingredient in liquid hand soap. In this part, the researchers will access the combined guava and bilimbi extract liquid soap with the highest antibacterial activity and a determined concentration.

Figure 2. Commercially Available Antibacterial Liquid Hand Soap - A La Maison Lavender Aloe

Cultured Bacteria

The researchers utilized specific bacteria, namely Staphylococcus aureus; gram-positive bacteria, and Escherichia coli; gram-negative bacteria. The bacteria mentioned were obtained from the Philippine National Collection of Microorganisms (PNCM), located at the University of the Philippines - Los Baños.

Additionally, the obtained bacteria were incubated for three (3) days and were sub-cultured in Nutrient Agar for every three (3) days to maintain the culture and its active form, this is to prolong the life of the said bacteria. Subcultures were incubated and refrigerated until use.

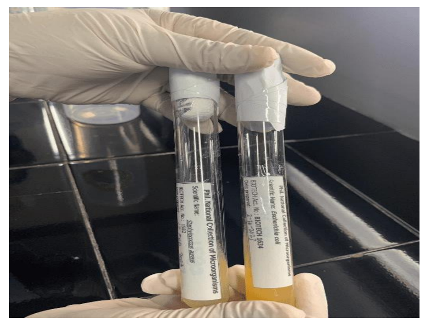

Figure 3. Culture of S. aureus and E. coli

Preparation of Culture Media

The Nutrient Agar (NA) was utilized to subculture and grow the Escherichia coli and Staphylococcus aureus. By the Millipore package, the composition of the Nutrient Agar (NA) is as follows: meat extract, yeast extract, and peptone. It was prepared by suspending 28 grams in one liter of distilled water. It was boiled to dissolve completely and sterilized by autoclaving for 15 minutes at 121 °C. The solution was poured into the petri dish for 10mL. The agar was then cooled and solidified before covering it with bond paper and set aside in the laboratory refrigerator.

Figure 4. (A) Nutrient agar in tubes (B) Petri Plates

Preparation of Leaf Extracts

For the materials, the researchers prepared three sets with different concentrations. These are the following: the first set consists of 50% of bilimbi extract and 50% of guava extract; the second set consists of 100% of bilimbi extract; and the third set consists of 100% of guava extract.

Table 1. Soap Composition per mL

Soap Prototype	Soap compositions		
	Soap base	Bilimbi extract	Guava extract
SET PG (Pure Guava)	40 mL		4mL
SET PB (Pure Bilimbi)	40 mL	4 mL	
SET GB (Combined Guava and Bilimbi)	40 mL	2 mL	2 mL

Data Gathering and Procedures

Preparation of Leaves for Pulverization

The fresh guava and bilimbi leaves were cleaned by washing them three times with tap water to remove any traces of sand or dust. It was then air-dried for 24 hours. Right after that, it was oven-dried at 60°C for 15 minutes by utilizing the school-provided hot-air oven. After drying, the leaves were ground into a fine powder using a blender, mortar & pestle, and strainer.

Figure 5. Washing of (A) P. guajava (B) A. bilimbi leaves

Figure 6. Air Drying of (A) A. bilimbi (B) P. guajava Leaves for 24 hours

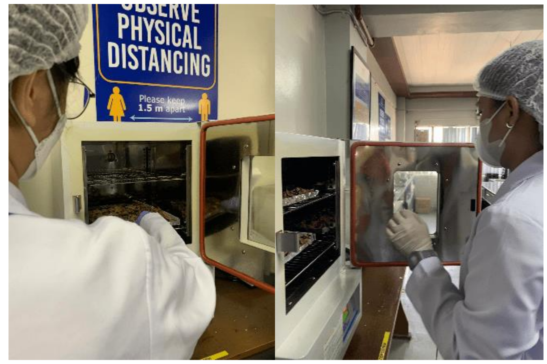

Figure 7. Oven Drying of P. guajava and A. bilimbi Leaves

Figure 8. Pulverization of (A) P. guajava (B) A. bilimbi leaves

Preparation of Leaf Extract

The extract of powdered leaves was prepared by soaking the 290 grams of guava leaves in 2 L of 95% ethanol for 48 hours with occasional stirring. The mixture was then filtered and the filtrate obtained was concentrated using a water bath at 60°C to obtain semi-solid extract. The concentrated crude extract was then collected and stored in an amber bottle and labeled.

For bilimbi leaves, 325 grams of dried bilimbi leaves were soaked in 2.5 L of 95% ethyl alcohol for 48 hours with occasional stirring. The mixture was then filtered and the filtrate obtained was concentrated using a water bath at 60°C to obtain semi-solid extract. The concentrated crude extract was then collected in an amber bottle and labeled. Both extracts were kept in the refrigerator at a temperature of 2-4 °C until used to make liquid soap.

Figure 9. Crude Extract of (A) Psidium guajava (B) Averrhoa Bilimbi

Preparation of Liquid Soap Base

In the preparation of the soap base, 700 mL of coconut oil and 280 mL of glycerin were heated in a separate beaker at 72 °C for 15 minutes. After that, the temperature was lowered to 60 °C and the mixture was stirred continuously for 25 to 30 minutes. For the preparation of lye water, 175 grams of KOH were dissolved in 980 mL distilled water and stirred until the mixture became homogeneous. Once the lye water was completely mixed and became clear, the lye water solution was slowly added to the heated coconut oil. The coconut oil and lye-water solution were poured into the heated glycerin.

The solution of lye water with coconut oil and glycerin was heated again at 70 °C and stirred continuously for three to four hours. The mixture will solidify and will serve as the soap paste. Let the hard soap paste sit for 24 hours or 1 day before adding distilled water. About 1000 mL of distilled water was then added to the soap paste and was then stirred until the soap paste dissolved. 10 grams of borax was added to neutralize and preserve the soap base solution. A lavender-scented oil was then added to the separated sets of soap prototypes with a measurement of 0.5 mL per prototype.

Figure 10. Making of the Soap Base

Preparation of Mueller Hinton Agar (MHA)

By the Millipore package, the composition of the Mueller Hinton Agar (MHA) is as follows: beef infusion solids, starch, casein hydrolysate, and agar. It was prepared by suspending 38 grams in one liter of distilled water. It was heated to dissolve completely and then autoclaved for 15 minutes at 121 °C. Using a serological pipette, at least 10 mL of agar was poured into Petri dishes. The agar was then cooled and solidified before covering it with bond paper and set aside in the laboratory refrigerator.

Figure 11. MHA in Petri plates

Antibacterial Susceptibility Test

The disc-diffusion method was used to evaluate the antibacterial properties of the prepared antibacterial liquid soap. Each filter paper disc was soaked in different concentrations of liquid soap (50% bilimbi leaf extract 50% guava leaf extract, 100% bilimbi leaf extract, and 100% guava leaf extract) for 24 hours. Additionally, 10 filter paper discs were also soaked in the negative control containing the soap base without any extracts and the positive control containing the commercial soap, A La Maison Soap. This is to demonstrate the validity of the results. Three (3) paper discs were then placed in MHA agar plates that contained swabbed E. coli and S. aureus. After 24 hours, zones of inhibition are then measured in millimeters using a Vernier caliper.

Figure 12. Different Concentrations of Liquid Soap with Soaked Filter Paper Disc

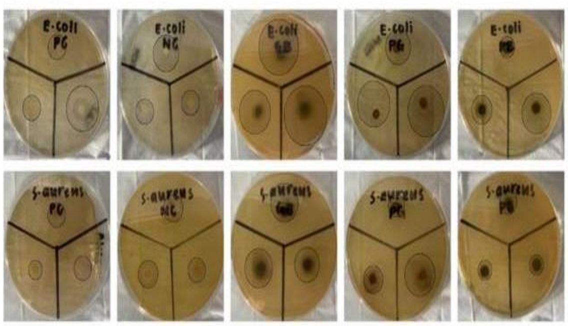

Figure 13. MHA agar with paper discs after 24 hours of incubation

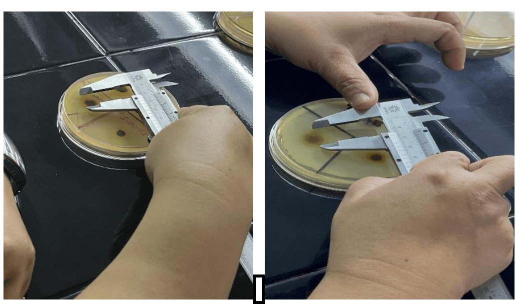

Figure 14. Measuring the zone of inhibition

Foam Retention

In order to assess the foam retention of the different soap prototypes, the researchers placed 100mL of each (Pure Guava, Pure Bilimbi, Combined Guava and Bilimbi, Negative Control: Plain Soap, Positive Control: A La Maison) into a beaker. They then used a one-speed electric mixer to stir each soap mixture for 5 seconds and create foam, which was timed to determine the length of time it took for the foam to disappear.

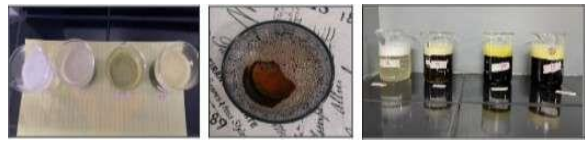

Figure 15. Foam Retention Test of the Prototypes

Statistical Analysis

Kruskal-Wallis Testing (Likert) was performed by using the Statistical Package for the Social Sciences (SPSS). This is to determine the significant difference of more than two groups. This test was chosen because the collected data did not follow a normal distribution. A confidence level of 90% was set for the results, meaning that p-values less than 0.05 were considered to indicate significance. The Kruskal-Wallis test was applied to the triplicate measurements of the zone of inhibition for each concentration of liquid soap to identify the concentration that exhibits the greatest and least inhibitory effect.

III. RESULTS & DISCUSSION

Phytochemical Compositions of the Leaf Extracts

The Qualitative Phytochemical Analysis was carried out to identify the alkaloids, tannins, saponins, flavonoids, glycosides, sterols, and triterpenes was carried out using the method of Botanical Taxonomy and Discovery (BTD) Manual. About 10 mL each of the crude extracts of P. guajava and A. bilimbi was brought to the DOST - Standard and Testing Division (STD). The tables presented below will support the study in terms of the antibacterial properties of each plant extracts.

Table 2. Plant constituents of Averrhoa bilimbi and Psidium guajava

Test parameters	Result		Test method
	A. bilimbi	*P. guajava*	
Sterols	+	+	Liebermann-Burchard Test
Triterpenes	+	+	
Flavonoids	+	+	Shinoda Test
Alkaloids	+	+	Mayer's Test
Saponins	+	+	Froth's Test
Glycosides	+	+	Fehling's Test
Tannins	+	+	Ferric chloride Test

The qualitative analysis of leaf extracts from Averrhoa bilimbi and Psidium guajava indicates the presence of several plant components such as sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins. These compounds are responsible for the antibacterial properties exhibited by both plants.

In Averrhoa bilimbi, the sterols and triterpenes were identified using the Liebermann-Burchard Test. Triterpenes act as antibacterial agents by inhibiting bacterial cell division and destroying the bacterial membrane. Flavonoids, tested using the Shinoda test, disrupt the cell wall and rupture bacterial cells, thereby exerting antibacterial effects. Alkaloids in Averrhoa bilimbi's leaf extracts destroy the bacterial cell wall and inhibit the development of bacterial DNA cells. Mayer's test was used to identify alkaloids in these extracts. Tannins, also present, inhibit bacterial growth and inactivate essential enzymes, leading to bacterial death.

Similarly, in Psidium guajava, the leaf extract also contains sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides, and tannins. The test parameters used for determining the phytochemical properties are the same as those for Averrhoa bilimbi. These compounds exhibit antibacterial properties by inhibiting bacterial growth and disrupting and lysing bacterial cell walls. Saponins, flavonoids, glycosides, and triterpenes in Psidium guajava are positively correlated with antibacterial activity, as noted by Kumar et al. (2021). The tannins present in the leaf extracts of Psidium guajava contribute to the inhibition of bacterial growth and deprive essential compounds required for bacterial growth, according to Das et al. (2020).

Physicochemical Parameters of Antibacterial Liquid Hand Soap

2.1 Color

The pure extract liquid soap derived from guava and bilimbi exhibits a vibrant, planter- like green color, which is also observed in the liquid soap created by combining both extracts. The intensity of the color is determined by the quantity of plain soap added, indicating that the shade can be adjusted by varying the amount of plain soap incorporated into the mixture.

Figure 16. Soap color reference: perceived versus actual color

2.2 Odor

The pure extract liquid soap made from guava and bilimbi possesses a fresh and musky fragrance, as does the combination of these extracts. To enhance the scent of the liquid soap, researchers introduced lavender essential oil, resulting in a more pleasing aroma.

2.3 Foam Retention

In accordance with the study of Ordoyo et al. (2019), foam retention capacity is one of the important characteristics of the cleansing efficacy of the liquid soap. It was also mentioned that the increased lathering ability of the soap could increase the bacterial reduction.

Table 3. Plant constituents of Averrhoa bilimbi and Psidium guajava

Trials	Experimental			Controlled	
	Pure Guava	Pure Bilimbi	Combined Guava and Bilimbi	Negative (Plain Soap)	Positive (A La Maison)
1st trial	164 mins	124 mins	165 mins	172 mins	70 mins
2nd trial	160 mins	136 mins	158 mins	165 mins	58 mins
3rd trial	171 mins	136 mins	157 mins	173 mins	73 mins
Average	165 mins	132 mins	160 mins	170 mins	67 mins

2.4. Test for pH

The researchers conducted three trials for each soap prototype and recorded the average pH levels. The pH level of pure guava was found to be 10.5 on average, while that of pure bilimbi was 10.6. The pH level of a mixture of 50% guava and 50% bilimbi was also found to be 10.6 on average. The negative control, which was plain liquid soap, had an average pH level of 10.7. The positive control, A La Maison commercial soap, had the lowest average pH level of 10.5.

Based on Table 4, the pure guava and positive control A La Maison commercial soap) had the lowest pH level of 10.5, while the negative control (plain soap) had the highest pH level of 10.7.

Table 4. pH Level of the Experimental and Controlled Prototypes

Trials	Experimental			Controlled	
	Pure Guava	Pure Bilimbi	Combined Guava and Bilimbi	Negative (Plain Soap)	Positive (A La Maison)
1st trial	10.5	10.5	10.5	10.7	10.2
2nd trial	10.5	10.6	10.6	10.7	10.6
3rd trial	10.5	10.6	10.6	10.6	10.7
Average	10.5	10.6	10.6	10.7	10.5

Antibacterial Testing

Liquid soap must contain ingredients that can kill bacteria but not damage the human body's tissues and skin. Therefore, an adequate amount of antibacterial agent can be added to the liquid soap base to prevent the growth of the microorganism.

The antibacterial testing of the liquid soap prototypes was carried out using agar disk diffusion. After 24 hours of incubation, the diameter of the zones of inhibition was measured in millimeters using a Vernier caliper. The results obtained are shown in Table 5.

Experimental				Controlled	
Bacteria	Pure Guava	Pure Bilimbi	Combined Guava and Bilimbi	Negative (Plain Soap)	Positive (A La Maison)
E. coli	26	19	38	15	12
	24	15	24	14	12
	17	9	22	14	22
Average	22.33	14.33	28	14.33	15.33
S. aureus	18	8	19	17	12
	12	9	12	12	9
	8	8	17	17	10
Average	12.67	8.33	16	15.33	10.33

Combined guava and bilimbi have the highest average zone of inhibition against E. coli, with an average of 28 mm, while pure bilimbi and negative control (plain soap) have the lowest with an average of 14.33 mm. Combined guava and bilimbi have the highest average zone of inhibition against S. aureus, with 16 mm, followed by the negative control with 15.3 mm, and pure guava with 12.67 mm. The positive control has an average zone of inhibition of 10.33 mm, while pure bilimbi has the lowest with an average of 8.33 mm.

Table 6. Test for Significant Difference of Experimental and Controlled Set-ups

Bacteria	Class	Mean	Statistic	P-value	Decision	Remarks
E. coli	Pure guava	**11.83**	10.5990	0.0310	Reject Ho	Significant
	Pure bilimbi	**6.17**				
	Combined Guava and Bilimbi	**12.83**				
	Negative Control	**6.17**				
	Positive Control	**3**				
S. aureus	Pure guava	**13.17**	11.6250	0.0200	Reject Ho	Significant
	Pure bilimbi	**2.17**				
	Combined Guava and Bilimbi	**10.17**				
	Negative Control	**9.33**				
	Positive Control	**5.17**				

The p-value for the comparison of the liquid soap prototypes against E. coli is 0.0310, which is lower than the given significance level of 0.05. As a result, the null hypothesis is rejected, meaning that there is a significant difference between the prototypes. The p-value for the comparison of the liquid soap prototypes against S. aureus is 0.0200, which is lower than the given significance level. This indicates that there are significant differences between the prototypes, leading to the rejection of the null hypothesis.

The results suggest that the differences observed in antibacterial activity between the prototypes are unlikely to have occurred by chance. The liquid soap prototypes exhibit measurable differences in their ability to inhibit the growth of E. coli and S. aureus compared to each other. Additionally, an appreciable proportion of tested combinations exhibited synergistic properties. The variations in effectiveness against different bacterial strains could imply that one or both of the plant extracts used in the prototypes have varying degrees of effectiveness.

IV. DISCUSSION

The P. guajava and A. bilimbi leaves were successfully extracted using maceration extraction using ethanol as solvent. From 290 grams of powdered Psidium guajava leaves, they were able to recover 20.44% of the extract, while from 325 grams of powdered Averrhoa bilimbi leaves, they recovered 6.52% of the extract. Saponins, flavonoids, glycosides, and triterpenes in Psidium guajava are positively correlated with antibacterial activity, as noted by Kumar et al. (2021). The tannins present in the leaf extracts of Psidium guajava contribute to the inhibition of bacterial growth and deprive essential compounds required for bacterial growth, according to Das et al. (2020).

Both Averrhoa bilimbi and Psidium guajava possess a wide range of polyphenolic compounds, including flavonoids and tannins. These compounds are considered probable causes of the antibacterial properties observed in the leaf extracts. The qualitative phytochemical analysis further suggests that using ethanol as a solvent for leaf extraction enhances the expression of phenolic compounds, which are known to contribute to the antibacterial activity of the extracts. Ethanol is found to be more effective than an aqueous solvent for capturing these beneficial compounds, as reported by Iwansyah et al. (2021).

The pH testing is necessary to determine whether the soap is safe to use on the skin or not, Cheng E. (2020). In another study, Wijayawardhana et al. (2020) stated that the pH under the physiochemical property of the soap must not be above the normal range of acceptable pH in soap making because an increase in pH in soap could also increase bacterial growth.

In the study by Cheng, E. (2020), It is stated that handmade soap has an alkaline pH level, which provides it with its cleansing function. Handmade soap for skin should ideally fall within a pH range of 8 to 10 to ensure safety, as pH levels above 11 can be too harsh and cause skin irritation. Conversely, a pH below 8 is not practical for handmade soap due to reduced cleansing power. In this study, the pure guava liquid soap, positive control (A La Maison commercial soap), and negative control (soap base) all meet the pH standards set for handmade soap.

The manufactured liquid soap without crude extracts (negative control) had the longest foam retention capacity, potentially due to the amount of fatty acids retained since no other bioactive ingredients were included. The foaming property of the soap is caused by the fatty acids found in coconut oil (Oghome et al. 2012). Several studies have demonstrated that longer foam retention results in better microbial reduction (Fischler et al., 2007; Fuls et al., 2008; Jensen et al., 2015; Lowbury & Lilly, 1973; Ojajärvi, 1980).

The average diameter of the zone of inhibition formed in the combined guava and bilimbi against E.coli was considered as very strong activity. This is supported by Monares et al. (2022), stating that the diameter of the inhibition zone between 20-30 mm is categorized as very strong.

According to the study of Loh et al. (2018), the greater the zone of inhibition diameter, the more it is susceptible to bacteria. The least zone of inhibition diameter may be due to insufficient measurement and concentration of the leaf extract added to the plain liquid soap. Additionally, the process of mixing the liquid soap and the leaf extracts may degrade the antibacterial agents present in Averrhoa bilimbi leaf extract.

CONCLUSION

The phytochemical analysis results of both leaf extracts confirmed that both plants were rich in Sterols, Triterpenes, Flavonoids, Alkaloids, Saponins, Glycosides, and Tannins. These extracts were then used to create different concentrations that were incorporated as an ingredient in antibacterial liquid soap.

In addition, the experimental setups included Pure Guava, Pure Bilimbi, and a combination of Guava and Bilimbi. All of these experimental setups met the standard pH requirement for liquid soap, which is not to exceed 11. Their average pH values were 10.5, 10.6, and 10.6 respectively, ensuring that they are safe for application to human skin. The formulated liquid soaps from the three experimental setups showed higher foam retention capacity compared to the commercially available liquid soap. The negative control, which was the plain liquid soap, had the highest foam retention capacity, lasting for 170 minutes. On the other hand, the commercially available liquid soap had an average foam retention of 67 minutes. Foam retention capacity is one of the important characteristics of the cleansing efficacy of liquid soap. It was also mentioned that the increased lathering ability of the soap could increase bacterial reduction (Ordoyo et al., 2019).

The research demonstrates that the liquid soap containing combined guava and bilimbi extract exhibited superior antibacterial properties against both E. coli and S. aureus compared to the commercially available liquid soap. Nonetheless, the study also found that bilimbi alone did not exhibit much antibacterial activity against both bacteria. However, when bilimbi was combined with guava, there was a potential improvement in its antibacterial properties against E. coli and S. aureus.

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