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Evaluation of the Antibacterial Potential of the Pomelo (*Citrus maxima*) Peel Extract Liquid Hand Soap

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Abstract Pomelo (*Citrus maxima*) has been noticed for its antibacterial activity due to the presence of flavonoids, carotenoids, and phenolic acids, all of which have the potential human health benefits. This study aimed to formulate an antibacterial liquid hand soap made of pomelo (*C. maxima*) peel extract and evaluate its antibacterial potential. This study utilized a quantitative research design to determine the estimated number of colony-forming units and compare if there is a significant difference before and after using the liquid hand soaps. The antibacterial potential of the hand soap was measured using the standard plate count method. Also, a product evaluation of the formulated liquid hand soap was done using an adapted five-point Likert scale questionnaire. Results revealed a noticeable decrease in the estimated number of colony-forming units after using the hand soaps. Furthermore, mixed measures factorial ANOVA gave a p-value of 0.265, which means no significant difference in the bacterial count between the two liquid hand soaps, as it is greater than the 0.05 level of significance. Therefore, the pomelo (*C. maxima*) peel extract and commercial liquid hand soap exhibited antibacterial activity in terms of their bacterial count by the standard plate count test. Moreover, the results for the product evaluation showed that between the two liquid hand soaps, the pomelo (*C. maxima*) peel extract liquid hand soap resulted in a higher average mean for the senior high school and college students and employees' perceptions than the commercial liquid hand soap. However, more research is needed to improve the concentration of the pomelo (*C. maxima*) peel crude extract to achieve better and more comprehensive results for commercial use.

Keywords: *antibacterial activity, bacterial count, liquid hand soap, pomelo (C. maxima) peel extract, product evaluation*

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1. Introduction

Bacterial infections have a significant effect on the health of individuals [1], and the development of antibacterial resistance has emerged as a worldwide health issue, causing effective mortality rates in human populations [2]. Although various therapies and antibiotics have been established, microbes have developed adaptive mechanisms to counteract the effect of antimicrobial medications. As a result, new antibacterial products are urgently needed to prevent the spread of infections that have developed resistance to conventional antibiotics and to provide therapies for chronic and severe diseases [3].

One fruit popular in the Philippines and has antibacterial properties is the pomelo (*C. maxima*), which originated in southern China. [4] states that *C. maxima* highlighted that it is utilized as a remedy for several ailments. It has been observed that pure chemicals and crude organic extracts of *C. maxima* leaves, seeds, peels, pulp, fruits, and roots are attractive. *C. maxima* have already been tested for their pharmacological action. It has been shown to have analgesic, anti-inflammatory, anticancer, CNS activity, anti-diabetic activity, hypocholesterolemic, antioxidant activity, anti-diarrheal, hepato-protective, antibacterial, analgesic, and anti-inflammatory properties.

The peel of the pomelo (*C. maxima*) has been highlighted for its antibacterial characteristics, which have the potential for commercial applications. Since it is readily available in the Philippines, producing a product from it could be suitable for a newly formulated antibacterial liquid hand soap using pomelo (*C. maxima*) peel extract. Thus, the study aims to formulate a liquid hand soap with pomelo (*C. maxima*) peel extract as an organic antibacterial ingredient. Specifically, to assess the quality of the soap base and the bacterial count before and after using the pomelo (*C. maxima*) peel extract liquid hand soap.

2. Materials and Methods

2.1. Preparation of Pomelo (*C. maxima*) Peel Powder

This study adopted the same method as [5]. Two kilograms of fresh Pomelo (*C. maxima*) peel were thoroughly washed with hot water and salt to remove dirt and unwanted particles. Air-drying was done for one week to remove the moisture of fresh Pomelo (*C. maxima*) peels. After being air-dried for one week, the Pomelo (*C. maxima*) peel was dried in a dehydrator for three days. The dried Pomelo (*C. maxima*) peels were then pulverized using a blender.

2.2. Preparation of Pomelo (*C. maxima*) Peel Extract

The pomelo (*C. maxima*) pulverized peels were extracted with 95% ethanol as its solvent. The maceration method done by [6] was adopted for peel extraction. About 100 grams of Pomelo (*C. maxima*) peels were mixed with 1,000 mL of 95% ethanol (1 g:10 mL) in a beaker. The beaker was then covered with aluminum foil and was allowed to stand at room temperature for 72 hours. The ethanolic extract was then filtered using filter paper, decanted using a cheesecloth, served as a filtrate, and then subjected to a rotary evaporator with reduced pressure and controlled temperature. The final extract was then stored in the refrigerator until the extract was used in the preparation of the pomelo (*C. maxima*) peel extract liquid hand soap.

2.3. Preparation of Treatment

The Pomelo (*C. maxima*) Peel crude extract was first diluted with ethanol and distilled water. 5% 30 mL Crude Extract = 1.5 g crude extract (30 mL x 0.05). Then, a small amount of ethanol was added (1 mL at a time with stirring until dissolved). When a crude extract was dissolved, enough distilled water was added to dilute the extract until a 30 mL total was obtained. The diluted crude extract of the Pomelo (*C. maxima*) Peel was added to the soap base on a clean beaker, depending on the concentration needed to formulate the liquid hand soap, and it was stirred thoroughly. The treatment for the liquid hand soap is 30 mL (5%) + 95 mL soap base.

2.4. Preparation of the Soap Base

For the formulation of liquid hand soap, the hot process method by [7] was used in the study. 45.5 mL of coconut oil was heated in a beaker at 70°C for 15 minutes, and 18.2 mL of glycerin was heated separately. Both coconut oil and glycerin were then heated at 70°C and gently stirred for another 15 minutes. The temperature was constantly checked using a thermometer. 11.5 grams of KOH was weighed using an analytical weighing scale, and 38 mL of distilled water was then prepared. The KOH was mixed with distilled water and stirred using a stirring rod to dissolve the KOH. The mixture served as the lye-water solution. Once the lye water was thoroughly mixed until it became clear, the solution was slowly added to the heated coconut oil. The coconut oil and lye-water solution was poured into the heated glycerin in a beaker. The solution was then heated at 70°C for 50 minutes. After this, the solution was continuously stirred using a stirring rod. This mixture served as the soap base. The liquid hand soap was then neutralized and preserved by adding 1 g of borax powder to the resulting mixture. The soap treatment was prepared by adding 30 mL of Pomelo peel extract to the 150 mL soap base. A 10 mL lavender, lemon, sweet orange, and peppermint essential oil was added to the mixture.

2.5. Preparation for Hand Swab Test and Bacterial Count

In reference to the study conducted by [5], the same method was utilized in this study in testing the number of bacteria present in the hands, which was the hand swab test. In doing the hand swab test before using the two different soaps, Pomelo (*C. maxima*) peel extract liquid hand soap and commercial liquid hand soap, the disposable sterile cotton swabs were first moistened using distilled water and were then swabbed on the hands of the participants. The participants were then hand swabbed again after using the Pomelo (*C. maxima*) peel extract and commercial liquid hand soaps. The six participants rubbed their hands with soap for 20 seconds in reference to [8]. The sterile cotton swab stick that was used to swab the surface of the hands, specifically the palm and between the fingers, was then placed directly in the sterilization container and was allowed to rest on a zip lock inside an icebox. Once the samples have been fully collected, the isolated samples undergone the Standard Plate Count test to determine the number of bacteria present in each trial.

2.6. Product Evaluation Procedure

For the product evaluation procedure, an adapted five-point Likert scale questionnaire from [9] was utilized in this study. This survey questionnaire assessed the participants' perceptions in terms of color, smell, and lather. In choosing the participants, there were three clusters of participants aged 18 and above. Specifically, 20 Senior High School Students, 20 College Students, and 20 Employees.

2.7. Statistical Tools

This study used mixed measures factorial ANOVA to compare the Standard Plate Count test results before and after using the Pomelo (*C. maxima*) peel extract and the commercial liquid hand soaps.

3. Results and Discussion

Table 1 presents the standard plate count test results for the Pomelo (*C. maxima*) Peel Extract and commercial liquid hand soaps, and there is a noticeable decrease in the estimated average number of colony-forming units after using them. This may be due to the time interval for hand washing with soap. A similar study by [10] states that washing hands with soap longer significantly removes bacteria from hands. With the given results, it may also seem reasonable to assume that hand washing with

Pomelo (*C. maxima*) peel extract and commercial liquid hand soap also effectively reduces the bacterial count. As stated in the study by [11], Pomelo (*C. maxima*) peel extract can eradicate bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. With this reference, trials 1 and 2 using the pomelo liquid hand soap showed no decrease in the number of colony-forming units, maybe because the bacteria present on the hands of the participants are a kind of bacteria that cannot be eradicated by the antibacterial properties of the Pomelo (*C. maxima*) peel extract.

Table 1. Standard Plate Count Test Results

		Estimated Average No. Of Colony Forming Units (1:10 dilution)	
Hand Soap	**Trial**	**Before**	**After**
Pomelo	1	80	30
	2	10	10
	3	10	10
Commercial	1	50	30
	2	160	10
	3	40	30

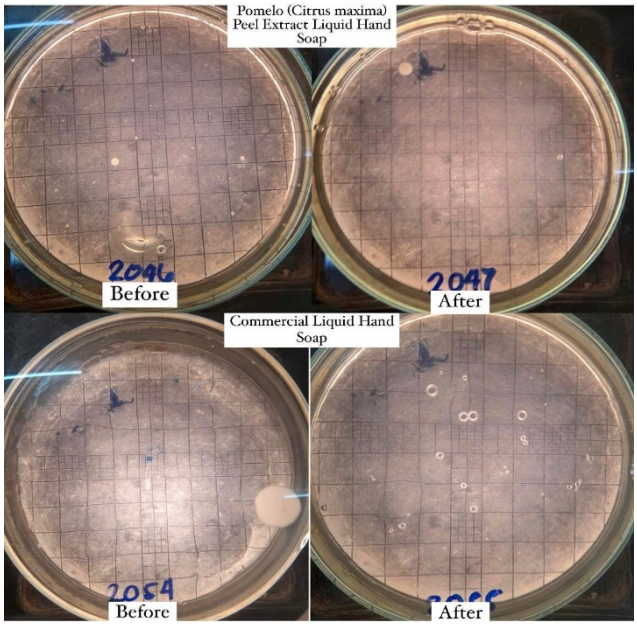

Figure 1. Estimated Average No. Of Colony Forming Units Before and After Using the Pomelo (*Citrus maxima*) and the Commercial Liquid Hand Soaps

Table 2. Mixed Measures Factorial ANOVA Table Examining the Difference in Bacterial Count Before and After Using Pomelo (*C. maxima*) Peel Extract and the Commercial Liquid Hand Soaps

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Intercept	18408.333	1	18408.333	12.843	.023
Hand Soaps	2408.333	1	2408.333	1.680	$.265^{NS}$
Error	5733.333	4	1433.333		

Significant at <0.05 level.

Differences in the bacterial count before and after using Pomelo (*C. maxima*) peel extract and the commercial liquid hand soaps were examined using mixed measures factorial ANOVA and are summarized in Table 2. The mixed measures factorial ANOVA yielded an F value of 12.843 and a computed probability value of 0.265^{NS}, which is higher than the 0.05 level of significance. This led to the failure of rejecting the null hypothesis. This means there is no significant difference in the bacterial count before and after using Pomelo (*C. maxima*) peel extract and commercial liquid hand soaps. This suggests that the two liquid hand soaps were not significantly different from one another.

From the data, it can be ascertained that the two liquid hand soaps are effective at a certain level. However, it is much better to test the Pomelo (*C. maxima*) peel extract liquid hand soap with more participants because, in the study conducted by [10], 240 samples were collected to get a more accurate result. Also, the results can be supported by the fact that the Pomelo Peel is known to have carotenoids, flavonoids, and phenolic acids, which were associated with its antibacterial and skin protective properties [12].

With the given results in the table below, the mean of the senior high school students is 4.70 regarding absorbability and their overall impression, which means that they favor it the most. The college students and the employees, on the other hand, favor the pleasant skin sensation given by the pomelo (*C. maxima*) peel extract liquid hand soap more as its mean is 4.70 for the college students and 4.75 for the employees, which means that they perceive the skin sensation given by the pomelo (*C. maxima*) peel extract liquid hand very highly acceptable.

Table 3. Product Evaluation of the Three Clusters of Participants for the Pomelo (*Citrus maxima*) Peel Extract Liquid Hand Soap

Product Evaluation	**SHS Students**			**College Students**			**Employees**		
	Mean	**SD**	**QI**	**Mean**	**SD**	**QI**	**Mean**	**SD**	**QI**
1. The product is pleasant in the eyes.	4.50	0.50	VHA	4.30	0.73	VHA	4.45	0.60	VHA
2. The product is very well-tolerated.	4.60	0.49	VHA	4.15	0.59	HA	4.60	0.60	VHA
3. The product has an intensive care effect.	4.65	0.48	VHA	4.45	0.60	VHA	4.60	0.68	VHA
4. The product provides a very pleasant skin sensation.	4.60	0.49	VHA	4.70	0.57	VHA	4.75	0.44	VHA
5. The product absorbs perfectly.	4.70	0.46	VHA	4.40	0.50	VHA	4.70	0.57	VHA
6. The product possesses a very pleasant smell.	4.65	0.67	VHA	4.60	0.60	VHA	4.60	0.68	VHA
7. My overall impression of the product is very positive.	4.70	0.46	VHA	4.50	0.61	VHA	4.55	0.51	VHA
Overall Mean & SD	4.63	0.49	VHA	4.44	0.60	VHA	4.61	0.58	VHA

Legend: VHA, HA, AA, LA, VLA.

Thus, when it comes to the pomelo (*C. maxima*) peel extract liquid hand soap's tolerability was least favored by the college students as its mean is 4.15, meaning they only perceive it as highly acceptable. Moreover, when it comes to the color of the pomelo (*C. maxima*) peel extract liquid hand soap, it was least favored by the senior high school students and employees as the mean for the senior high school in terms of color is 4.50 and 4.45 for the employees.

Moreover, the results show that the overall mean of the three clusters of participants is close to each other as the overall mean of the senior high school students is 4.63, 4.44 for the college students, and 4.61 for the employees which can all be interpreted as very high acceptability. With the given results, the three clusters of participants' mean ranged between 4.21 – 5.00, which means that the participants perceived the Pomelo (*C. maxima*) peel extract liquid hand soap positively. Based on the scoring procedure of the product evaluation, the mean scores can be interpreted as very high, which means that they perceive the product as very acceptable.

Furthermore, the result of this study is similar to [13], where the Lemongrass Laundry Detergent Product was interpreted as very high as its overall mean range for appearance is 4.48 and 4.93 for scent, which means the product is good as it falls between the mean range of 4.20 – 5.00. With this reference, it can be established that the pomelo (*C. maxima*) peel extract liquid hand soap passed the overall acceptability of the product. Moreover, the study of [14] stated that a product's most critical positive characteristics are when it absorbs quickly, is soft on hands, smells and feels clean, and does not dry on hands. With the given results in Table 3, it can be seen that the Pomelo (*C. maxima*) peel extract met the expected positive characteristics of a product which means that the product is good and is perceived well by all the participants.

With the given result, the mean of the senior high school students is 4.70 regarding color, which means that they favor it the most. The college students favor the skin sensation given by the commercial liquid hand soap and its absorbability as the mean for the skin sensation given by the product, and its absorbability is 4.35. The employees favor the color, its tolerability, and the skin sensation given by the commercial liquid hand soap, as all its means are 4.35, meaning they perceive it highly acceptable. Thus, when it comes to the commercial liquid hand soap's scent, it was least favored by the senior high school students and employees as the mean for the senior high school students in terms of smell is 3.35, which means that they perceive it as averagely acceptable and 3.85 for the employees, meaning they only perceive it as highly acceptable. Moreover, the tolerability of commercial liquid hand soap was least favored by college students as its mean in terms of commercial liquid hand soap's tolerability is 4.15.

Moreover, the results show that the overall mean of the three clusters of participants is also close to each other, as the overall mean of the senior high school students is 4.19 which can be interpreted as high acceptability. Thus the overall mean for the college students 4.30, and 4.21 for the employees which can be interpreted as very high acceptability. With the given results, the senior high school students mean ranged between 3.41 – 4.20, whereas the college students and the employees' mean ranged between 4.21 – 5.00, which means that all the participants perceived the commercial liquid hand soap positively. However, based on the scoring procedure of the product evaluation, the mean scores of the senior high school can be interpreted as high. In contrast, the college students and employees' mean scores can be interpreted as very high.

In addition, the result of this study is also similar to [13], where the Lemongrass Laundry Detergent Product was interpreted as very high as its overall mean range for appearance is 4.48 and 4.93 for scent, which means the product is good as it falls between the mean range of 4.20 – 5.00. With this reference, it can be established that the commercial liquid hand soap passed the overall acceptability of the product. Moreover, the study of [14] stated that a product's most critical positive characteristics are when it absorbs quickly, is soft on hands, smells and feels clean, and does not dry on hands. With the given results in Table 4, it can be seen that the commercial liquid hand soap also met the expected positive characteristics of a product which means that the product is good and is perceived well by all the participants.

Furthermore, Tables 3 and 4 show that the overall mean score of the Pomelo (*C. maxima*) peel extract liquid hand soap is much higher than the commercial liquid hand soap. With the given results, it can be ascertained that the perceptions of the three clusters of participants for the Pomelo (*C. maxima*) peel extract liquid hand soap are more positive than the commercial liquid hand soap.

Table 4. Product Evaluation of the Three Clusters of Participants for the Commercial Liquid Hand Soap

Product Evaluation	SHS Students			College Students			Employees		
	Mean	SD	QI	Mean	SD	QI	Mean	SD	QI
1. The product is pleasant in the eyes.	4.70	0.47	VHA	4.30	0.66	VHA	4.35	0.96	VHA
2. The product is very well-tolerated.	4.35	0.59	VHA	4.15	0.49	HA	4.35	0.73	VHA
3. The product has an intensive care effect.	4.30	0.66	VHA	4.30	0.57	VHA	4.20	0.60	HA
4. The product provides a very pleasant skin sensation.	4.30	0.73	VHA	4.35	0.59	VHA	4.35	0.73	VHA
5. The product absorbs perfectly.	4.30	0.66	VHA	4.35	0.49	VHA	4.15	1.01	HA
6. The product possesses a very pleasant smell.	3.35	1.23	AA	4.25	0.79	VHA	3.85	0.85	HA
7. My overall impression of the product is very positive.	4.00	0.65	HA	4.40	0.60	VHA	4.20	0.87	HA
Overall Mean & SD	4.19	0.71	HA	4.30	0.60	VHA	4.21	0.82	VHA

Legend: VHA, HA, AA, LA, VLA.

4. Conclusion

The results revealed a noticeable decrease in the average number of bacteria colonies after using the two liquid hand soaps. This means that the two liquid hand soaps were effective in decreasing bacterial count after using the liquid hand soaps. Furthermore, there is no significant difference in the bacterial count before and after using the Pomelo (*C. maxima*) peel extract and the commercial liquid hand soaps. This means that the two liquid hand soaps were not significantly different from one another and were effective at a certain level. Moreover, based on the product evaluation of the overall acceptability of the Pomelo (*C. maxima*) peel extract and commercial liquid hand soaps, it can be concluded that both of the products are acceptable.

5. Recommendations

Given that this study only has one treatment with a concentration of 5% crude extract of the pomelo (*C. maxima*) peel, it is recommended to explore more treatments with a concentration from least to highest to have a more comprehensive results for commercial use.

For the standard plate count test, more participants are recommended to have a wider scope and more conclusive results.

It is further recommended to have more participants in the product evaluation and compare the over-all acceptability of the two liquid hand soaps.

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