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The Effect of Hand Washing Soap with Soursop Leaf and Guava Leaf Extract on Staphylococcus aureus Bacteria

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A B S T R A C T

This study tested a handwash formulation containing a combination of soursop leaf extract and guava leaf extract as active ingredients. These two extracts are known to have antibacterial properties, and the study results show that the formulated handwash has physical and chemical properties according to SNI standards. The formulated handwash has a brown color with a distinctive aroma of extract, homogeneous, pH 10.00, foam height of 100 mm, and viscosity ranging from 700-20,000 cPs with pseudoplastic flow properties. In addition, handwashing with a combination of soursop and guava leaf extract also showed significant antibacterial activity against Staphylococcus aureus bacteria. The average diameter of the inhibition zone for handwash with different formulas showed powerful results. Thus, combining soursop leaf extract and guava leaf extract can be an effective alternative in maintaining hand hygiene and preventing disease transmission through contact with microorganisms on the hands. Further research needs to be done to optimize the use of natural ingredients such as soursop leaf extract and guava leaf extract in health care products to provide more comprehensive benefits to maintain the health and cleanliness of the body.

Kata kunci:

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Ekstrak daun sirsak
Ekstrak daun jambu biji
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A B S T R A K

Penelitian ini menguji formulasi handwash yang mengandung kombinasi ekstrak daun sirsak dan ekstrak daun jambu biji sebagai bahan aktif. Kedua ekstrak ini diketahui memiliki sifat antibakteri, dan hasil penelitian menunjukkan bahwa handwash yang diformulasikan memiliki sifat fisik dan kimia yang sesuai dengan standar SNI. Handwash yang diformulasikan memiliki sediaan berwarna cokelat dengan aroma khas ekstrak, homogen, pH 10,00, tinggi busa 100 mm, dan viskositas berkisar antara 700-20.000 cPs dengan sifat alir pseudoplastis. Selain itu, handwash dengan kombinasi ekstrak daun sirsak dan ekstrak daun jambu biji juga menunjukkan aktivitas antibakteri yang signifikan terhadap bakteri Staphylococcus aureus. Diameter rata-rata zona hambat untuk handwash dengan formula yang berbeda menunjukkan hasil yang sangat kuat. Dengan demikian, kombinasi ekstrak daun sirsak dan ekstrak daun jambu biji dapat menjadi alternatif efektif dalam menjaga kebersihan tangan dan mencegah penularan penyakit melalui kontak mikroorganisme pada tangan. Penelitian lebih lanjut perlu dilakukan untuk mengoptimalkan penggunaan bahan alami seperti ekstrak daun sirsak dan ekstrak daun jambu biji dalam produk perawatan kesehatan, guna memberikan manfaat yang lebih luas dalam upaya menjaga kesehatan dan kebersihan tubuh.

INTRODUCTION

Hands, as a member of the human body that functions as a medium of contact with various objects, have the potential as a medium for transmitting microorganisms into the human body, which in turn can cause various diseases. Staphylococcus aureus found most often on the skin of the hands, is a type of bacteria that can be a source of infection. Staphylococcus aureus has the characteristics of being spherical and gram-positive and generally forms in irregular groups. Therefore, one of the actions that can be taken to prevent the risk of harmful microorganisms and reduce the potential for causing disease is to maintain hand hygiene by regularly washing hands (Barbuti et al., 2023; Howden et al., 2023; Loftus et al., 2023; Rani et al., 2023).

One method commonly used to maintain hand hygiene is through handwash, which can be obtained in various formulations using synthetic and natural chemical-based ingredients. One of the synthetic chemical substances often used in handwash is triclosan, which has antibacterial properties. The mechanism of triclosan's antibacterial action involves damage to the cytoplasmic membrane, synthesis of ribonucleic acid (RNA), fatty acids, and bacterial cell proteins (Chen et al., 2023; Kavya et al., 2023). However, using triclosan as an active ingredient in handwash also has the potential to cause bacteria to become insensitive to this substance. It can hurt the environment and humans, both in the short and long term (Altwiley et al., 2023; Ardila & Bedoya-García, 2023).

As an alternative to replacing handwashes containing triclosan, handwashes containing natural ingredients can be an option that is a safer and more environmentally friendly solution. Some natural ingredients reported to have enjoyable antibacterial activity for further study in the context of handwash include soursop leaves (*Annona muricata L.*) and guava leaves (Psidium guajava L.). Soursop leaf (*Annona muricata L.*) is a natural product rich in secondary metabolites, such as flavonoids, saponins, and tannins. Flavonoids in soursop leaves have an antibacterial mechanism that involves damage to the bacterial cell wall and inhibits enzyme activity (Aisyah et al., 2023; AKANJI, 2023; Vindhya et al., 2023). Saponins can also act as antibacterials with their ability to cause the lysis of bacterial cells. In addition, guava leaves (*Psidium guajava L.*) also contain secondary metabolites such as tannins and saponins, which have an antibacterial mechanism by damaging the bacterial cell membrane (Gideon, 2023; Ndako et al., 2023; Ngwanguong et al., 2023; Rajad et al., 2023). In developing handwashes based on natural ingredients, the potential of these natural ingredients needs to be further explored through comprehensive research to understand the antibacterial effects in detail and the potential side effects associated with their use. Thus, using handwash based on natural ingredients can be a safer and more sustainable choice in maintaining hand hygiene and preventing the spread of pathogenic microorganisms that can cause disease.

This study focuses on developing handwashes that contain natural chemicals with a combination antibacterial approach, in which two types of extracts from two different plants, namely soursop leaves (*Annona muricata L.*) and guava leaves (*Psidium guajava L.*), are used simultaneously. To inhibit bacterial growth.

METHODS

Chemicals and Materials

Samples of soursop leaves (Annona muricata L.) and guava leaves (Psidium guajava L.) came from Herbarium Bogorinase, National Research and Innovation Agency, Bogor, West Java. The materials used in this study were Carbopol 940 (Alchemy), Sodium laureth sulfate (Finar), Coconut oil, KOH (Pupick med), Stearic acid (Merck), DMDM Hydantion (Lonza), Butyl hydroxy toluene (Tekkim), Disc paper; Aluminum foil, Staphlococcus aureus bacteria, 70% ethanol (OneMed), Mueller Hinton Agar (Merck), Sodium Agar (Merck), Aquadest (OneMed), Hydrochloric Acid (Merck), Magnesium (Merck), Ferric Chloride (Vickers), Chloroform (Merck), Ammonia (Merck), Sulfuric acid (Merck), Dragendorff reagent (Merck), Acetic anhydrous acid (Merck), triclosan. The tools used in this study are, Analytical balance (OHAUS PX224 Analytical Balance), pH meters (PH-02), viscometer, measuring cups 100mL, 50mL, 10mL (pyrex), Beaker glass 250mL, 100mL (pyrex), erlenmeyer 100mL (pyrex), stir sticks, magnetic stirrer (SH-2), petri dishes, drop pipette, circular loop needle, Bunsen, test tubes (pyrex), test tube racks, Incubator (Memmert), Autoclave (GEA medical LS-351J), Bio Safety Cabinet (abl-bsc120), Oven (DHG 9053A).

Sample preparation

Determination of plants from soursop leaves (Annona muricata L.) and guava leaves (Psidium guajava L.) as active ingredients were carried out at the National Research and Innovation Agency, Bogor, West Java. Simplicia is made based on the Indonesian Herbal Pharmacopoeia; in short, Soursop leaves and guava leaves are washed thoroughly, air dried without direct sunlight, and macerated with 70% ethanol until transparent, concentrated using a rotary evaporator (Gueboudji et al., 2023; Kemenkes, 2017; Septiana et al., 2023).

Phytochemical Screening

Phytochemical screening analysis was carried out to identify the chemical content of the sample secondary metabolites, particularly Flavonoids, Tannins, and Saponins, which are known to have antibacterial potential. The Flavonoid test tested a viscous extract of 1 mg in ethanol by adding Mg and concentrated HCl. A change in color to dark red or orange indicated a positive result within 3 minutes. Bangle extract was used as a comparison. The tannin test was carried out by testing a viscous extract of 1 mg in ethanol with the addition of 1% FeCl3 solution; the formation of a bluish-black or blackish-green color indicated a positive result. As a comparison, tea leaf extract was used. The saponin test was carried out by testing the viscous extract of 1 mg in distilled water (1:1), boiling, cooling, and shaking vigorously for 1 minute. Positive results were indicated by foam formation, which remained stable after adding 1 N HCl and incubating for 10 minutes. Bangle is used as a comparison (Dubale et al., 2023; Giri et al., 2023; Thamer et al., 2023).

Handwash formulation containing Soursop Leaf Extract and Guava Leaves

The handwash formula for soursop leaf and guava leaf extract consists of several chemicals, as seen in Table 1. This formulation method involves the following steps: Carbopol

940 is put into a beaker glass and mixed with distilled water, then swells for 24 hours (mass 1). Stearic acid was dissolved in distilled water and heated over a water bath before being put into a porcelain cup. Coconut oil is put into a beaker glass of as much as 30 ml. KOH is first dissolved in distilled water, then added little by little while stirring and heated at 50^{0}C until a soap paste (mass 2) is formed. Mass 1 and mass two are mixed in a hot mortar slowly and stirred until homogeneous. Sodium laureth sulfate is added and stirred until homogeneous. The dissolved stearic acid was added and stirred until homogeneous, then DMDM Hydantoin was added and stirred until homogeneous. Soursop leaf extract (Annona muricata L.) and guava leaf extract (Psidium guajava L.), previously dissolved with Butylated Hydroxytoluene, were added and stirred until homogeneous. After all the ingredients are homogeneous, distilled water is added until the volume reaches 100 ml, then the solution is put into a pump bottle container.

Table 1
Handwash preparation formulation of soursop leaf extract and guava leaf

Material	Formula % (b/v)			
	1	2	3	4
Soursop Leaf Extract	-	3	7	10
Guava Leaf Extract	-	15	20	25
Coconut oil	30	30	30	30
Potassium hydroxide (KOH)	16	16	16	16
Carbopol 940	1	1	1	1
Sodium Lauryl Sulfat	1	1	1	1
Stearic acid	0,5	0,5	0,5	0,5
DMDM Hydantion	0,5	0,5	0,5	0,5
Butylated Hydroxytoluene (BHT)	1	1	1	1
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

Handwash evaluation

Evaluation of Soursop Leaf and Guava Leaf Extract Hand Soap. Hand washing soap preparations of soursop leaf extract (Annona muricata L.) and guava leaf extract (Psidium guajava L.) were evaluated after they were formed and stored at room temperature (28-30°C). The evaluation includes weekly stability tests for six weeks, including organoleptic tests, pH tests, foam height tests, viscosity and flow properties tests, and homogeneity tests. In the stability test, organoleptic observations were made of the color and smell of the preparation visually. The pH of the preparation was measured by calibrating a pH meter using buffer solutions 4, 7, and 10, and the results were recorded. Foam height was measured after shaking 1 g of the preparation with 10 ml of distilled water for 20 seconds. The viscosity of the preparation was measured by placing 250 ml of the preparation in a beaker glass and measuring the viscosity value using a viscometer at a certain speed (rpm). The homogeneity of the preparation was observed by dripping the preparation on a glass slide and examining the parts that were not well mixed (Bahuguna & Kashyap, 2016; Conover & Gibson, 2016; Ghurghure et al., 2019; Kusarkar et al., 2022; Patel et al., 2017).

Antibacterial activity test by disc diffusion

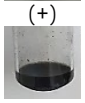
Handwash diffusion test activity containing soursop leaf extract (Annona muricata L.) and guava leaf extract (Psidium guajava L.) was evaluated using a sterile cotton swab smeared on the surface Muller Hinton Agar media that had been inoculated with bacteria. Followed by the administration of a blank disk on the surface of the media, as well as the administration of 3 variations of extract concentration and one negative control of 20 µL on a blank disk. After that, the media was incubated for 24 hours at 37°C with tightly closed (Cebi & Erarslan, 2023; Feizi et al., 2023; Mariyammal et al., 2023; Mohapatra et al., 2023; Singh et al., 2023).

RESULTS AND DISCUSSION

Sample Analysis

Determination of soursop leaves (Annona muricata L.) and guava leaves (Psidium guajava L.) was carried out at BRIN (National Research and Innovation Agency) Bogor to verify the authenticity of the plants used. The determination results show that the soursop leaves (Annona muricata L.) belonged to the Annonaceae family, and the guava leaves (Psidium guajava L.) used to belong to the Myrtaceae family. The viscous extract obtained after the evaporation process was brown, with a weight of soursop leaf ethanol extract of 243.11 g and a yield of 48.622%. Meanwhile, the weight of the guava leaf extract was 249.36 g, and the yield of the guava leaf ethanol extract was 49.872%. The yield of soursop leaf and guava leaf extracts complied with the requirements of the Indonesian Herbal Pharmacopoeia, which stipulates a yield of not less than 7.2%, indicating satisfactory yield results (Menteri Kesehatan RI, 2008).

Table 2
Phytochemical screening test of soursop and guava leaf extracts

Compound	Parameter	Annona muricata extract	Guava Leaf Extract
Flavonoid	Red/orange solution	 (+)	 (-)
Tanin	Black/bluish black/greenish black solution	 (+)	 (+)
Saponin	Stable foam	 (+)	 (+)

Phytochemical Screening

The results of the phytochemical screening of Annona muricata extract (Table 2) showed the presence of flavonoids, tannins, and saponins. Flavonoids were detected by a color change from green to red due to the hydrolysis of O-glycosyl by concentrated HCl (Gaikwad et al., 2023; Shah et al., 2023). H+ will replace the hydrolyzed glycosyl from an acid with strong electronegativity. Furthermore, Mg will reduce the flavonoid compounds, forming red or orange complex compounds. Tannins were detected by a change in color from green to black, while saponins were detected by foam formation after shaking, and the foam did not disappear when HCl was added.

Phytochemical screening results showed that Guava Leaf Extract contains tannins and saponins (Table 2). Tannins are characterized by a color change from green to black, forming a black color in the extract after adding FeCl3 because tannins will form complex compounds with Fe3+ ions (Mailoa et al., 2013, 2014; Mondal et al., 2001). There is a Fe3+ ion as the central atom, and tannins have an O atom with a lone pair of electrons that can coordinate with the central atom as a ligand. Furthermore, saponins were characterized by foam formation after shaking, and when added with HCl, the foam did not disappear. The principle of the saponin test is that there is a hydrolysis reaction of saponin compounds into aglycones and glycones, which are characterized by the formation of stable froth.

Formula Evaluation

Evaluation and stability of handwash preparations based on organoleptic tests showed that combining soursop and guava leaf extracts resulted in a high-viscosity handwash. The color of the handwash produced in each formulation is slightly different (Figure 1), where the control formulation (FI) is white, while F2, F3, and F4 are brown. The brown color of the handwash indicated the presence of soursop and guava leaf extracts, which was evident before the addition of soursop and guava leaf extracts. The base color of the handwash was white. In addition, the results of the organoleptic test on the odor of the handwash combination of soursop and guava leaf extracts showed a distinctive odor, which depended on the concentration of the extract added to the preparation.

Figure 1. Handwash preparations

Organoleptic examination (Table 3) of formulas I, II, III, and IV also met the specifications required by the Indonesian National Standard (SNI), including the color and characteristic odor. Furthermore, the results of overall observations of handwash preparations showed that the four formulas made did not change color, smell, and were stable during storage for 6 weeks at room temperature.

Table 3
Organoleptic Test

Time (Week)	Formula											
	I			II			III			IV		
	In	BA	BT	In	BA	BT	In	BA	BT	In	BA	BT
0	-	-	+	+	+	+	+	+	+	+	+	+
1	-	-	+	+	+	+	+	+	+	+	+	+
2	-	-	+	+	+	+	+	+	+	+	+	+
3	-	-	+	+	+	+	+	+	+	+	+	+
4	-	-	+	+	+	+	+	+	+	+	+	+
5	-	-	+	+	+	+	+	+	+	+	+	+
6	-	-	+	+	+	+	+	+	+	+	+	+

Description: W (Color), Smell (BA), Shape (BT)

The pH test for handwash preparations of soursop and guava leaf extracts showed a pH value ranging from 10.00 to 10.79 (Table 4), indicating that the pH tended to change. The four handwash formulas stored at room temperature (20-25^0 C) experienced insignificant increases and decreases, which may be due to the influence of room temperature, which was not constant when making measurements using a digital pH meter which is very sensitive to changes in temperature. Nevertheless, the pH test results for six weeks showed that all handwash formulas met the criteria for good handwash and still met the skin pH standard by SNI, which allows for handwash pH between 8-11. Furthermore, the results of the ANOVA statistical test showed that the formula's effect on pH during storage was not significant at the 95% confidence level, so the formula's difference did not affect the pH value.

Table 4
pH Test

Time (Week)	Formula (pH)			
	I	II	III	IV
0	10,04	10,04	10,04	10,02
1	10,00	10,63	10,79	10,69
2	10,00	10,62	10,69	10,56
3	10,03	10,32	10,38	10,43
4	10,02	10,02	10,01	10,01
5	10,02	10,02	10,02	10,02
6	10,04	10,04	10,11	10,11

The handwash foam height test showed varying results for each formula, with foam height values ranging from 20-119 (Table 5). Nevertheless, these results are still by the

value required by SNI, namely 13-220 mm. However, based on the results of the ANOVA statistical test, the effect of the formula on foam height

Table 5
Foam height test

Time (Week)	Formula (cm)			
	I	II	III	IV
0	9,8	7	2	2,5
1	11,2	8	3,5	3
2	11,9	9,5	3	3,2
3	12	10	4	3,9
4	11,9	9,6	7,5	2
5	11,5	8	7,6	2
6	11	7,8	6,5	2

The results of the viscosity test showed that the viscosity of the handwash preparations increased with increasing concentration of the extract (Table 6). This shows that the higher the concentration of the extract, the higher the viscosity because adding the extract reduces the amount of water in the preparation and makes it thicker. These results are by the quality standards for handwash preparations set by SNI, where a suitable viscosity ranges from 500-20,000 cPs or 0.5-20 Pa.S. Thus, all handwash formulations containing soursop and guava leaf extracts met the quality standard requirements. In addition, the results of the ANOVA statistical test showed a significant difference in the viscosity between the different formulas during storage, with a significance value (<0.05) at the 95% confidence level. This shows that the formula affects the viscosity of handwash preparations.

Table 6
Test viscosity

Formula	Week To (cPs)						
	0	1	2	3	4	5	6
I	8,81	8,81	8,81	8,81	8,81	8,81	8,81
II	23,73	23,73	23,73	23,73	23,73	23,73	23,73
III	24,29	24,29	24,29	24,29	24,29	24,29	24,29
IV	25,71	25,71	25,71	25,71	25,71	25,71	25,71

The rheogram results showed that the flow properties of the handwash preparations containing soursop and guava leaf extracts in the four formulas were non-Newtonian with a pseudoplastic flow type, in which the flow properties were not affected by time (Figure 2). This can be seen from the decreasing curves to the left of the increasing curves, which indicates that the preparation has a lower viscosity at each shear speed value. In addition, the difference between the descending and ascending curves indicates a breakdown of the structure, which does not reshape immediately. The ideal rheological property for topical preparations is pseudoplastic because it can be poured easily and returns to its original state quickly.

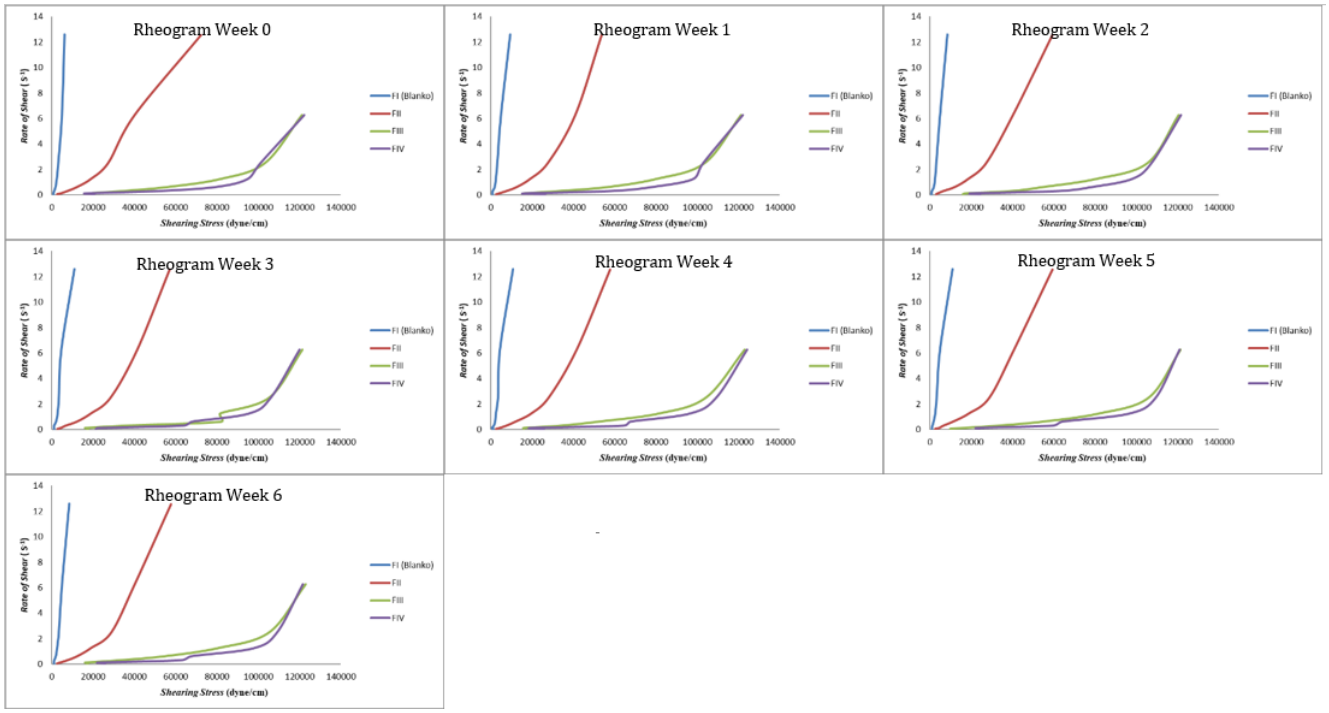


Figure 2. Rheogram

The homogeneity test results showed that all handwash formulations containing a combination of soursop and guava leaf extracts were homogeneous. This can be observed from the absence of coarse grains or clumping particles on the glass plate. These results are consistent with previous research, which states that preparation is homogeneous if no

coarse grains or lumps are visible. During six weeks of storage, all handwash preparation formulas remained homogeneous. The homogeneity test results for formulas I, II, III, and IV met the specifications set by SNI, namely homogeneous, so it can be concluded that the handwash preparations were stable.

Antibacterial activity

This study found that handwash preparations containing a combination of soursop and guava leaf extracts had antibacterial activity against Staphylococcus aureus, with the result that the diameter of the inhibition zone ranged from 23.73 mm - 25.71 mm (Figure 3). The inhibition zone diameter test results showed that handwash with a combination of soursop and guava leaf extracts had an average diameter of inhibition zone which was categorized as very strong, namely >20 mm (Devagi et al., 2020; Soltanian et al., 2019)

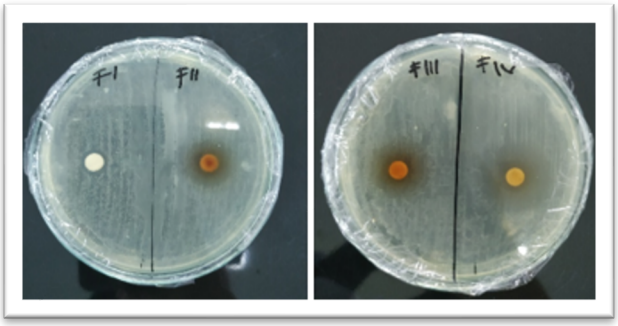


Figure 3. Antibacterial activity from handwash contain soursop and guava leaf extracts

Based on the statistical analysis of ANOVA, it was found that there was a significant difference in the effect of the formula on the inhibition during storage because the significance value (<0.05) was at the 95% confidence level. This shows that the different formulas affect the inhibition of handwash containing a combination of soursop and guava leaf extracts during storage.

CONCLUSIONS AND SUGGESTIONS

The conclusion of this study stated that handwash preparations containing a combination of soursop and guava leaf extracts were able to inhibit the growth of Staphylococcus aureus bacteria. The largest diameter of the inhibition zone was obtained in Formula II with a value of 25.71 mm, indicating a very strong inhibitory effect. In addition, this handwash preparation also meets the physical quality requirements with the characteristics of a brown color, a distinctive odor of the extract, a homogeneous preparation, and a pH in the range of 10.00 - 10.79. The resulting foam height ranges from 2-11.9 cm/20-119 mm, and the viscosity ranges from 692-19,800 cPs and has pseudoplastic flow properties. The results of this study provide the potential for the use of a combination of soursop and guava leaf extract handwash preparations as an alternative hand sanitizer product that is effective and meets physical quality standards.

Conflict of Interest Statement

This research does not include any conflict of interest

REFERENCES

Aisyah, N., Zulkifli, L., & Rasmi, D. A. C. (2023). Isolation of endophytic bacteria and fungi from soursop (Annona muricata L.) and bioactivity test as antimicrobial against Eschericia coli, Staphylococcus epidermidis, and Candida albicans. *Jurnal Pijar Mipa*, *18*(2), 254–259.

AKANJI, O. C. (2023). Antibacterial activity of Annona muricata leaves′ extracts. *International Journal of Frontier Research in Science*, *2*(1), 24–28.

Altwiley, D., Brignoli, T., Duggan, S., & Massey, R. C. (2023). Triclosan-resistant small-colony variants of Staphylococcus aureus produce less capsule, less phenol-soluble modulins, and are attenuated in a Galleria mellonella model of infection. *Microbiology*, *169*(1), 001277.

Ardila, C. M., & Bedoya-García, J. A. (2023). Bacterial resistance to antiseptics used in dentistry: A systematic scoping review of randomized clinical trials. *International Journal of Dental Hygiene*, *21*(1), 141–148.

Bahuguna, M., & Kashyap, S. (2016). Formulation and evaluation of hand wash. *World Journal of Pharmaceutical Research*, *5*(7), 1559–1577.

Barbuti, M. D., Myrbråten, I. S., Morales Angeles, D., & Kjos, M. (2023). The cell cycle of Staphylococcus aureus: An updated review. *MicrobiologyOpen*, *12*(1), e1338.

Cebi, N., & Erarslan, A. (2023). Determination of the Antifungal, Antibacterial Activity and Volatile Compound Composition of Citrus bergamia Peel Essential Oil. *Foods*, *12*(1), 203.

Chen, X., Mou, L., Qu, J., Wu, L., & Liu, C. (2023). Adverse effects of triclosan exposure on health and potential molecular mechanisms. *Science of The Total Environment*, *879*, 163068.

Conover, D. M., & Gibson, K. E. (2016). A review of methods for the evaluation of handwashing efficacy. *Food Control*, *63*, 53–64.

Devagi, P., Suresh, T. C., Sandhiya, R. V., Sairandhry, M., Bharathi, S., Velmurugan, P., Radhakrishnan, M., Sathiamoorthi, T., & Suresh, G. (2020). Actinobacterial-mediated fabrication of silver nanoparticles and their broad spectrum antibacterial activity against clinical pathogens. *Journal of Nanoscience and Nanotechnology*, *20*(5), 2902–2910.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*, 51–62.

Feizi, H., Tahan, V., & Kariman, K. (2023). In vitro antibacterial activity of essential oils from Carum copticum and Ziziphora clinopodioides plants against the phytopathogen Pseudomonas syringae pv. syringae. *Plant Biosystems-An International Journal Dealing with All Aspects of Plant Biology*, *just-accepted*, 1–10.

Gaikwad, S., Gadiya, S., & Tungar, S. (2023). Phytochemical Analysis of Black Turmeric Curcuma Caesia Roxb. *Journal of Medicinal Plants*, *11*(1), 41–43.

Ghurghure, S. M., Dhange, A. A., Kamalapure, N. R., Kate, S. N., Katkar, A. R., Katta, A. V, Kattimani, C. U., & Kattimani, S. G. (2019). Formulation and Evaluation of Herbal Hand Wash Gel

using Nerium oleander. *Research Journal of Topical and Cosmetic Sciences*, *10*(1), 1–6.

Gideon, M. (2023). *Novel Optimization of Psidium guajava Antibacterial Activity for Drug Discovery and Development*.

Giri, B. R., Baral, R., Bhatt, H., Khadka, A., Tamrakar, R., Timalsina, G., & Gyawali, R. (2023). Phytochemical Screening, Free-Radical Scavenging Activity, in vitro Alpha-Amylase Inhibitory Activity, and in vivo Hypoglycemic Activity Studies of Several Crude Drug Formulations Based on Selected Medicinal Plants of Nepal. *Pharmaceutical Chemistry Journal*, 1–10.

Gueboudji, Z., Kadi, K., Mahmoudi, M., Hannachi, H., Nagaz, K., Addad, D., Yahya, L. Ben, Lachehib, B., & Hessini, K. (2023). Maceration and liquid–liquid extractions of phenolic compounds and antioxidants from Algerian olive oil mill wastewater. *Environmental Science and Pollution Research*, *30*(2), 3432–3439.

Howden, B. P., Giulieri, S. G., Wong Fok Lung, T., Baines, S. L., Sharkey, L. K., Lee, J. Y. H., Hachani, A., Monk, I. R., & Stinear, T. P. (2023). Staphylococcus aureus host interactions and adaptation. *Nature Reviews Microbiology*, 1–16.

Menteri Kesehatan RI. (2008). Farmakope Herbal Indonesia Edisi I. *Jakarta: Dirjen Pelayanan Farmasi Dan Alat Kesehatan*.

Kavya, B., King, B., Rigsbee, A. S., Yang, J. G., Sprinkles, W., Patel, V. M., McDonald, A. A., Amburn, S. K., & Champlin, F. R. (2023). Influence of outer membrane permeabilization on intrinsic resistance to the hydrophobic biocide triclosan in opportunistic Serratia species. *Heliyon*.

Kemenkes, R. I. (2017). Farmakope Herbal Indonesia Edisi II. *Jakarta: Kementrian Kesehatan RI*.

Kusarkar, P., Kupkar, M., & Dudhgaonkar, T. (2022). A Study on Formulation and Evaluation of Herbal Hand Sanitizer and Herbal Handwash. *Asian Journal of Pharmaceutical Research*, *12*(3), 199–202.

Loftus, R. W., Dexter, F., & Brown, J. R. (2023). Staphylococcus aureus Transmission in the Anaesthesia Work Area Has Greater Risk of Association with Development of Surgical Site Infection when Resistant to the Prophylactic Antibiotic Administered for Surgery. *Journal of Hospital Infection*.

Mailoa, M. N., Mahendradatta, M., Laga, A., & Djide, N. (2013). Tannin extract of guava leaves (Psidium guajava L) variation with concentration organic solvents. *International Journal of Scientific and Technology Research*, *2*(9), 106–110.

Mailoa, M. N., Mahendradatta, M., Laga, A., & Djide, N. (2014). Antimicrobial activities of tannins extract from guava leaves (Psidium guajava L.) on pathogens microbial. *International Journal of Scientific & Technology Research*, *3*(1), 236–241.

Mariyammal, V., Sathiageetha, V., Amalraj, S., Gurav, S. S., Amiri-Ardekani, E., Jeeva, S., & Ayyanar, M. (2023). Chemical profiling of Aristolochia tagala Cham. leaf extracts by GC-MS analysis and evaluation of its antibacterial activity. *Journal of the Indian Chemical Society*, *100*(1), 100807.

Mohapatra, B., Mohapatra, S., & Sharma, N. (2023). Biosynthesized Ag–ZnO nanohybrids exhibit strong antibacterial activity by inducing oxidative stress. *Ceramics International*.

Mondal, K. C., Banerjee, D., Jana, M., & Pati, B. R. (2001). Colorimetric assay method for determination of the tannin acyl hydrolase (EC 3.1. 1.20) activity. *Analytical Biochemistry*, *295*(2), 168–171.

Ndako, J. A., Oludipe, E. O., Junaid, S. A., Echemita, R. F., Fajobi, V. O., Dojumo, V. T., Ndako, P. J., & Omole, A. O. (2023). *Antibacterial Potency of Fractions from Psidium guajava Leaves and Bark Crude Extracts against Bacterial Isolates Responsible for Foodborne Infection*.

Ngwanguong, T. E., Teke, G. N., Tamekou, S. L., Njuabe, M. T., & Kuiate, J.-R. (2023). In-vitro anti-salmonella activity of methanol and aqueous extracts and their associations of Psidium guajava and Carica papaya leaves. *Investigational Medicinal Chemistry and Pharmacology*, *Vol. 6 No. 1*.

Patel, A., Kushwah, P., Pillai, S., Raghuvanshi, A., & Deshmukh, N. (2017). Formulation and evaluation of Herbal Hand wash containing Ethanolic extract of Glycyrrhiza glabra root extract. *Research Journal of Pharmacy and Technology*, *10*(1), 55–57.

Rajad, S., Karodi, R., Dhanake, K., Kohakde, S., & Bendre, S. (2023). Formulation And Evaluation of Polyherbal Mouth Ulcer Gel Containing Bombax Ceiba Thorn Extract and Psidium Guajava Leaf Extract. *Journal of Coastal Life Medicine*, *11*, 845–857.

Rani, Z., Nasution, H. M., Kaban, V. E., Nasri, N., & Karo, N. B. (2023). Antibacterial activity of freshwater lobster (Cherax quadricarinatus) shell chitosan gel preparation against Escherichia coli and Staphylococcus aureus. *Journal of Applied Pharmaceutical Science*, *13*(2), 146–153.

Septiana, E., Rachman, F., Prasetyoputri, A., Izzati, F. N., Rahmawati, S. I., Hapsari, Y., Wulandari, D. A., & Putra, M. Y. (2023). The impact of extraction method and solvent on biological activities of garlic extract. *AIP Conference Proceedings*, *2606*(1), 020020.

Shah, T., shahab Khan, M., & Alam, B. (2023). Phytochemical screening of Salvia moorcroftiana plant extracts qualitatively. *Phytopharmacology Research Journal*, *2*(1), 30–40.

Singh, C., Anand, S. K., Upadhyay, R., Pandey, N., Kumar, P., Singh, D., Tiwari, P., Saini, R., Tiwari, K. N., & Mishra, S. K. (2023). Green synthesis of silver nanoparticles by root extract of Premna integrifolia L. and evaluation of its cytotoxic and antibacterial activity. *Materials Chemistry and Physics*, 127413.

Soltanian, S., Mohamadi, N., Rajaei, P., Khodami, M., & Mohammadi, M. (2019). Phytochemical composition, and cytotoxic, antioxidant, and antibacterial activity of the essential oil and methanol extract of Semenovia suffruticosa. *Avicenna Journal of Phytomedicine*, *9*(2), 143.

Thamer, F. H., Thamer, N., Alhamzi, A., Al-Ansi, N., Al-Sadi, S., & Al-Shibeh, A. (2023). Antioxidant Capacity, Total Phenol Contents and Phytochemical Screening of Citrullus colocynthis Crust, Pulp and Seeds Extracts. *Am. J. Biochem. Biotechnol*, *19*(1), 12–19.

Vindhya, P. S., Suresh, S., Kunjikannan, R., & Kavitha, V. T. (2023). Antimicrobial, antioxidant, cytotoxicity and photocatalytic performance of Co doped ZnO nanoparticles biosynthesized using Annona Muricata leaf extract. *Journal of Environmental Health Science and Engineering*, 1–19.

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