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HANDSANITIZER FROM KITOLOD LEAF EXTRACT: OPTIMIZED ANTIBACTERIAL GEL AGAINST *Staphylococcus aureus* WITH SNI 2588:2017 QUALITY ANALYSIS

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ABSTRACT: This research aims to test the effectiveness of hand sanitizer based on kitolod leaf extract as a natural alternative for cleaning hands. The research method includes extraction of kitolod leaves, phytochemical testing, making hand sanitizer, antibacterial test, swab test, and analysis based on SNI 2588:2017 standards. Extraction of kitolod leaves using 96% ethanol and phytochemical tests showed the presence of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. In the antibacterial test against *Staphylococcus aureus*, the hand sanitizer formula with 32% kitolod extract showed an inhibition zone of 19.7 ± 0.35 mm. The market hand sanitizer with 70-95% alcohol showed the highest inhibition zone (29.7 ± 5.15 mm). Quality testing of Formula 1 hand sanitizer with SNI 2588:2017 standards, shows that the product meets the established quality requirements. This product has a pH of 6.44 ± 0.45, the expected pH standard to avoid skin irritation. The TPC test results also show that this product's number of bacterial colonies is below the set limit of 2.75x102 ± 5.03 colonies/g, confirming the product's safety as a hand sanitizer.

Keywords: Antibacterial, Hand Sanitizer, Kitolod Leaf Extract, Phytochemical Testing, SNI 2588:2017.

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INTRODUCTION

Along with the increasing busyness of people, especially in urban areas, and the number of instant products that are fast and practical, there is an innovation in waterless hand sanitizers known as antiseptic hand sanitizers or hand sanitizers (Manullang & Yogopriyatno, 2022). In an era increasingly focused on efficiency, hand sanitizer has surged as the primary alternative to hand soap. This change is due to various factors, including increased busyness, practicality, and the notion that hand sanitizer delivers comparable results to soap (Noval et al., 2020). The broader use of hand sanitizer is primarily inspired by its ease and speed in cleaning hands. They are considered more practical, especially when access to water and soap is limited, such as in public places, public transportation, or during travel (Taringan & Arsad, 2022).

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Hand sanitizers on the market generally contain alcohol group compounds with concentrations ranging from 50% to 70% (Najiyah, 2021). This concentration is proven effective as an antiseptic that can inhibit or kill bacteria. Nonetheless, the use of alcohol in large quantities is necessary due to its role as the main ingredient. However, it is essential to keep in mind that alcohol can dissolve the fat and serum layers of the skin and can potentially irritate the skin of those sensitive to alcohol (Maharani, 2014). In response to these concerns, natural ingredients are considered safer alternatives and are the focus of development in hand sanitizer manufacturing (Sasmita, 2019). The public's growing interest in re-adopt a natural approach, often referred to as "back to nature," seems to be reflected in health and beauty maintenance efforts (Widyani, 2020). This is evident in the number of products that use active ingredients from plants for health care, beauty (Handayani, 2022; Raslina et al., 2018), and disease prevention (Munarsih et al., 2022).

Research by Ariningsih et al. (2014), showed that natural materials containing secondary metabolite compounds, such as flavonoids, alkaloids, saponins, and tannins, have the potential as natural antiseptic substances. Secondary metabolite compounds have been widely used as dyes, food aromas, and traditional medicines (Yustiana, 2021). The existence of these compounds provides a strong foundation for further research in utilizing the natural antiseptic properties of these natural ingredients in the development of hand sanitizers that are more friendly to the skin but still effective in cleaning and protecting from germs (Herdiana et al., 2023). One of the natural ingredients that take center stage in this innovation is kitolod leaves. Previous research has revealed that kitolod leaves contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, triterpenoids, and steroids (Gupta et al., 2020). The unique combination of these components provides a solid basis for making kitolod, which leaves a potential ingredient in manufacturing hand sanitizers.

Based on research conducted by Ramayani (2022), which has been conducted previously, antibacterial testing proves that kitolod leaf extract can inhibit the growth of *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a gram-positive and commensal bacterium that colonizes 30% of healthy individuals in various parts of the body (Raheem et al., 2022). *Staphylococcus aureus* is a significant cause of skin, soft tissue, respiratory, bone, joint, and endovascular disorders (Gupta et al., 2020). Transmission of *Staphylococcus aureus* can occur through close or direct contact from one person to another, sharing personal items, food contamination, and fomite contamination such as door algae (Ramayani, 2022).

This study differs from previous studies in that it conducted antibacterial activity testing against *S. aureus* bacteria and included a swab test as part of the study. The swab test is a significant additional step to ensure the cleanliness and effectiveness of hand sanitizers in real-world situations. In addition, applying parameters based on SNI 2588:2017 confirms the commitment of hand sanitizer manufacturers to meet nationally established quality standards. The importance of the swab test and adherence to the SNI 2588:2017 standard provides a new dimension to this study. The swab test allows researchers to measure the

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effectiveness of hand sanitizer in removing germs on various surfaces directly. At the same time, the SNI standard provides clear guidance to ensure the product is safe and effective. This research aims to test the effectiveness of hand sanitizer based on kitolod leaf extract as a natural alternative for cleaning hands. With this innovation, it is hoped that kitolod leaf-based hand sanitizers can provide a more holistic and effective solution to hand hygiene. This innovation enriches the hand sanitizer formulation with potential natural ingredients and affirms the commitment to meet the highest quality standards. Thus, these steps create an effective product and give users the confidence of guaranteed safety and hygiene.

METHOD

Materials and Tools

Materials

In the process of extracting kitolod leaves, the materials needed are the kitolod leaves themselves, 96% ethanol, methanol, concentrated HCl, magnesium powder, 0.05 N ammonia, 2 N H_2SO_4, anhydrous acetic acid, 1% FeCl3, and distilled water. As for the manufacture of hand sanitizers, materials such as Carbopol as a thickener, TEA (Triethanolamine) for pH adjustment, glycerol, apple essences, Na-Benzoate, and distilled water as a solvent to mix all ingredients evenly.

Tools

In the extraction process of kitolod leaves, the tools used include an oven for drying kitolod leaves, scales to weigh the ingredients appropriately, test tubes to carry out the phytochemical test process and compound identification, pipettes to take liquid volume, and a spectrophotometer for measuring the absorbance value of bacteria. Meanwhile, in making hand sanitizers, tools such as a hotplate for heating the solution, an Erlenmeyer for mixing ingredients, a pH meter for measuring acidity, and a petri dish for antibacterial tests are used.

Kitolod Leaf Extraction

Kitolod leaves from Wedomartani Village, Ngemplak District, Sleman Regency, DI Yogyakarta, which are dark green, are processed into simplisia by drying them using an oven at 40-50°C. After that, the kitolod leaves were pollinated to become crude plant powder. a 4.5 kg crude plant powder was then macerated using 45 L of 96% ethanol. The filtrate from the maceration was then evaporated to produce a thick extract. The resulting thick extract was then subjected to phytochemical tests.

Phytochemical Test

Flavanoid Test

A flavonoid identification test was conducted using the cyanidin test. A 0.1-gram extract sample was heated for 5 minutes in 5 mL of 30% methanol. Then, the filtrate was added with a few drops of concentrated HCl and magnesium powder. The formation of red color indicates the presence of flavonoid compounds (Hidayah et al., 2022).

Steroid/Titerpenoid Alkaloid Test

Identification of alkaloids and steroids/triterpenoid derivatives was carried out using the Culvenor-Fitzgerald method. A 4-gram extract sample was

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pulverized and crushed with chloroform to form a paste. Then, 10 mL of 0.05 N ammonia was added and crushed again. After that, it was filtered into a test tube. 2 N H_2SO_4 was added and shaken vigorously. The filtrate was left until two layers (chloroform and sulfuric acid) were formed. The two layers were separated with a pipette. The sulfuric acid layer is used for alkaloid identification by adding Wagner's reagent. If a brown precipitate forms or the solution becomes cloudy, it indicates the presence of alkaloid compounds. The chloroform layer is used to identify the presence of steroid and triterpenoid compounds. The chloroform layer formed is transferred to a cup, then five drops of anhydrous acetic acid are added and allowed to dry. After drying, three drops of H_2SO_4 were added. If there is a change in red or purple color, it indicates the presence of triterpenoids. If it turns blue, it indicates the presence of steroids**.**

Saponin Test

The alkaline test was conducted to confirm the identification of saponins. A finely-dried sample of bamboo leaves was put into a test tube, and distilled water was added. After heating at 100°C for three minutes and cooling, it was shaken energetically for one minute. A foam that forms at least 1 cm in height and remains stable after standing for 15 minutes indicates the presence of saponins.

Tannin Test

Tannin is identified by adding 0.1 grams of extract into 5 mL of distilled water in a test tube, then boiling for 5 minutes. After boiling, the residue and filtrate were separated by filtration. $FeCl_3$ 1% solution is added to the resulting filtrate. If a dark blue or blackish green color forms, indicating the presence of tannin.

Preparation of Hand Sanitizer

Making hand sanitizer with kitolod leaf extract follows the following steps. First, the ingredients weighed according to the formulation are mixed gradually. Water (distilled water) as much as 50 mL is poured while stirring, then slowly adding glycerol to mix evenly. Next, kitolod leaf extract was added to the mixture while stirring until evenly distributed, followed by apple essences and Na-Benzoate. After mixing the liquid ingredients, Carbopol is added slowly while stirring until it is well dissolved and forms the desired gel consistency. The stirring process should continue until the mixture is evenly distributed and the desired consistency is achieved. Next, the pH of the mixture is checked to ensure it conforms to the desired standard. pH correction is done if required by adding an appropriate amount of TEA.

Table 1. Formulation of Kitolod Leaf Extract Hand Sanitizer.

Compositions	F1 (%)	F2 (%)	F3 (%)
Kitolod Leaf Extract	32	16	8
Carbopol	1.5	1.5	1.5
TEA	1.1	1.12	1.12
Glycerol	25.2	25.2	25.2
Apple Essences	1	1	1
Na-Benzoat	0.1	0.1	0.1
Aquades ad	100	100	100

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Antibacterial Test against *Staphylococcus aureus* ATCC 25923

Preparation of Staphylococcus aureus ATCC 25923 Bacterial Culture

Bacteria from the culture were taken and dissolved in an aseptic 0.9% NaCl solution (Rondhianto et al., 2015). The turbidity of the suspension was visually compared with the McFarland 5 standard, and then the absorbance value was measured using a spectrophotometer in the wavelength range of 400-600 nm (Rizki et al., 2022). The results showed that the highest absorbance value reached 0.5209 at a wavelength of 450 nm. By looking at the turbidity level comparable to the McFarland 5 standard, it can be concluded that the suspension of *Staphylococcus aureus* bacteria amounted to 1,500 bacteria.

Antibacterial Test

One to two ounces of pure culture was suspended for 24 hours. Then, 1 ml of the suspension was pipetted into a petri dish, followed by thawed NA media in the petri dish containing the bacteria. After that, it was homogenized to form a figure-eight pattern and allowed to freeze. Millipore paper treated with dental extract, positive control, and hand sanitizer formulations with various concentrations is placed aseptically into the Petri dish. This process was followed by incubation at 35ºC for 24 hours (Ramayani, 2022). Microbial growth was recorded, and the transparent zone around the millipore paper plate was observed and measured.

Swab Test

Media Preparation

To prepare Nutrient Agar (NA) media, 6 grams of Nutrient Agar was weighed and then dissolved in an Erlenmeyer until it reached a volume of 160 mL. This solution was heated on a hotplate until boiling. Next, 15 mL of NA solution was pipetted and put into a test tube, then closed and sterilized in an autoclave for 1 hour at 121˚C.

Swab Test

For testing, 5 mL of sterile distilled water was prepared in a test tube, and a swab rod was inserted into the sterile distilled water. Swabbing was performed on the hand area before and after contact with the sample and standard. Next, the swab rod is inserted into sterile distilled water again. 1 mL of the test swab was put into a petri dish, added with NA media, and homogenized. After that, incubation was carried out at ±35˚C for 48 hours (Ramayani, 2022). Finally, the results were calculated and recorded for further evaluation.

Analysis Based on SNI 2588:2017

pH Test

To start the pH measurement, the calibrated pH meter was turned on. Next, the electrode of the pH meter is inserted into the product to be measured, and the standard hand sanitizer is used for comparison. After that, the measured pH readings are recorded to obtain information about the acidity or basicity of the two products.

Total Plate Numbers Test

1) Preparation of Media and Solvent Controls

To make media control and solvent control, the initial preparation involved 2 Petri dishes. PCA media as much as 15 mL was poured into one of the Petri

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dishes for media control. Next, 1 mL of BPW was pipetted into a petri dish containing 15 mL of PCA media for solvent control. Both Petri dishes were homogenized by making a figure-eight motion on the table 12 times. After that, both Petri dishes were incubated at 25˚C twice for 24 hours, and the growing colonies were counted and recorded.

2) Sample Examination

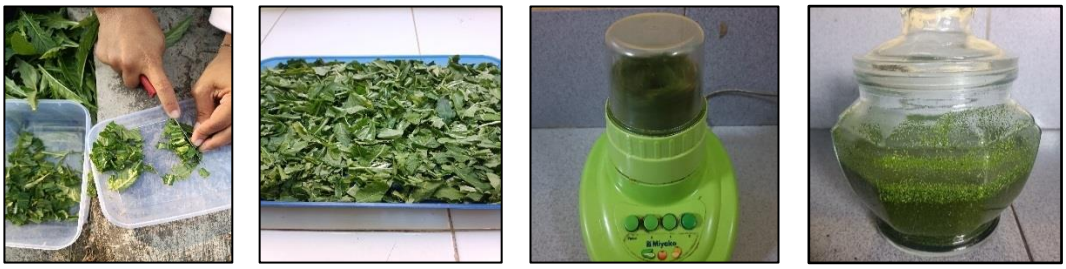
For sample examination, 1 mL of Handsanitizer product was pipetted into a sterile test tube containing 9 mL of BPW to perform a 10-1 dilution. Then, 1 mL of the 10-1 dilution was pipetted into a test tube containing 9 mL of BPW for dilution 10-2. This is followed by taking 1 mL from dilution 10-2 and put into a test tube containing 9 mL BPW for dilution 10-3. Furthermore, 1 mL of each dilution was pipetted into a sterile cup, and 15 mL of PCA media was done in duplicate. The whole was then incubated for 48 hours to observe colony growth.

Data Analysis

All quantitative data were statistically analyzed using the One-Way ANOVA method at 95% confidence level and Tukey HSD post hoc test. Statistical analysis was performed using the IBM SPSS 25 application program.

RESULT AND DISCUSSION

Hand sanitizer begins with washing, drying, and pulverizing kitolod leaves, which are then made into extracts through the maceration process. After the maceration and filtering process, the extract was concentrated using a rotary evaporator. The result was a thick green extract with a yield of 13.78%. This extract is used for phytochemical tests and as an ingredient in Hand Sanitizer. Phytochemical tests were carried out to identify the presence of alkaloids, flavonoids, saponins, and tannins in the extract. Phytochemical screening of extracts and hand sanitizer formulas is carried out to identify compounds' content. The findings of phytochemical screening are by Mareintika`s research (2021), showing that the extracts and hand sanitizer gel preparations contain alkaloids, flavonoids, saponins, and tannins, which are thought to have antibacterial properties. Table 2 shows the results of phytochemical screening.



Picture 1. Cutting Kitolod Leaves After Washing, Preparation of Kitolod Leaf Drying, Kitolod Leaf Dry Powdering, and Kitolod Leaf Maceration.

Table 2. Phytochemical Screening Results.

Phytochemical Screening Results				
Flavonoids	**Alkaloids**	**Saponins**	**Tannins**	**Steroids/Triterpenoids**
++	++	++	+	+^{s}

++: Very Strong Result; +: Strong Result; -: Negative; ^{T}: Triterpenoids; ^{S}: Steroids

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Phytochemical test results indicated the presence of flavonoids, saponins, steroidal alkaloids, and tannins. Flavonoids, as antibacterial agents, form extracellular protein complexes that disrupt the integrity of the bacterial cell membrane (Donadio et al., 2021). Saponins are active ingredients that increase membrane permeability, resulting in cell hemolysis (Ondevilla et al., 2023). The interaction of saponins with bacteria or fungi can cause damage or lysis of these cells. Tannins have antibacterial properties by shrinking the cell wall or cell membrane, disrupting the permeability of bacteria, which ultimately inhibits the ability of bacterial cells to perform their life functions, inhibiting their growth (Chen et al., 2022; Sulistyarsi et al., 2023). In the context of their antibacterial mechanism, Steroidal alkaloids may act by several potential mechanisms. For example, some steroidal alkaloids have been shown to have the ability to disrupt bacterial cell wall synthesis or inhibit vital enzymes necessary for bacterial growth (Elafify & Shi, 2022). They can also interfere with the replication process of bacterial DNA or interact with cellular transport mechanisms essential for bacterial life. Steroidal alkaloids in kitolod leaves could strengthen their antibacterial properties through these mechanisms.

The presence of natural antiseptic compounds in kitolod leaf extract makes it an active component in Hand Sanitizer. In addition, the components involved in making hand sanitizer include gelling agent (Carbopol 940), humectant (glycerin), pH regulator (Tri Ethanol Amin), and solvent (aquadest). The gelling agent serves as the basis of gel formation that forms the texture of the gel mass. Humectants are used to keep the product moisturized and increase the moisture content of the skin layer during use. The pH regulator is responsible for balancing the acidity or basicity of the gel, while the solvent plays a role in dissolving the ingredients to form a uniform hydrogel preparation.

Antibacterial Test against *Staphylococcus aureus* ATCC 25923 Culture and Hand Swab Test

The preparation of hand sanitizer was carried out using different amounts of extracts, namely 32% in formula 1, 16% in formula 2, and 8% in formula 3. Various amounts of extracts aim to find the optimal formulation for inhibiting bacterial growth, tested through antimicrobial and swab tests. The following are the observation results of the antimicrobial and swab tests (Table 3).

Table 3. Results of Antibacterial Test against *Staphylococcus aureus* and Swab Test.

Samples	Antibacterial (mm Zone of Inhibition)	Bacteria Count on Hand/Swab Test (colonies/gram)			
		24 Hours		48 Hours	
		Before	After	Before	After
Kitolod Extract	19.7 ± 0.35^{b}	-	-	-	-
Formula 1	17.5 ± 0.53^{b}	151.3 ± 9.01^{c}	82 ± 4.00^{b}	355.3 ± 6.02^{c}	164.7 ± 10.69^{c}
Formula 2	7.5 ± 1.33^{a}	35 ± 6.00^{a}	111 ± 16.82^{c}	127 ± 7.00^{b}	317 ± 20.66^{d}
Formula 3	4.3 ± 0.58^{a}	35 ± 4.00^{a}	58 ± 7.21^{a,b}	110 ± 17.03^{a,b}	90 ± 6.56^{b}
Market Hand Sanitizer	29.7 ± 5.15^{c}	70.33 ± 8.02^{b}	35 ± 5.57^{a}	91.3 ± 6.11^{a}	39 ± 5.57^{a}
Media Control	0 ± 0.00^{d}	0 ± 0.00^{d}	0 ± 0.00^{d}	0 ± 0.00^{d}	0 ± 0.00^{e}

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Samples	Antibacterial (mm Zone of Inhibition)	Bacteria Count on Hand/Swab Test (colonies/gram)			
		24 Hours		48 Hours	
		Before	After	Before	After
Solvent Control	0 ± 0.00[d]	0 ± 0.00[d]	0 ± 0.00[d]	0 ± 0.00[d]	0 ± 0.00[e]

In interpreting the Tukey test data, the letters used refer to significant differences between the groups in the test. Here, different letters indicate significant differences between the groups. The antibacterial test results showed that the hand sanitizer from the market showed the highest zone of inhibition (29.7 ± 5.15 mm), followed by kitolod extract (19.7 ± 0.35 mm), which showed significant antibacterial activity. Formula 1 (17.5 ± 0.53 mm) also showed a considerable zone of inhibition, although lower than the kitolod extract. Formula 2 (7.5 ± 1.33 mm) and Formula 3 (4.3 ± 0.58 mm) showed smaller inhibition zones, with Formula 3 showing the most minor antibacterial activity among the other formulations.

It should be noted that hand sanitizers from the market show the most potent antibacterial activity because the market hand sanitizer used for comparison contains 70-95% alcohol. Alcohol acts by coagulating proteins and lipids, depending on the availability of water to permeabilize cell membranes. Kitolod leaf extract showed potent antibacterial activity despite having a lower inhibition zone. Meanwhile, formulations with lower concentrations of kitolod extract (Formula 2 and Formula 3) had smaller inhibition zones. These results suggest that the concentration of kitolod leaf extract affects the antibacterial activity of the product, with decreased activity in formulations containing lower extract concentrations. There are four levels of classification of bacterial growth inhibition, including inhibition zones of more than 20 mm said to be very strong, inhibition zones of 10-20 mm said to be strong, inhibition zones of 5-10 mm said to be moderate(Dikarulin et al., 2022) . Inhibition zones of less than 5 mm are said to be weak (Fikroh et al., 2020). The inhibition test results show that the comparison hand sanitizer has extreme activity. In contrast, kitolod leaf extract and formula one substantially inhibit the growth of *S. aureus* ATCC 25923 bacteria. The media control and solvent control showed no inhibition zone, confirming that the antibacterial activity is related to the active ingredient content in the hand sanitizer formulation and not due to the media or solvent used.

The swab test results illustrate the effectiveness of four hand sanitizer formulas (Formula 1, Formula 2, Formula 3, and Market Hand sanitizer) on the number of bacteria on the hands. In the 24-hour observation before using the hand sanitizer, Formula 1 showed an average of 151.3 ± 9.01 bacterial colonies, which then decreased significantly to 82 ± 4.00 colonies after using the hand sanitizer. At 48 hours, the average Formula 1 before use was 355.3 ± 6.02 bacterial colonies, which dropped significantly to 164.7 ± 10.69 colonies after using the hand sanitizer. This illustrates the ability of Formula 1 to reduce the number of bacteria after using hand sanitizers in both time spans. Formula 2 shows that in the 24-hour observation before using the hand sanitizer, the average number of bacteria was 35 ± 6.00 colonies, which increased significantly to 111 ± 16.82 colonies after using the hand sanitizer. At 48 hours, the average before use was

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127 ± 7.00 colonies, which increased significantly to 317 ± 20.66 colonies after using the hand sanitizer. This shows that although Formula 2 reduces the number of bacteria in some cases, it also aggravates the bacterial condition of the hands in some instances. Formula 3 shows a slightly different change. In the 24-hour observation before using the hand sanitizer, the average number of bacteria was 35 ± 4.00 colonies, which increased slightly to 58 ± 7.21 colonies after using the hand sanitizer. At 48 hours, the average before use was 110 ± 17.03 colonies, which dropped to 90 ± 6.56 colonies after using the hand sanitizer. Formula 3 showed a slight decrease at 48 hours after using the hand sanitizer but still showed an increase in some cases.

Market hand sanitizer shows that in the 24-hour observation before using the hand sanitizer, the average number of bacteria was 70.33 ± 8.02 colonies, slightly increasing to 91.3 ± 6.11 colonies after using the hand sanitizer. At 48 hours, the average before use was 91.3 ± 6.11 colonies, which increased to 39 ± 5.57 colonies after using the hand sanitizer. The Market hand sanitizer showed a slight decrease 48 hours after using the hand sanitizer, although it still showed an increase in the initial observation. This indicates that Formula 1 showed the most consistent decrease in bacterial counts at 24 and 48 hours after hand sanitizer application, followed by Formula 3, Market hand sanitizer, and Formula 2, which showed less favorable results in some cases.

Formula 1 showed the most significant reduction in the number of bacteria between 24 and 48 hours after using the hand sanitizer due to the higher concentration compared to the other formulas. These results indicate that Formula 1 has the potential to be an effective formula in reducing the number of bacteria on the hands. The next step is to conduct quality testing based on the Indonesian National Standard (SNI) 2588: 2017. This test will focus on pH and total plate count (TPC) parameters to evaluate the quality and safety of Formula 1 hand sanitizer. The pH measurement will provide an overview of the acidity or basicity of the hand sanitizer. In contrast, the TPC will provide information on the total number of bacteria in the product sample. The results of this quality testing will help ensure that Formula 1 is effective in reducing the number of bacteria on hands and meets the quality standards set by SNI 2588:2017 for hand sanitizer products.

Quality Test of the Best Formula Hand Sanitizer (F3) Based on SNI 2588:2017

For several important reasons, Kitolod leaf extract hand sanitizer needs to be tested for quality according to SNI 2588:2017 standards. First, pH testing is essential because it determines the acidity or basicity of the product. Maintaining an appropriate pH can avoid irritation to the user's skin. In addition, too acidic or alkaline products can also interfere with comfort during use. Furthermore, Total Plate Number (TPC) testing is essential because it shows the number of bacterial colonies that can grow in the sample the SNI 2588:2017 standard limits the number of bacterial colonies in hand sanitizer products. TPC testing helps ensure that the product does not contain excess bacteria, which could be a potential risk of infection or health hazard to the user.

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Conformity of kitolod leaf extract hand sanitizer to these standards is a significant quality indicator before the product is distributed to the market or widely used by the public. This ensures that the product is safe, non-irritating, and can provide protection from bacterial contamination of the user's skin. The following are the results of hand sanitizer quality testing based on SNI 2588: 2017 (Table 4).

Table 4. Quality Requirements According to SNI 2588:2017 and Test Results.

Criteria	Units	Requirements	Results
pH	-	4-10	6.44 ± 0.45
TPC	Colonies/g	Maks. $1x10^3$	$2.75x10^2$ ± 5.03

The test results showed that the product met the established quality standards, with a dosage pH of 6.44 ± 0.45. This occurs because the carbopol gel base is initially dissolved in distilled water as free acid. To stabilize the pH, neutralizing agents in negatively charged bases were added. The addition of tri ethanol amine as a gel neutralizing agent is carried out until it reaches pH 6.44 ± 0.45 by controlling hand sanitizer products on the market. The importance of keeping the pH of the hand sanitizer not too acidic or alkaline is to prevent skin irritation or other discomfort when used.

Hand sanitizer testing using the Total Plate Count (TPC) method showed results for 48 hours. The greater the dilution, the number of bacterial colonies decreases. The optimum time for bacterial colony growth in samples is 48 hours at 35±1˚C. The test was conducted by pouring the sterilized media into a sterile petri dish and homogenizing it with the sample to spread the bacteria on the surface of the oxygen-rich medium and inside it. The 10^{-1} dilution yielded $27.5x10^1$ colonies/g, then reduced to $2.75x10^2$ in the 10^{-2} dilution. TPC analysis was made with serial dilutions from 10-1 to 10^{-3}, aiming to reduce the density of bacteria grown. The TPC method ensures the safe use of products on the skin, especially hand sanitizers that are often applied to the hands. Besides being used on food and beverages, the TPC method is also effective in testing hand sanitizers.

CONCLUSION

Kitolod leaf extract used in hand sanitizer manufacture exhibits vigorous phytochemical activity, containing compounds such as flavonoids, saponins, steroid alkaloids, and tannins that potentially have antibacterial properties. This active component plays a vital role in the ability of hand sanitizers to inhibit the growth of bacteria, especially *S. aureus* ATCC 25923. From the results of antibacterial and swab tests, Formula 1 showed the most consistent effectiveness in reducing the number of bacteria between 24 and 48 hours after using hand sanitizer, followed by Formula 3, Hand Sanitizer Market, and Formula 2. Using kitolod leaf extract in Formula 1 significantly reduces the number of bacteria on the hands after using hand sanitizer. In addition, testing the quality of Formula 1 hand sanitizers with SNI 2588:2017 standards shows that the product meets the established quality requirements. This product has a pH of 6.44 ± 0.45, the expected pH standard, to avoid skin irritation. TPC test results also show that the number of bacterial colonies in this product is below the set limit of $2.75x10^2$ ±

5.03 colonies/g, confirming the product's safety for use as a hand sanitizer. From these results, it can be concluded that Formula 1 has the potential to be an effective hand sanitizer in reducing the number of bacteria on the hands and has met the quality standards set by SNI 2588:2017. The next step is to consider Formula 1 as a viable option for further development as a safe and effective hand sanitizer product.

ADVICE

Further research is needed to optimize formulations, understand the mechanism of action of active compounds, and test product safety and effectiveness in human users. These follow-up studies could expand knowledge about the potential use of kitolod leaf extract in sanitary products and the broader health and environmental implications.

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REFERENCES

Ariningsih, U., Aziz, A., & Musyaddad, H. (2014). Produksi Handsang "*Handsanitizer* Berbahan Utama Pelepah Pisang" sebagai Program Percontohan UMKM. In *Pekan Ilmiah Mahasiswa Nasional Program Kreativitas Mahasiswa - Pengabdian Kepada Masyarakat* (pp. 1-7). Jakarta, Indonesia: Indonesian Ministry of Research, Technology and Higher Education.

Chen, W., Xia, S., & Xiao, C. (2022). Complex Coacervation Microcapsules by Tannic Acid Crosslinking Prolong the Antifungal Activity of Cinnamaldehyde Against *Aspergillus brasiliensis*. *Food Bioscience, 47*(1), 1-20. https://doi.org/10.1016/j.fbio.2022.101686

Davis, W., & Stout, T. (1971). Disc Plate Method of Microbiological Antibiotic Assay: I. Factors Influencing Variability and Error. *Applied Microbiology, 22*(4), 659-665. https://doi.org/10.1128/am.22.4.659-665.1971

Dikarulin, S. A., Listyaningrum, D. A. D., Ananda, B. S., Putri, T. A. Amirta, R., Yuliansyah., Kusuma, I. W., Ramadhan, R., Rosamah, E., Suwinarti, W., & Arung, E. T. (2022). Uji Aktivitas Antibakteri Ekstrak Propolis Lebah *Heterotrigona itama* dari Beberapa Lokasi Budidaya di Kalimantan Timur terhadap Bakteri *Propionibacterium acnes*. *Ulin : Jurnal Hutan Tropis, 6*(2), 121-125. http://dx.doi.org/10.32522/ujht.v6i2.7824

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. *Pharmaceutics, 13*(5), 1-23. https://doi.org/10.3390/pharmaceutics13050660

Elafify, M., & Shi, C. (2022). Response of Foodborne Pathogens to Phytochemicals. In *Stress Responses of Foodborne Pathogens* (pp. 367-398). Berlin, Germany: Springer Nature.

Fikroh, R. A., Matsjeh, S., & Anwar, C. (2020). Aktivitas Antibakteri Turunan Kalkon Tersubstitusi Bromo terhadap Bakteri *Bacillus subtilis* dan *Escherichia coli*. *Al-Kimia, 8*(1), 72-82. https://doi.org/10.24252/al-kimia.v8i1.12024

Gupta, N., Jain, V., Joseph, M. R., & Devi, S. (2020). A Review on Micropropagation Culture Method. *Asian Journal of Pharmaceutical Research and Development, 8*(1), 86-93. http://dx.doi.org/10.22270/ajprd.v8i1.653

Handayani, D. (2022). Uji Formulasi Krim Ekstrak Rimpang Kunyit (*Curcuma longa* Linn) terhadap Penyembuhan Luka Sayat pada Tikus Jantan (*Rattus norvegicus*). *Thesis*. Universitas Binawan.

Herdiana, N., Sugiharto, R., & Winanti, D. (2023). *Rempah dan Minyak Atsiri Daun*. Lampung: University of Lampung

Hidayah, M. A., Tunjungsari, G. P., Fikroh, R. A., Mashadi, H., Handayani, T., & Nurohmah, B. A. (2022). Antihyperuricemia Potential in Ethanol Extract of Siamese Bamboo (*Thyrsostachys siamensis*) Leaves on Male White House Mice (*Mus musculus* L.). *Walisongo Journal of Chemistry, 5*(2), 120-129. https://doi.org/10.21580/wjc.v5i2.10666

Maharani, R. K. (2014). Formulasi Sediaan Gel Antiseptik Tangan Minyak Atsiri Daun Kemangi (*Ocimum basilicum* L.) dengan Basis HPMC dan Aktivitas Antibakteri terhadap *Staphylococcus aureus*. *Skripsi*. Universitas Muhammadiyah Surakarta.

Manullang, C. N., & Yogopriyatno, J. (2022). Rancang Bangun *Hand Sanitizer* Otomatis sebagai Upaya Meminimalisir Penyebaran Covid-19 di Kelurahan Kandang Limun. *Jurnal Pengabdian Raflesia Administrasi Publik, 1*(1), 37-46.

Mareintika, R. (2021). Uji Efek Pemberian Antibakteri Ekstrak Daun Kitolod (*Isotoma longiflora* (L) Presl.) terhadap *Staphylococcus aureus*. *Jurnal Medika Hutama, 2*(4), 1084-1088.

Munarsih, E., Handayani, R., Hilma., Hasanah, M., & Romsiah. (2022). Pelatihan Pembuatan *Hand Sanitizer* sebagai Upaya Bersama Melawan COVID-19 di Desa Teloko. *Jurnal Abdimas Mandiri, 6*(2), 119-124. https://doi.org/10.36982/jam.v6i2.2322

Najiyah, P. S. A. (2021). Uji Efektivitas Ekstrak Daun Cengkih (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) sebagai *Hand Sanitizer* Alami dalam Menghambat Bakteri *Staphylococcus aureus*. *Thesis*. Universitas Siliwangi.

Noval, N., Nastiti, K., Nugraha, D. F., Rahmadani., & Alawiyah, T. (2020). Produk Inovasi *Hand Sanitizer* dari Akar Bajakah sebagai Upaya Pencegahan di Masa Pandemi Covid-19. *Logista : Jurnal Ilmiah Pengabdian Kepada Masyarakat, 4*(2), 305-312. https://doi.org/10.25077/logista.4.2.305-312.2020

Ondevilla, J. C., Hanashima, S., Mukogawa, A., Miyazato, D. G., Umegawa, Y., & Murata, M. (2023). Effect of the Number of Sugar Units on the Interaction Between Diosgenyl Saponin and Membrane Lipids. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1865*(5), 1-20. https://doi.org/10.1016/j.bbamem.2023.184145

Raheem, H. Q., Hussein, E. F., Rasheed, A. H., & Imran, N. K. (2022). Antibacterial Action of Silver Nanoparticles against *Staphylococcus aureus* Isolated from Wound Infection. *Research Journal of Pharmacy and Technology*, *15*(6), 2413–2416. https://doi.org/10.52711/0974-360X.2022.00401

Ramayani, S. L. (2022). Potensi Gel Handsanitizer Ekstrak Daun Kitolod (*Isotoma longiflora* L.) sebagai Antibakteri terhadap Bakteri *Staphylococcus aureus*. *Jurnal Jamu Kusuma, 2*(1), 8-13. https://doi.org/10.37341/jurnaljamukusuma.v2i1.26

Raslina, H., Dharmawibawa, I. D., & Safnowandi, S. (2018). Diversity of Medicinal Plants in National Park of Rinjani Mountain in Order to Arrange Practical Handout of Phanerogamae Systematics. *Bioscientist : Jurnal Ilmiah Biologi*, *4*(1), 1-6. https://doi.org/10.33394/bioscientist.v4i1.210

Rizki, S. A., Latief, M., Fitrianingsih., & Rahman, H. (2022). Uji Aktivitas Antibakteri Ekstrak N-Heksan, Etil Asetat, dan Etanol Daun Durian (*Durio zibethinus* Linn.) terhadap Bakteri *Propionibacterium acnes* dan *Staphylococcus epidermidis*. *Jambi Medical Journal : Jurnal Kedokteran dan Kesehatan, 10*(3), 442-457.

Rondhianto., Wantiyah., & Widyaputri, A. I. (2015). Perbedaan Penggunaan *Povidone Iodine* 1% dengan Nacl 0,9% sebagai Dekontaminasi Oral terhadap Kolonisasi *Staphylococcus aureus* pada Pasien *Post* Operasi dengan General Anesthesia di Ruang Mawar RSUD Dr. Abdoer Rahem Situbondo. *Jurnal Keperawatan, 6*(1), 34-43. https://doi.org/10.22219/jk.v6i1.2850

Sasmita, W. A. (2019) Pemanfaatan Cairan Pelepah Pisang dan Lidah Buaya sebagai *Hand Sanitizer* Alami. *Skripsi*. Poltekkes Kemenkes Yogyakarta.

Sulistyarsi, A., Rahayu, T., Primiani, C. N., & Pujiati. (2023). Phytochemical Analysis of Indonesian Gadung Mango Leaf (*Mangifera indica* L. var. Gadung) and Their Antibacterial Activity. *Biodiversitas Journal of Biological Diversity*, *24*(11), 6295-6304. https://doi.org/10.13057/biodiv/d241153

Taringan, S. F. N., & Arsad, N. (2022). Edukasi Pembuatan *Hand Sanitizer* Bahan-bahan Alami sebagai Upaya Pencegahan Penyebaran Covid-19. *JPKM : Jurnal Pengabdian Kesehatan Masyarakat, 3*(2), 109-119. https://doi.org/10.37905/jpkm.v3i2.15288

Widyani, R. (2020). *Book Chapter : Rumah Kita Dosen Indonesia (Inovasi Pembelajaran)*. Yogyakarta: Deepublish Publisher.

Yustiana, E. D. (2021). *Skrining* Fiktokimia Metabolit Sekunder Ekstrak Daun Keji Beling (*Strobilanthes crispus* BL). *Thesis*. STIKES Muhammadiyah Klaten.