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IN VITRO ANTIBACTERIAL ACTIVITY OF RAED® SABUN MANDIAN SEMAMBU BODY WASH PRODUCT AGAINST SKIN BACTERIA

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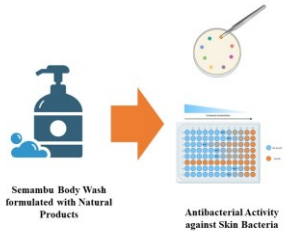
Graphical abstract



Abstract

Common bacteria associated with skin infections include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. Antibacterial body washes containing natural products are preferred for preventing skin infections without adverse effects, and Raed Sabun Mandian Semambu® is formulated with such natural ingredients. The aim of this study was to determine antibacterial activity of Raed Sabun Mandian Semambu® against bacteria that cause skin infection. Antibacterial activity was determined using diameter of zone of inhibition, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The time-kill assay was conducted at 0.5x MIC and 1x MIC for *S. aureus* and *S. epidermidis*, and at 0.5x MIC, 1x MIC, and 2x MIC for *S. pyogenes*, with incubation at 0, 2, 4, 6, 8, 10, and 24 hours. Results show inhibition zone diameters at product concentrations of 25%, 50%, 75%, and 100%. The MIC values indicate that the product inhibited the growth of all tested bacteria. The MBC/MIC ratio demonstrates bactericidal effect on all bacterial strains. The time-kill assay revealed bactericidal effect at 1x MIC for *S. aureus* and *S. epidermidis*, and at 2x MIC for *S. pyogenes*. In conclusion, Raed Sabun Mandian Semambu® exhibits a bactericidal effect on all tested bacteria.

Keywords: Antibacterial body wash, Bactericidal Effect, Natural Products, Semambu, Skin Infection

Abstrak

Bakteria biasa yang dikaitkan dengan jangkitan kulit termasuk *Staphylococcus aureus*, *Staphylococcus epidermidis*, dan *Streptococcus pyogenes*Pencuci badan antibakteria yang mengandungi produk semula jadi lebih digemari kerana dapat mencegah jangkitan kulit tanpa kesan sampingan, dan Raed Sabun Mandian Semambu® diformulasikan dengan bahan semula jadi sedemikian. Tujuan kajian ini adalah untuk menentukan aktiviti antibakteria Raed Sabun Mandian Semambu® terhadap bakteria yang menyebabkan jangkitan kulit. Aktiviti antibakteria ditentukan berdasarkan ukuran diameter zon perencatan, kepekatan perencatan minimum (MIC) dan kepekatan pembunuh minimum (MBC). Ujian pembunuhan masa

dijalankan pada 0.5x MIC dan 1x MIC untuk *S. aureus* dan *S. epidermidis*, serta pada 0.5x MIC, 1x MIC dan 2x MIC untuk *S. pyogenes*, dengan inkubasi pada 0, 2, 4, 6, 8, 10, dan 24 jam. Keputusan menunjukkan diameter zon perencatan pada kepekatan produk 25%, 50%, 75% dan 100%. Nilai MIC menunjukkan bahawa produk ini menghalang pertumbuhan semua bakteria yang diuji. Nisbah MBC/MIC menunjukkan kesan bakterisid ke atas semua strain bakteria. Ujian pembunuhan masa menunjukkan kesan bakterisid pada 1x MIC bagi *S. aureus* dan *S. epidermidis*, serta pada 2x MIC bagi *S. pyogenes*. Kesimpulannya, Raed Sabun Mandian Semambu® menunjukkan kesan bakterisidal terhadap semua bakteria yang diuji.

Kata kunci: Pencuci badan antibakteria, Kesan bakterisidal, Produk semulajadi, Semambu, Jangkitan kulit

1.0 INTRODUCTION

Antibacterial body wash is a type of antibacterial product widely used in daily life to prevent infections. These products are applied to the skin with the intention of slowing or inhibiting bacterial growth on the body's surface, thereby helping to prevent skin infections [1]. Antibacterial body washes and antiseptics often contain various active ingredients, such as chlorhexidine gluconate, triclosan, and chloroxylenol, which possess antimicrobial properties effective against a range of bacteria [2].

Antibacterial products are classified as over-the-counter antimicrobial drugs and do not require a prescription, as their active ingredients have been proven to inhibit bacterial growth [1]. Antibacterial body washes, like hand washes and soaps, are formulated for use with water and are rinsed off after application. Common active ingredients in antibacterial and antiseptic products include chloroxylenol, triclosan, chlorhexidine gluconate, sodium laureth sulfate, and cocamidopropyl betaine. Chloroxylenol and chlorhexidine gluconate are antimicrobial agents effective against a broad spectrum of gram-positive and gram-negative bacteria, fungi, and viruses. They are frequently used in hand soap and surgical instrument products due to their ability to reduce bacterial counts on the skin [3]. Triclosan, a synthetic antimicrobial agent, is effective against a variety of bacteria and fungi and is typically found in personal care products such as soap, toothpaste, and deodorant [4]. Sodium laureth sulfate and cocamidopropyl betaine function as surfactants that help cleanse the skin by removing dirt and oil [5], as well as eliminating unpleasant odors. Cocamidopropyl betaine is also well-known for its cleansing and foaming properties in body washes and is commonly found in shampoo products [6].

However, prolonged use of antiseptic and antibacterial products may lead to antimicrobial resistance. In contrast, regular soap and water are generally sufficient for the natural removal of bacteria from the skin [1].

Staphylococcus aureus, Staphylococcus epidermidis, and *Streptococcus pyogenes* are among the most well-known and widespread bacterial pathogens found on the skin, capable of causing skin infections. *S. aureus* is a primary opportunistic pathogen in humans, responsible for various skin infections, ranging from minor invasive infections to life-threatening conditions such as septicemia and endocarditis [7]. Although less virulent than *S. aureus, S. epidermidis* is a common cause of infections in hospitalized patients and is often associated with implanted medical devices [8]. *S. pyogenes*, also known as Group A *Streptococcus*, can lead to a range of skin and soft tissue infections, including impetigo and cellulitis, as well as more severe conditions such as toxic shock syndrome and rheumatic fever [9].

In this study, the natural body wash product, Raed Sabun Mandian Semambu® was used as a commercial body wash with anti-parasitic properties. However, this product is formulated with several natural ingredients, including neem essential oil, frankincense essential oil, anise essential oil, clove essential oil, chamomile essential oil, and pro-vitamin B5, which have been shown to provide additional benefits such as antibacterial and antimicrobial properties. Neem leaf oil, clove oil, and anise oil possess strong antibacterial and antimicrobial effects that can inhibit the growth of harmful bacteria on the skin, thus reducing the risk of skin infections [10]. Additionally, chamomile and frankincense essential oils have natural anti-inflammatory properties, which can help reduce redness, irritation, and swelling of the skin, making them beneficial for conditions such as eczema or rosacea [11, 12].

2.0 METHODOLOGY

2.1 Test Material

The material used in this study is a body wash product, Raed Sabun Mandian Semambu®. This product is formulated with various natural ingredients, including neem essential oil, frankincense essential oil, anise essential oil, clove essential oil, and chamomile essential oil, along with several other components that serve as stabilizers and emulsifiers. Vancomycin was used as a positive control.

2.2 Test Organisms

The bacteria used in this study were *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. The bacteria were clinical isolates previously isolated from Hospital Canselor Tuanku Muhriz, Kuala Lumpur. All bacterial cultures were prepared at the Novel Antibiotics Laboratory, Faculty of Health Sciences, Kuala Lumpur Campus, Universiti Kebangsaan Malaysia.

2.3 Determination of Minimal Inhibitory Concentration (MIC)

The broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) using a 96-well microtiter plate [13]. To ensure accuracy and prevent contamination, only a single bacterial species was allowed per microtiter plate. For the MIC determination, 50 µL of Mueller-Hinton Broth (MHB) was added to all test wells of the 96-well microtiter plate using a micropipette. Then, 50 µL of the test product, Raed Sabun Mandian Semambu®, was added to the first well, and a two-fold serial dilution was performed down to well 10. After dilution, 50 µL from well 10 was discarded. Following this, bacterial inoculum was added to wells 1 through 10. Subsequently, 50 µL of bacterial inoculum was added to well 11, containing only MHB, to serve as a positive control to confirm bacterial growth. In well 12, 50 µL of the test product was added to MHB to act as a negative control. For the positive control, the same procedure was repeated, replacing Raed Sabun Mandian Semambu® with vancomycin. Vancomycin was used as positive control because it is considered as last resort of treatment in *S. aureus* infection [7]. Once all test materials were prepared, the 96-well microtiter plate was sealed with a sterile lid, and the edges were wrapped with parafilm to prevent evaporation. The plate was then incubated at 37°C overnight. After incubation, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reagent was added to each well containing test samples as it is an indicator for bacterial growth. Then, the plate was incubated at 37°C on a shaker incubator at 80 rpm for 30 minutes. The MIC was determined by observing the color change in each well. A shift from the yellow color of the MTT reagent to purple indicated bacterial growth, while wells that remained yellow indicates no bacterial growth.

2.4 Determination of Minimal Bactericidal Concentration (MBC)

The streak plate method was used to determine the Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. For this method, 10 µL of the test sample from wells that did not show a yellow color change with MTT was cultured onto Mueller-Hinton Agar (MHA) plates and incubated at 37°C for 24 hours.

2.5 Time-Kill Assay

The time-kill assay was conducted as described previously [14]. In this assay, the concentrations of Raed Sabun Mandian Semambu® used were 0.5x MIC and 1x MIC for *Staphylococcus aureus* and *Staphylococcus epidermidis*, while *Streptococcus pyogenes* was tested with 0.5x MIC, 1x MIC, and 2x MIC. For the positive control, vancomycin was used at a concentration of 1x MIC. A growth control was also included, where the inoculum was placed in Mueller-Hinton broth without any antibacterial agent. Universal bottles containing the treatment groups were incubated in a shaking incubator at 37°C, with sampling times set at 0, 2, 4, 6, 8, 10, and 24 hours. At the beginning of incubation (time=0 hour), sample was collected to determine the original concentration of respective bacteria used in this experiment. At every incubation time point, 10 µL of the solution from each treatment group in the universal bottles was collected and subjected to a 10-fold serial dilution, ranging from 10^{-1} to 10^{-10}. This dilution was performed by adding 10 µL of the test solution into an Eppendorf tube containing 90 µL of normal saline. Subsequently, 10 µL from this tube was transferred to the next Eppendorf tube, and the dilution process was repeated until a concentration of 10^{-10} was reached. To determine the colony-forming units (CFU/mL), the spread plate method was used. This involved taking 10 µL from selected dilutions and spreading it onto agar plates, which were then incubated at 37°C.

3.0 RESULTS AND DISCUSSION

3.1 MIC, MBC, and MBC/MIC Ratio of Raed Sabun Mandian Semambu®.

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of Raed Sabun Mandian Semambu ®. Table 1 summarises the values of MIC, MBC, and MBC/MIC ratio Raed Sabun Mandian Semambu ® against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. The MIC value of this product against *S. aureus* and *S. epidermidis* was 100% while *S. pyogenes* was 25%

Table 1 The value of MIC, MBC, and MBC/MIC Ratio of Raed Sabun Mandian Semambu® against bacterial strains tested

Bacterial strains	MIC (%)	MBC (%)	MBC/MIC Ratio
Staphylococcus aureus	100	100	1
Staphylococcus epidermidis	100	100	1
Streptococcus pyogenes	25	50	2

The Minimum Bactericidal Concentration (MBC) of the Raed Sabun Mandian Semambu® product was determined using the streak plate technique. The MBC values for *S. aureus* and *S. epidermidis* were similar at 100%, while the MBC for *S. pyogenes* was 50%. An MBC/MIC ratio of 4 or lower indicates that the test substance exhibits bactericidal effects, whereas a ratio of 4 or higher suggests bacteriostatic activity [15]. The calculations based on the obtained MBC and MIC values indicate that the study product demonstrates bactericidal properties against all three bacterial strains tested. According to Table 1, Raed Sabun Mandian Semambu® product exhibited better bactericidal effects against *S. aureus* and *S. epidermidis*, with a lower MBC/MIC ratio of 1. The results of the study on the MIC of the Raed Sabun Mandian Semambu® product against *S. aureus*, *S. epidermidis*, and *S. pyogenes* indicate that the product exhibits varying antibacterial activity across these bacterial strains. The MIC value for *S. pyogenes* was the lowest at 25%, meaning that at this concentration, the product effectively inhibits the growth of *S. pyogenes*. Raed Sabun Mandian Semambu® showed the same MIC value of 100% against *S. aureus* and *S. epidermidis*, demonstrating that the product has a better inhibitory effect on *S. pyogenes* at a lower concentration compared to the other two strains.

The inhibitory effects observed against all bacterial strains are believed to be linked to the natural ingredients formulated in Raed Sabun Mandian Semambu®, including neem essential oil (highest concentration), followed by frankincense essential oil, anise essential oil, clove essential oil, and chamomile essential oil. Several studies have documented the antimicrobial activities of these natural compounds. For instance, previous study [16] demonstrated that the MIC of *Azadirachta indica* (neem leaves) was 4.2 mg/mL against Gram-positive bacteria, including *Streptococcus* strains. Previous study [17] investigated the antimicrobial activity of *Boswellia serrata* (frankincense) essential oil against several pathogenic bacteria, including *S. aureus* and *S. epidermidis*. The study found that Boswellia serrata essential oil demonstrated antimicrobial activity against both bacteria, with MIC values of 0.125 mg/mL and 0.25 mg/mL, respectively. Additionally, another study assessed the antimicrobial activity of clove extract in vitro and in vivo against pathogenic microorganisms, showing that clove extract exhibited antibacterial activity against *S. aureus* with MIC of 2 mg/mL [18]. Other research has explored the antimicrobial activity of *Pimpinella anisum* (anise) essential oil against various bacteria, including *S. aureus, S. epidermidis*, and *S. pyogenes*, finding MIC values ranging from 0.05% to 0.1% [17]. While no study has explicitly demonstrated that *S. pyogenes* can be inhibited at lower concentrations than *S. aureus* and *S. epidermidis*, these findings suggest that the natural components of Raed Mandian Semambu® exhibit antibacterial activity against all three bacterial strains tested.

Furthermore, the results of MBC Raed Sabun Mandian Semambu® indicate that the study product exhibits bactericidal effects at the same concentrations as the MIC values for *S. aureus* and *S. epidermidis*, and at higher concentrations than the MIC for *S. pyogenes*. The MBC for the product against both *S. aureus* and *S. epidermidis* was found to be 100%, indicating that at the original concentration of Raed Sabun Mandian Semambu®, it can effectively kill both *S. aureus* and *S. epidermidis*. In other words, the use of Raed Sabun Mandian Semambu® yields the best results when undiluted. In contrast, at a lower concentration of 50%, the product also demonstrated bactericidal effects against *S. pyogenes*.

An MBC/MIC ratio of 4 or lower indicates bactericidal effects, while a ratio of 4 or higher indicates bacteriostatic activity [15]. The calculated MBC/MIC ratios reveal that the study product exhibits bactericidal effects against all three bacterial strains. The MBC/MIC ratios for *S. aureus* and *S. epidermidis* were both 1, demonstrating the product's strong bactericidal effects against these two strains. The MBC/MIC ratio for *S. pyogenes* was 2, indicating that the product also exhibits good bactericidal effects against this bacterium. Overall, the MBC values obtained suggest that the study product, Raed Sabun Mandian Semambu®, exhibits good bactericidal effects against *S. pyogenes* as it can kill this bacterium at lower concentrations. Although the MBC values for *S. aureus* and *S. epidermidis* are higher at 100% concentration, the MBC/MIC ratios are lower when compared to *S. pyogenes*. Therefore, this study product demonstrates good antibacterial activity against all bacterial strains tested.

3.2 Time-Kill Assay of Raed Sabun Mandian Semambu®.

Figure 1 illustrates the time-kill curve for *S. aureus* tested with Raed Sabun Mandian Semambu® at concentrations of 0.5x MIC and 1x MIC. The graph shows a gradual decline in bacterial activity at each hourly interval across the different concentrations of the test product. No bacterial growth was observed at the 10-hour and 24-hour marks for the 1x MIC concentration of the product. The time-kill curve indicates that at the 1x MIC concentration, the reduction in CFU/ml was ≥3 log10 CFU/ml. In contrast, the time-kill curve for the 0.5x MIC concentration demonstrated a reduction in CFU/ml of 2.4 log10 CFU/ml, which is lower than that observed at the 1x MIC concentration.

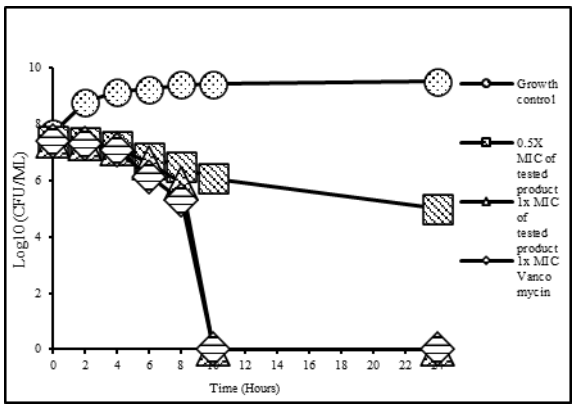


Figure 1 Tme-kill Curve of Raed Sabun Mandian Semambu® against *Staphylococcus aureus*

Figure 2 presents the time-kill curve for the product Raed Sabun Mandian Semambu® at concentrations of 0.5x MIC and 1x MIC against *S. epidermidis*. The time-kill curve indicates a gradual decline in bacterial activity at each concentration tested. Additionally, this curve shows that no bacterial growth occurred at the 10-hour and 24-hour marks for the 1x MIC concentration. Based on the curve, at the 1x MIC concentration of the test product, there was a reduction in CFU/ml of ≥3 log10 CFU/ml. In comparison, the 0.5x MIC concentration exhibited a decrease in CFU/ml of 2.12 log10 CFU/ml, which is lower than that observed at the 1x MIC concentration.

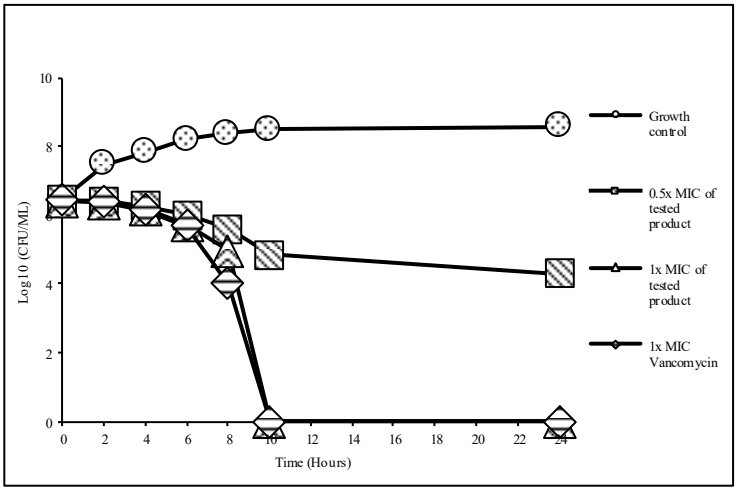


Figure 2 Time-kill Curve of Raed Sabun Mandian Semambu® against *Staphylococcus epidermidis*

Figure 3 illustrates the time-kill curve for the product Raed Sabun Mandian Semambu® tested at concentrations of 0.5x MIC, 1x MIC, and 2x MIC against *S. pyogenes*. The curve indicates a decrease in bacterial activity at each tested concentration, with no bacterial growth observed at the 24-hour mark for the 2x MIC concentration. Consequently, the reduction in CFU/ml for the 2x MIC concentration was ≥3 log10 CFU/ml. Furthermore, the decrease in CFU/ml for the 1x MIC concentration was higher than that of the 0.5x MIC, with reductions of 2.09 log10 CFU/ml for 1x MIC and 2.07 log10 CFU/ml for 0.5x MIC.

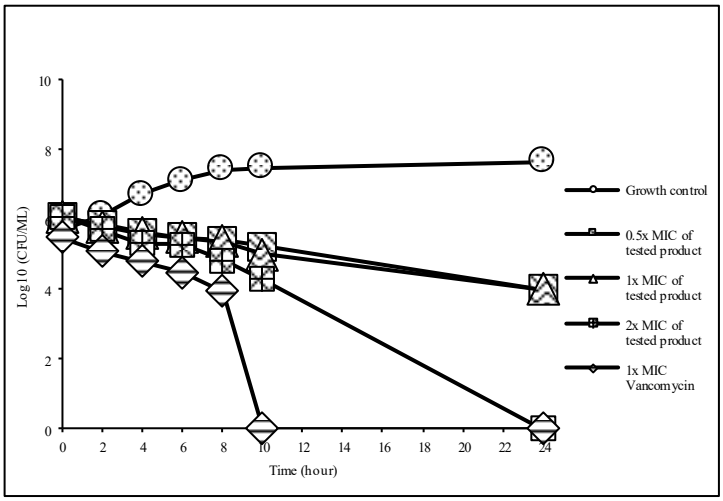


Figure 3 Time-kill Curve of Raed Sabun Mandian Semambu® against *Streptococcus pyogenes*

The time-kill assay was conducted to study and understand the interactions between antimicrobial agents and the microorganisms involved in this research. This assay is often performed to evaluate whether the antimicrobial activity of a particular agent or product is concentration-dependent or time-dependent [19]. The time-kill assay was performed on bacteria that have demonstrated antibacterial activity to validate the results obtained from the determination of MIC and MBC values [14]. This study employed three different MIC concentrations, namely 0.5X, 1X, and 2X for *S. pyogenes* because MBC/MIC ratio for *S. pyogenes* was 2 while two different concentrations (1X and 0.5X) was employed *S. epidermidis* and *S. aureus* because MBC/MC ration was 1.

The time-kill curve for *S. aureus* treated with Raed Sabun Mandian Semambu®, at concentrations of 1x MIC and 0.5x MIC, indicated a reduction in CFU/ml at each tested time point, decreasing until the 24-hour mark, with no resurgence in CFU/ml observed within this period. This implies that the tested product exhibits a prolonged antibacterial effect lasting up to 24 hours. Furthermore, the reduction in CFU/ml at 1x MIC was ≥3 log10 CFU/ml, with no bacterial growth observed at both the 10-hour and 24-hour marks. In the time-kill assay, a bactericidal effect is defined as a reduction of more than 3 log10 CFU/ml, equivalent to 99.9% bacterial death [20]. This indicates that at the 1x MIC concentration, the study product demonstrated bactericidal effects against *S. aureus*. Conversely, at the 0.5x MIC concentration, the reduction in CFU/ml was 2.4 log10 CFU/ml, which is lower compared to the 1x MIC concentration. The

difference in log reduction of *S. aureus* at these two concentrations demonstrates that the bactericidal effect exhibited is concentration-dependent.

Similarly, the time kill curve for *S. epidermidis* treated with Raed Mandian Semambu® also showed a decrease in CFU/ml over 24 hours, with no bacterial growth starting from the 10-hour mark. This indicates that the product has an antibacterial effect comparable to that of *S. aureus*, also lasting 24 hours. Additionally, the reduction in CFU/ml at 1x MIC was ≥3 log10 CFU/ml, which was higher compared to 0.5x MIC (2.12 log10 CFU/ml). As with *S. aureus, S. epidermidis* also displayed a bactericidal effect at the 1x MIC concentration, with a CFU/ml reduction 3 log10 CFU/ml. The difference in log reduction of *S. epidermidis* confirms that the bactericidal effect is likewise concentration-dependent.

When comparing the results of the time-kill assay with the MIC and MBC values for this tested product against *S. aureus* and *S. epidermidis*, there is a correlation and correspondence among the three conducted assays. At the 1x MIC concentration, which corresponds to both the MIC and MBC values, the reduction in colonies of *S. aureus* and *S. epidermidis* was the highest, with ≥3 log10 CFU/ml observed from the mortality curves for these bacteria. This confirms the presence of a bactericidal effect at the original concentration of the study product against *S. aureus* and *S. epidermidis*.

The time-kill assay conducted with Raed Sabun Mandian Semambu® against *S. pyogenes* exhibited slight differences from the time-kill curves for *S. aureus* and *S. epidermidis*. Three different concentrations were used for the time-kill assay against *S. pyogenes*; 0.5x MIC, 1x MIC, and 2x MIC. The mortality curve for *S. pyogenes* at these concentrations indicated a decrease in CFU/ml over a 24-hour period, with only the 2x MIC showing no bacterial growth at the 24-hour mark. This demonstrates that the product exhibits antibacterial activity against *S. pyogenes*, which can be maintained for up to 24 hours. The reduction in CFU/ml at the 2x MIC concentration was the highest at ≥3 log10 CFU/ml, followed by 1x MIC (2.09 log10 CFU/ml) and 0.5x MIC (2.07 log10 CFU/ml). Comparing the results of the time-kill assay with the MIC value for this product against *S. pyogenes*, a relationship and correspondence between the results of the two assays is evident. The MIC value at 1x MIC is 25%, and the reduction in colonies of *S. pyogenes* observed from the time-kill assay curve indicates a significant inhibitory activity at this concentration. The 2x MIC concentration used in the time-kill assay is 50%, which also corresponds to the MIC value, demonstrating a colony reduction against *S. pyogenes* similar to that seen for *S. aureus* and *S. epidermidis* at a concentration of 100%, indicating a CFU/ml reduction greater than 3 log10 CFU/ml. This substantiates that the antibacterial activity of the study product is most effective at the 2x MIC concentration against *S. pyogenes*.

Overall, the results from the time-kill assays demonstrate that the compounds in Raed Sabun Mandian Semambu® exhibit good antibacterial activity against all three strains of skin pathogens; *S. aureus*, *S. epidermidis*, and *S. pyogenes*. The time-kill curves for all tested bacteria indicate that this study product can exhibit bactericidal effects against *S. aureus, S. epidermidis*, and *S. pyogenes*. For *S. aureus* and *S. epidermidis*, the 1x MIC concentration shows the highest reduction in CFU/ml compared to the 0.5x MIC concentration. For *S. pyogenes*, the highest reduction in CFU/ml was observed at 2x MIC, which is comparable to the reduction seen in *S. aureus* and *S. epidermidis* at the 1x MIC concentration. This suggests that bacterial growth diminishes as the concentration of active ingredients in the product increases. Consequently, Raed Sabun Mandian Semambu® is anticipated to demonstrate superior antibacterial activity at its original concentration of 100%, indicating its potential as an effective antibacterial bath soap product. The bactericidal benefits of Raed Mandian Semambu®, as demonstrated in this study, have the potential to effectively inhibit and kill bacterial skin pathogens, thereby reducing the risk of skin infections and promoting skin health. This study also has limitation as it was only examined on specific strains of *S. pyogenes*, *S. aureus*, and *S. epidermidis*. As each genus and species demonstrate variability in genetics, further study should also include larger panel of clinical isolates and comparative characterization of susceptible versus non-susceptible strains to better define the specificity and potential application range of the compound. Furthermore, mechanism of antibacterial activity of the product at molecular level should also be examined.

4.0 CONCLUSION

Overall, based on the determination of the minimum inhibitory concentration (MIC), Raed Sabun Mandian Semambu® demonstrates the ability to inhibit the growth of *S. aureus* and *S. epidermi*dis at a concentration of 100%, while at a lower concentration of 25%, it shows inhibitory effects against *S. pyogenes*. This indicates that the product is more effective at inhibiting the growth of *S. pyogenes* compared to *S. aureus* and *S. epidermidis*. From the determination of the minimum bactericidal concentration (MBC) revealed that this product exhibits good bactericidal effects against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. At a concentration of 100%, the product is capable of killing both *S. aureus* and *S. epidermidis*. Meanwhile, it demonstrates bactericidal effects against *S. pyogenes* at a lower concentration of 50%. According to the results of the time-kill assay, the highest reduction in CFU/ml for *S. aureus, S. epidermidis*, and *S. pyogenes* was ≥3 log10 CFU/ml. The time-kill assay indicates that Raed Sabun Mandian Semambu® exhibits good bactericidal effects against all three studied bacteria. Thus, Raed

Mandian Semambu® is believed to have the potential to become an effective antibacterial bath soap product.

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Conflict of Interest

All authors declare no conflict of interests.

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