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RESEARCH ARTICLE

In vivo effect of bromelain from *Ananas comosus* on *Staphylococcus aureus* skin infection in an animal model

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Abstract

The skin is the largest organ in the body and aids in protection against foreign microorganisms. Any physical changes to the skin structure would eventually promote microbial colonization that leads to soft tissue and skin infections. This study was conducted to establish the effectiveness of bromelain extraction from *Ananas comosus* in treating *Staphylococcus aureus* skin infections in BALB/c mice. A total of 18 male mice aged 9 - 12 weeks were divided into three groups: bromelain-treated, iodine-treated, and negative control groups. The skin infection was inflicted on the dorsal area of the mouse by first removing the hair and applying the tape-stripping method before inoculating it with *S. aureus*. The microbial samples from the infected skins of the mice were collected on days 1, 4, and 7 after infection using a Q-Swab apparatus and streaked on blood agar. The samples were incubated at 37°C for 24 h and the *S. aureus* colony formation unit (CFU) from each group was calculated. The results indicated that mice treated with bromelain had a lower average number of *S. aureus* CFU ($2.55x10^{-2}$ CFU/mL) compared with iodine-treated mice ($1.56x10^{-2}$ CFU/mL) and had a significantly different count compared with the negative control group ($8.24x10^{-2}$ CFU/ml) on day 7 post-infection. Data were analyzed using one-way ANOVA and Student's t-test for significant differences with $p<0.05$. In conclusion, the findings of this study showed that bromelain can be developed and applied as an antimicrobial agent against *S. aureus* skin infection.

1. INTRODUCTION

Skin comprises about 12% - 16% of body weight, making it the largest organ compared to other body parts (1,2). However, when these barriers are disrupted, microorganisms can start to invade and replicate onto the skin. Skin and soft tissue infections are classified into primary and secondary infections. For instance, primary infection occurs when bacteria replicate on healthy skin, whereas secondary infection occurs due to pre-existing trauma or skin disease (3,4). Gram-positive microorganisms are the most common microorganisms that cause skin and soft tissue infection (SSTI), particularly *Staphylococcus aureus* (*S. aureus*) (5,6). The initial outbreak of *S. aureus* in patients with low methicillin immunity occurred in 1960 (7,8). However, over the years, the rates of *S. aureus* infection have rapidly increased from 3% to 20%. Methicillin-Resistant *S. aureus* (MRSA) has become a major contributor to hospital- and community-acquired infections and has been significantly linked to morbidity and mortality (9). While the prevalence of MRSA differs across countries and regions, Asia ranks among the areas with the highest incidence globally.

S. aureus invasion and toxins involves employing various strategies, including adhesion via microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), biofilm formation, immune evasion

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through protein A, and the production of enzymes and toxins that damage host tissues and trigger an exaggerated immune response. MRSA strains are defined by their resistance to beta-lactam antibiotics, but they differ significantly in their epidemiology, genetic background, and virulence factors. Community-Associated MRSA (CA-MRSA) is characterized by staphylococcal cassette chromosome mec (SCCmec) type IV or V elements, potent toxin production, and typically affects healthy individuals in community settings with skin and soft tissue infections. Healthcare-Associated MRSA (HA-MRSA) features larger SCCmec elements (type I, II or III), multidrug resistance, and primarily affects hospitalized or immunocompromised individuals, leading to various invasive infections. Understanding these mechanisms and distinctions is crucial for devising effective strategies for the prevention, diagnosis, and treatment of *S. aureus* infections.

Antibiotic resistance is a growing global threat that extends far beyond the challenges posed by MRSA. As bacteria evolve to withstand our current arsenal of antibiotics, the treatment of infections, especially common ones like skin and soft tissue infections (SSTIs), is becoming increasingly difficult to treat. This situation underscores the urgent need to explore and implement alternative therapies. The World Health Organization (WHO) has highlighted that SSTIs, ranging from minor abscesses to severe necrotizing fasciitis, significantly contribute to the global disease burden. A report by the WHO has estimated that antimicrobial resistance (AMR) is responsible for hundreds of thousands of deaths annually, with a substantial proportion related to common infections like SSTIs. Projections suggest that, if unchecked, AMR could lead to up to 10 million deaths per year by 2050.

This experimental study was conducted to determine the effect of enzymes derived from pineapple. *Ananas comosus* (*A. comosus*), also known as pineapple, has been introduced in Asia Pacific since the 16th century and has become one of the most widely used commercialized plants (10). The extraction of *A. comosus* produces bromelain, an enzyme originating from the *Bromeliaceae* family (11). Bromelain can be extracted from the leaf, stem, flesh, and crown of the fruit, but it is mostly found in the stem and flesh parts (12,13). This bromelain enzyme exhibits anti-helminthic and anti-fungal activities against infectious skin diseases, and its therapeutic effects can be enhanced by the use of antibiotics (14,15). In European and Western countries, bromelain has been marketed as a food supplement that is available in pharmacies. This is because bromelain can be safely be absorbed into the human intestines without losing its therapeutic effect. Although bromelain exerts low toxicity in the human body, for patient with kidney and liver disease, the use of bromelain is still recommended. Bromelain also contains carbohydrates, phosphatases, glucosidase, peroxidases, colored pigments, minerals, organic acids, and organic solvents (16,17). Among these components, protease is the most active component capable of exhibiting proteolytic activity and contributes to many beneficial applications (18,19).

The comparative analysis of bromelain concentration reveals that the pineapple stem is the richest source of this proteolytic enzyme, whereas the flesh and core contain lower levels. Bromelain aids wound healing and combats infections through multiple mechanisms: debridement of necrotic tissue, anti-inflammatory actions, potential antimicrobial effects, and tissue repair enhancement. Preliminary studies across various settings, including skin infections, diabetic ulcers, and inflammatory conditions, underscore its potential as an adjunctive therapy. Although further clinical trials and research are needed to establish standardized protocols and optimal dosing, bromelain remains a promising candidate in the search for alternative therapies to manage wound healing and combat infections.

Despite numerous studies exploring the biochemical properties of bromelain, significant research gaps remain in the *in vivo* evaluation of its therapeutic efficacy against *S. aureus*-induced skin infections. Previous investigations have often focused on *in vitro* models or limited animal studies that do not fully capture the potential of the enzyme for modulating infected skin flora and facilitating wound healing (20). Moreover, a notable disconnect between these studies and the global sustainable development goals, which emphasize responsible consumption and production by using renewable natural resources. This study aims to bridge these gaps by focusing on bromelain derived from the pineapple stem, a part of the plant that offers a higher concentration of this potent protease component. By leveraging the anti-inflammatory, antimicrobial, and wound-healing properties of stem-derived bromelain, this study seeks to elucidate its fundamental therapeutic effects against *S. aureus*-induced skin infections in a laboratory animal model.

The application of bromelain represents a practical and sustainable approach to enhancing healthcare, particularly for treating *S. aureus*-induced skin infections (21). This innovative treatment can be integrated into clinical practice in the future by developing standardized topical formulations and exploring combination therapies with antibiotics. Moreover, using bromelain derived from pineapple stem not only maximizes the therapeutic potential of the enzyme, but also supports responsible consumption and production for sustainable goals. This *in vivo* study is a pivotal step toward establishing bromelain as a viable, natural alternative in the fight against skin infections and antibiotic resistance, promising a future where natural resources play a central role in modern medicine.

2. MATERIALS AND METHODS

2.1. *S. aureus* Strain

The *S. aureus* culture (ATCC29213) was maintained at the Institute of Medical Science Technology, Universiti Kuala Lumpur. Bacteria were sub-cultured on nutrient agar slants and kept in the refrigerator at 4°C until required. A bacterial suspension was prepared by transferring a few colonies of 24-h-old bacterial cultures into 5 mL sterile normal saline.

2.2. Bromelain Preparation

The lyophilized pineapple stem-derived bromelain powder (B4882) was purchased from Sigma-Aldrich. A 0.1 g sample of bromelain powder was suspended in 5 mL of sterilized distilled water to yield a concentration of 20 mg/mL. The mixture was thoroughly stirred using a magnetic stirrer for approximately 30 minutes to optimize stirring conditions and achieve complete dissolution. This process ensured improved bromelain absorption before its application to the animal model.

2.3. Experimental Design and Procedure

12 male BALB/c mice aged 9 - 12 weeks were divided into three groups: the bromelain-treated group; the iodine-treated group; and the negative control group. This animal study was approved by the Animal Ethics Committee (AEC) at the Institute of Medical Science Technology, Universiti Kuala Lumpur (AEC/MESTECHUNIKL/ 2018/026).

Prior to the experiment, the animals were anesthetized and the hair on its dorsal area was removed. The exposed skin area was subjected to barrier disruption using the tape stripping method, which was repeated 10 times in succession. Consequently, the skin became damaged without any regular bleeding. Before the experiment, 1 L of phosphate-buffered saline (PBS) stock solution (containing of 8 grams NaCl, 0.2 grams KCl, 1.44 grams Na_2HPO_4, KH_2PO_4, and distilled water) was prepared. Bacterial infection was initiated by inoculating the damaged skin with 100 µL of PBS solution containing 10^7 cfu/mL of *S. aureus* from an overnight bacterial culture. Concentration of 10^7 cfu/mL of *S. aureus* is commonly used to infect mice because it is sufficiently high to establish a robust infection still being within a range that mimics clinical conditions in human (22). This concentration ensures consistency results, allowing for clear observation of infection progression and the effectiveness of any treatment tested (23). The first application of bromelain and iodine treatments was applied to the infected skins after 24 hours of bacterial inoculation. Topical applications of bromelain and iodine were administered to mice once every day. Mice were humanely sacrificed at the end of the experimental period (7^{th} day) for histological assessment. All animals were given free access to food and water ad libitum and maintained at room temperature with a 12 h light/dark cycle.

2.4. Bacterial Identification and Colony Formation Unit (CFU) Counts

The infected skin on the dorsal area of the mice was swabbed using Q-Swab with buffered peptone water as the collection medium on days 1, 4, and 7 of the experimental periods. The presence of *S. aureus* infection on the damaged skins of all mice was confirmed through catalase, coagulase, and Gram staining. Swabbed *S. aureus* samples were streaked onto blood agar medium (CM0055B, Oxoid™, UK) and incubated for 24 h. Subsequently, CFUs were measured using a colony counter machine (SC6 Plus, Weber Scientific, UK) and the average numbers were calculated accordingly.

2.5. Histological Assessment

All mice were euthanized in a carbon dioxide chamber. Skin tissue samples measuring approximately 2 cm by 2 cm were harvested and fixed in 10% formalin. After 24 h, the tissue samples were processed accordingly. The tissue samples were sectioned using a microtome instrument (BK-2478, Biobase, China) and mounted on glass slides before staining with Hematoxylin and Eosin staining solution (ab245880, Abcam, UK). Skin tissue samples were examined microscopically to evaluate histological changes, focusing on the epidermal epithelialization process observed 7 days post-treatment (4). A qualitative evaluation was conducted, with a single-blinded peer review of all slides to ensure unbiased assessment.

2.6. Statistical Analysis

The results were expressed as mean±standard error mean. The data from the CFU counts were analyzed using One-way ANOVA and Student's t-test for significant differences among the groups. $p<0.05$ is considered significantly different.

3. RESULTS AND DISCUSSION

3.1. CFUs Evaluation and Analysis

The colony-forming unit (CFU) counts of *S. aureus* on the damaged skin of mice revealed a progressive reduction throughout the experimental period, demonstrating the efficacy of the applied treatments. On day 1, topical application of bromelain resulted in a reduced mean CFU count compared with the iodine-treated group, highlighting its potential for early infection control intervention (Figure 1a). By day 4, mice treated with iodine exhibited the lowest mean CFU count ($5.57x10^{-2}$ CFU/mL), outperforming both the bromelain-treated group ($7.05x10^{-2}$ CFU/mL) and the negative control group ($1.29x10^{-1}$ CFU/mL) (Figure 1b). These findings underscore the antimicrobial effect of iodine during the early stages of treatment.

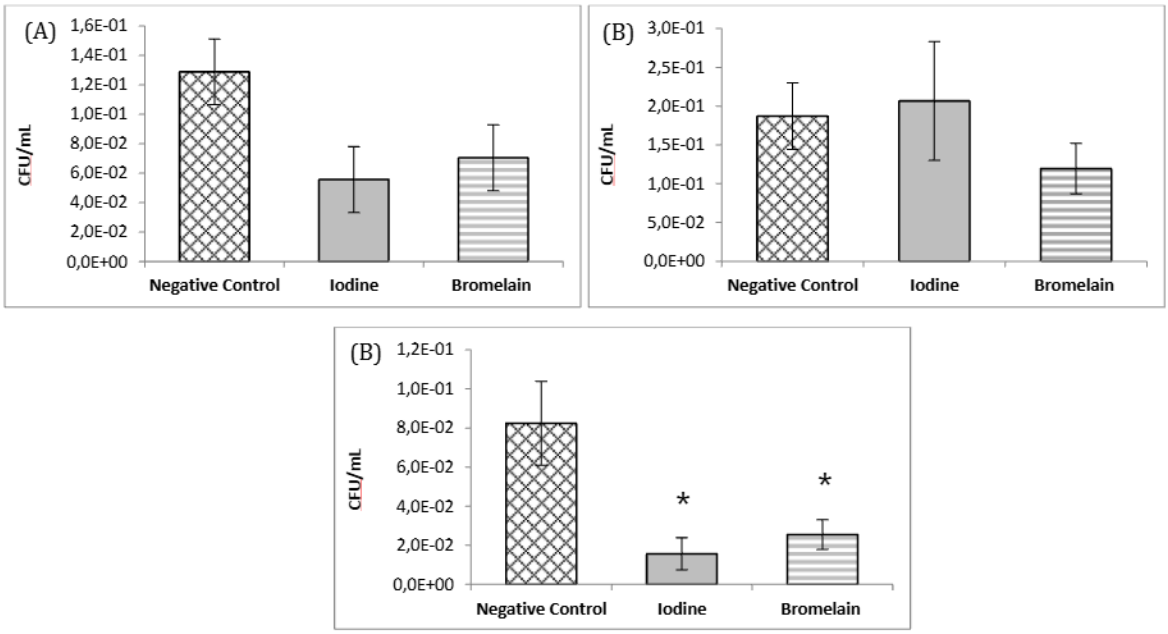

Figure 1. CFUs of *S. aureus* (CFU/mL) after treatment on (A) day 1, (B) day 4, and (C) day 7 of the experiment.
* = $p<0.05$ against negative control group.

By day 7, a statistically significant reduction in the CFU counts of *S. aureus* was observed in the bromelain-treated group compared to the negative control group. Bromelain-treated mice showed an average CFU count of 2.55×10^{-2} CFU/mL, which was markedly lower than that of the negative control group (8.24×10^{-2} CFU/mL) and comparable to the iodine-treated group (1.56×10^{-2} CFU/mL) (Figure 1c). These results suggest that the bromelain treatment effectively reduced the bacterial load over the course of study. The results are considered statistically significant with a *p*-value of 0.05, indicating a high likelihood that the treatment effect (the reduction in CFU counts) is circumstantial.

The similarity in CFU reduction between the bromelain and iodine groups suggests that the effect of bromelain is reproducible and robust, mirroring the antiseptic agent efficacy. Overall, the steady decline in CFU counts, as evidenced by the marked reduction by day 7, supports the conclusion that bromelain consistently reduces *S. aureus* load over time. This consistency across different treatment groups and over the observed period underscores bromelain's potential as a reliable alternative for managing skin infections. The progressive reduction in CFU counts suggests that bromelain not only inhibits bacterial growth but also supports the host's immune system in resolving the infection. These results indicate that bromelain is a viable alternative to iodine for the management of *S. aureus* skin infections. Bromelain's ability to achieve comparable outcomes by day 7 highlights its potential as a natural, effective treatment option, particularly for mild-to-moderate infections. Bromelain's proteolytic action reduces microbial load while minimizing tissue damage, making it an ideal candidate for topical applications in dermatological and wound-healing contexts.

The disruption of normal skin barriers significantly alters the skin's physiological properties, including water balance, electrolyte concentration, and pH levels. These changes create a conducive environment for microbial colonization, particularly by *Staphylococcus aureus* (*S. aureus*), a common commensal bacterium (2,24). Under normal conditions, *S. aureus* coexists with the host without causing any harm. However, its overpopulation can trigger inflammatory responses, leading to skin infections. Infections with *S. aureus* often manifest as crusty abscesses due to clotted plasma resulting from the action of the bacterial coagulase enzyme (8). Coagulase facilitates clot formation, which can protect *S. aureus* from immune defenses, contributing to its virulence. Additionally, *S. aureus* secretes enterotoxins that promote the release of inflammatory cytokines by interacting with keratinocytes and antigen-presenting cells (2). This results in localized inflammation, evident in the reddish lesions observed on the infected skin. Inflammation can also affect hair follicles, leading to folliculitis, a hallmark of *S. aureus* infections (6). Despite the natural resistance of mice to certain *S. aureus* toxins, such as alpha-hemolysin, infection can still cause significant histological and physiological changes, including hyperplasia. Epidermal proliferation is a response to tissue damage and inflammation, further altering the normal structure of the skin.

Bromelain, a proteolytic enzyme complex derived from *Ananas comosus* (pineapple), has promising antimicrobial and anti-inflammatory properties. The ability of the compound to degrade microbial cell wall proteins disrupts the structural integrity of bacteria, leading to cell membrane injury and eventual cell death (25). This mechanism involves the hydrolysis of peptide bonds within the bacterial cell wall, a process that weakens the external defenses of microbes (26). The enzymatic activity of bromelain, particularly through its glycoprotein components, has been widely recognized for its clinical applications, including its role as an antiseptic. In contrast, iodine works primarily by releasing free iodine, which rapidly penetrates microbial cells and disrupts their proteins, nucleotides, and membranes. This non-specific oxidative

damage is highly effective against a wide range of pathogens, including bacteria, viruses, and fungi. Unlike bromelain, iodine acts quickly by directly killing microbes upon contact. This makes it a powerful agent for the immediate reduction of microbial burden in wounds. Although iodine is effective in reducing infection, it does not actively contribute to the removal of necrotic tissue or modulation of inflammatory pathways in the same way bromelain does.

The use of bromelain topically on *S. aureus*-infected skin offers a natural alternative for reducing microbial growth. Its proteolytic activity not only diminishes the bacterial load but also alleviates inflammation by modulating the immune response of the host. Several molecular pathways have been implicated in the anti-inflammatory effects of bromelain. Bromelain may inhibit the activation of nuclear factor kappa B (NF-κB), a transcription factor that regulates the expression of various proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which can limit tissue damage and promote a more controlled and efficient healing process. There is evidence that bromelain may influence the activity of cyclooxygenase enzymes (particularly COX-2), which are responsible for the production of prostaglandins, the key mediators of inflammation and pain. Matrix metalloproteinases (MMPs) are enzymes that break down extracellular matrix components and are involved in tissue remodeling. Bromelain may help regulate MMP activity, ensuring that the breakdown of damaged tissue does not exceed what is necessary for proper remodeling. By facilitating the degradation of necrotic tissue, bromelain creates a more favorable environment for the migration of fibroblasts and keratinocytes, which are critical for tissue repair and re-epithelialization. Histological assessments have shown that bromelain treatment results in similar levels of neutrophil and leukocyte infiltration as iodine treatment, indicating comparable efficacy in reducing microbial infection by day 7 post-treatment. Furthermore, the effectiveness of bromelain is particularly notable for mild and transient skin infections, providing a targeted approach that minimizes prolonged inflammation and tissue damage.

The therapeutic potential of bromelain is further enhanced when combined with antibiotics. Bromelain broadens the antibacterial spectrum of antibiotics, improving their efficacy against *S. aureus* infections (27). This synergistic effect likely stems from the ability of bromelain to disrupt bacterial biofilms and enhance antibiotic penetration. As a result, combination therapy can effectively combat more resistant infections, thereby reducing the risk of chronic infection or recurrence. The current study demonstrated that bromelain treatment significantly reduced *S. aureus* CFU over time. Proteolytic activity of bromelain appears to disrupt bacterial colonies and biofilm formation, contributing to its antimicrobial effects. Earlier studies have reported that bromelain exhibits antimicrobial properties against various pathogens, including *S. aureus*. Previous research has indicated that bromelain can interfere with biofilm integrity, which is critical for managing chronic and unmanageable infections.

While bromelain is effective for mild and brief skin infections, iodine treatment remains more suitable for prolonged or severe cases. Iodine's broad-spectrum antimicrobial properties and ability to rapidly reduce microbial load make it a reliable choice for more extensive infections. However, the natural origin and specific enzymatic activity of bromelain provide an advantage in minimizing cytotoxicity and promoting healing in less severe conditions. The comparable outcomes observed between bromelain and iodine treatments in this study highlight the potential of bromelain as a natural alternative or adjunct to conventional therapies. Deepening knowledge about the biochemical properties and mechanisms of action of bromelain has illuminated its broad therapeutic potential. By advancing the understanding of how bromelain facilitates debridement, reduces inflammation, and combats microbial infections, researchers are paving the way for its integration into innovative treatment regimens. These developments, combined with sustainable production methods and promising clinical data, underscore the role of bromelain as a valuable tool in modern wound management and infection control.

3.2. Qualitative Histological Observation of Tissue Samples

In this preliminary qualitative histological observation of the infected skin samples, signs of acute inflammation were noted. In contrast to the normal skin structure, the morphology of the infected skin samples exhibited irregular thickness in both the dermis and epidermis layers (Figure 2a). None of the tissue samples showed signs of chronic inflammatory reaction, but cell infiltration was observed in the negative control group (Figure 2b), bromelain-treated (Figure 2c), and iodine-treated skin samples (Figure 2d).

The increase in collagen types I and III in bromelain-treated samples suggests that the enzyme is facilitates the deposition and maturation of the extracellular matrix, contributing to robust tissue repair. Bromelain may also modulate MMP activity, thereby promoting the removal of damaged tissue and preventing excessive degradation. A balanced MMP/TIMP (Tissue Inhibitors of Metalloproteinases) ratio is crucial for proper matrix remodeling and wound closure. Transforming Growth Factor-Beta (TGF-β) plays a multifaceted role in wound healing by promoting fibroblast proliferation, collagen synthesis, and immunomodulation. An increase in TGF-β levels in the wound microenvironment suggests that bromelain may enhance the signaling pathways that drive tissue repair. The increased expression and favorable modulation of these markers underscore the potential of bromelain to combat infection and actively promote tissue regeneration and repair. This multifaceted effect supports its therapeutic application in wound management and validates its role as a promising natural agent for enhancing the healing process.

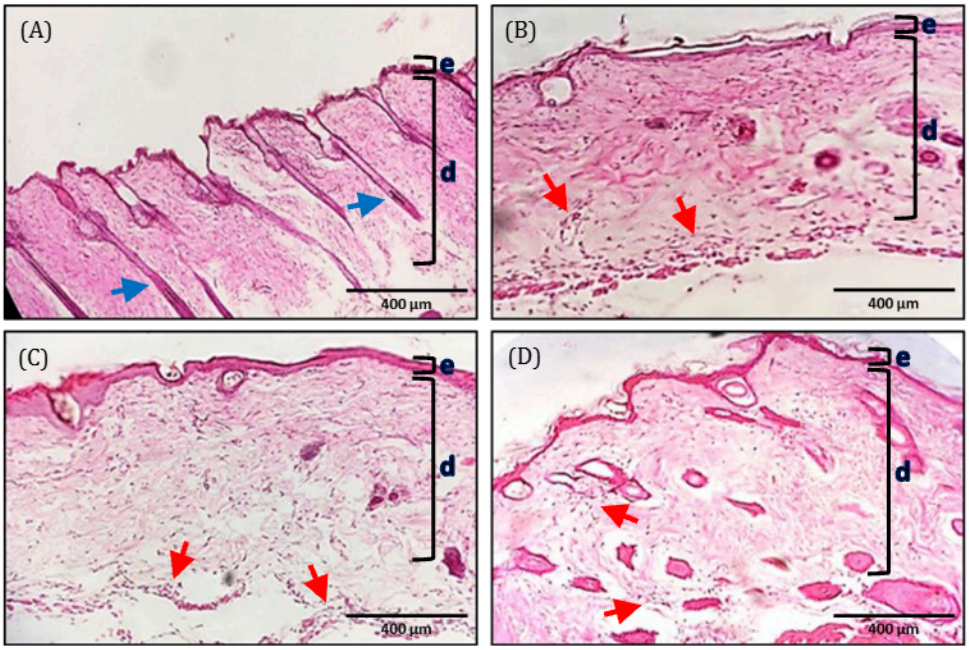

Figure 2: Histological assessment of *S. aureus*-infected skin samples harvested on day 7 demonstrating both the epidermal (a) and dermal (d) layers. Image (A) shows normal skin with clearly defined hair follicle structures (blue arrows). Images (B), (C), and (D) represent the negative control, bromelain-treated, and iodine-treated groups, respectively, with red arrows indicating cellular infiltration at the damaged site.

Bromelain can be extracted from various parts of the pineapple, including the stem and flesh, each of which contains distinct bioactive compounds (13). The pineapple stem, often considered a byproduct of agriculture, provides a sustainable and cost-effective source of bromelain. Among its key components, glycoproteins play a crucial role in the proteolytic activity and therapeutic applications of bromelain (16). The use of pineapple stems, a byproduct of agriculture, as a sustainable source of bromelain underscores a vital intersection between environmental stewardship and medical innovation. This approach not only reduces agricultural waste and promotes the efficient use of natural resources but also aligns with global initiatives to encourage responsible healthcare consumption and production. By integrating bromelain into medical treatments, humans can harness its therapeutic benefits while supporting sustainable practices and contributing to medical research in the future.

One key limitation of this preliminary work is the use of a single animal model, which may not fully capture the complexity of biological variability and immune responses. This limitation suggests that caution should be exercised when extrapolating these findings to clinical settings. Additionally, the lack of long-term observation in this study limits the understanding of the sustained effects, safety, and potential delayed outcomes associated with bromelain treatment. Future research is essential to further validate the efficacy of bromelain as a versatile therapeutic agent. Expanding investigations to include a broader range of pathogens and transitioning from animal models to human clinical trials will be crucial in defining modern medicine's role. Concurrently, refining the formulation of bromelain as a topical agent through studies on stability, delivery vehicles, and dosing regimens will help ensure its practical application in clinical settings. Together, these studies could pave the way for integrating this sustainable, natural product into the treatment protocols for various infections and wound-healing scenarios.

4. CONCLUSIONS

As one of the most recognizable fruits in the world, pineapple is easily found in the Southeast Asia region. With the abundance of available resource, extracting a high yield of bromelain can be achieved at a very low cost. Extracting bromelain from the stem of the fruit is inexpensive since the part is deemed as waste and by-products. Thus, optimizing this by-product from pineapple makes it not only economically beneficial but also environmentally friendly.

Bromelain extracted from *Ananas comosus* possesses *in vivo* antibacterial activity against *S. aureus* skin infection. The outcomes of this study suggest the use of bromelain as a cost-effective and natural-based compound for skin protection against possible infection. Although further investigation is needed to clarify the mechanism of action, this study indicated that bromelain can be explored for its antimicrobial properties.

Author contributions: SNAZ, MSSW, ENI, RI: Conceptualization, Validation. SNAZ, MSSW, RI: Methodology. SNAZ, ENI, RI: Formal analysis, Writing—review and editing. SNAZ, MSSW: Investigation. MSSW, RI: Resources. ENI, RI: data curation. SNAZ, RI: Writing—original draft preparation. MSSW, ENI, RI: Supervision. RI: Project administration, funding acquisition. All authors have read and approved to the published version of the manuscript.

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Ethics statement: Studies on the animal in this work has been approved by the Animal Ethic Committee (AEC) at Universiti Kuala Lumpur, Institute of Medical Science Technology (AEC/MESTECHUNIKL/ 2018/026).

Conflict of interest: None.

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