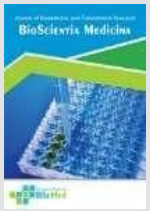
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Harnessing the Power of Nature: *Ananas comosus* Extract Gel as an Alternative Topical Treatment for Grade 2 Burn Wounds

Kharmi Juni Yanti[1*], Diah Adriani Malik[1], Novi Kusumaningrum[1]

[1]Department of Dermatology, Venereology and Aesthetics, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

ARTICLE INFO

Keywords:
Ananas comosus
Burn injury
Pineapple extract
Rattus norvegicus
Topical gel

***Corresponding author:**
Kharmi Juni Yanti

E-mail address:
kharmijuniyanti@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i7.1339

A B S T R A C T

Background: Burn injuries remain a significant global health challenge, often leading to complications such as infection and delayed healing. Conventional treatments can be costly and may have side effects, prompting exploration into natural therapeutic alternatives. *Ananas comosus* (pineapple), rich in the enzyme bromelain, has demonstrated anti-inflammatory and wound-healing properties, suggesting its potential as a topical agent for burn wounds. This study aimed to evaluate the efficacy of a topical gel formulated from *Ananas comosus* extract in promoting the healing of grade 2 burn wounds in a Wistar rat model by assessing its impact on wound diameter and the levels of pro-inflammatory cytokines Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6). **Methods:** A true experimental study with a post-test only control group design was conducted using 30 male Wistar rats. Grade 2 burn wounds were induced on the dorsum of the rats. The rats were randomly assigned to six groups (n=5 per group): a normal control (KN, no treatment), negative control (K-), a positive control (K+, silver plus alginate hydrogel), and three treatment groups receiving topical *Ananas comosus* extract gel at concentrations of 15% (P1), 20% (P2), and 25% (P3) twice daily for 14 days. Wound diameter was measured on days 1, 3, 6, 9, 12, and 14 using the ImageJ application. On day 15, tissue samples were collected for IL-1β and IL-6 quantification via ELISA. Data were analyzed using Kruskal-Wallis and Mann-Whitney U tests for wound diameter, and One-Way ANOVA with LSD post-hoc test for cytokine levels, with $p<0.05$ considered significant. **Results:** Significant differences in burn wound diameter and levels of IL-1β and IL-6 were observed among the groups ($p<0.05$). The P2 group (20% pineapple extract gel) exhibited the most significant reduction in wound diameter compared to the negative control and was comparable to the positive control group. This group also showed a marked decrease in IL-1β and IL-6 levels, with IL-1β levels similar to the positive control and IL-6 levels significantly lower than the untreated group and approaching those of the positive control. **Conclusion:** Topical application of 20% *Ananas comosus* extract gel effectively accelerated the healing of grade 2 burn wounds and reduced the levels of pro-inflammatory cytokines IL-1β and IL-6 in Wistar rats. These findings support the potential use of pineapple extract as an alternative herbal topical therapy for burn injuries. Further research is warranted to explore optimal formulations and clinical applications in humans.

1. Introduction

Burn injuries represent a substantial global public health concern, responsible for an estimated 180,000 deaths annually, with a disproportionately high burden in low and middle-income countries, particularly in regions like Southeast Asia. In Indonesia, the prevalence of burn injuries was reported as 0.7% in 2018, with grade 2 burns being a common occurrence. These injuries, resulting from thermal, chemical, electrical, or radiation sources, cause damage to the skin and underlying tissues, leading to significant morbidity, including prolonged hospitalization, disability, disfigurement, and profound psychological trauma. The severity of a burn

is determined by its depth and the total body surface area (TBSA) affected, which dictates the therapeutic approach and prognosis. Grade 2 burns, characterized by damage to the epidermis and varying portions of the dermis, present with erythema, pain, and blistering, and require meticulous management to prevent infection and promote optimal healing.[1,2]

The physiological process of burn wound healing is intricate and dynamic, conventionally divided into three overlapping phases: inflammation, proliferation, and remodeling. The initial inflammatory phase, typically lasting 1-4 days, is crucial for clearing debris and initiating repair, but if dysregulated or prolonged, it can impede healing and contribute to excessive scarring. Key mediators in this phase are pro-inflammatory cytokines such as Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6). IL-1β is a potent pyrogen and plays a pivotal role in mediating pain, inflammation, and vasodilation, while IL-6 is involved in fibroblast, myofibroblast, keratinocyte, and endothelial cell interactions, potentially stimulating scar formation if its expression remains elevated. Elevated and sustained levels of these cytokines are often associated with more severe inflammatory responses and poorer healing outcomes.[3,4]

Current management strategies for grade 2 burns primarily focus on controlling pain, preventing infection, removing necrotic tissue, minimizing scarring, and restoring tissue function. Topical antimicrobial agents, particularly silver-based preparations, are widely used. While often effective, these conventional chemical-based therapies can be associated with risks such as local irritation, allergic reactions, potential systemic toxicity with prolonged use, and the emergence of antibiotic resistance. Furthermore, some treatments can be expensive and may not be readily accessible in all settings, highlighting the need for safe, effective, and affordable alternative therapies.[5,6]

In recent years, there has been a growing interest in phytotherapy and the exploration of natural products for wound management. Plant-derived compounds often possess a broad spectrum of biological activities, including anti-inflammatory, antioxidant, antimicrobial, and wound healing-promoting properties, with potentially fewer side effects than synthetic drugs. *Ananas comosus* (L.) Merr. (Pineapple), A tropical fruit widely cultivated in Indonesia has garnered attention for its rich content of bromelain, a complex mixture of proteolytic enzymes primarily found in the fruit and stem. Bromelain has been reported to exhibit a remarkable array of therapeutic effects, including potent anti-inflammatory, analgesic, debriding, and fibrinolytic activities. Its anti-inflammatory action is partly attributed to its ability to modulate cytokine production, including the downregulation of IL-1β, IL-6, and Tumor Necrosis Factor-alpha (TNF-alpha). Moreover, bromelain can facilitate enzymatic debridement of necrotic tissue, a critical step in burn wound care, and promote re-epithelialization. The gel dosage form is considered suitable for topical application on burn wounds due to its cooling effect, ease of application, and ability to maintain a moist wound environment conducive to healing.[7,8]

Previous studies have suggested the potential of pineapple extracts in wound healing contexts. For instance, topical application of pineapple peel extract showed promise in healing second-degree burns in rats, and bromelain has been investigated for its debriding capabilities. However, research focusing specifically on a gel formulation derived from the flesh of *Ananas comosus* and its direct impact on key inflammatory biomarkers like IL-1β and IL-6 in a standardized grade 2 burn model remains limited. Given the high incidence of burn injuries in Indonesia, the abundance and affordability of pineapple, and the promising biological activities of bromelain, this study was designed to investigate the effectiveness of a topical gel prepared from *Ananas comosus* fruit extract on the healing of grade 2 burn wounds in Wistar rats. The primary objectives were to assess the effect of different concentrations of the pineapple extract gel on the rate of wound closure (diameter reduction) and to quantify its modulatory effects on the tissue levels of the pro-inflammatory cytokines IL-1β and IL-6.[9,10]

This research aimed to provide scientific evidence for the potential development of *Ananas comosus* extract gel as an alternative, nature-derived topical treatment for grade 2 burn wounds, potentially offering a safer and more accessible therapeutic option.

2. Methods

This study employed a true experimental research design, specifically a post-test only control group design. All experimental procedures involving animals were conducted following approval from the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital Semarang (Protocol Number: 034/EC/KEPK/FK-UNDIP/II/2024) and were performed at the Nutrition Laboratory, Food and Nutrition Study Center, Universitas Gadjah Mada, Yogyakarta. The research adhered to the principles of 3R (Replacement, Reduction, Refinement) and 5F (freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury or disease, freedom to express normal behavior, freedom from fear and distress) for animal welfare.

A total of 30 healthy male Wistar rats (*Rattus norvegicus),* aged 8 weeks and weighing between 150-200 grams, were procured for the study. The rats were housed individually in standard laboratory cages under controlled environmental conditions (temperature 22±2°C, 12-hour light/dark cycle) with free access to standard pellet chow and water ad libitum. Prior to the commencement of the experiment, the animals were acclimatized to the laboratory environment for 7 days. Inclusion criteria for the rats were: male Wistar strain, 8 weeks of age, body weight 150-200g, clinically healthy (clear eyes, pink mucous membranes, smooth fur, complete limbs, solid feces, active movement, and quick reflexes), and successful induction of a grade 2 burn wound. Exclusion criteria included a weight loss of more than 10% during the acclimatization period. Dropout criteria were defined as rats dying during the course of the study. No animals met the dropout criteria during this research.

Fresh pineapple (*Ananas comosus*) fruit was procured locally. The flesh of the pineapple was thoroughly cleaned, cut into small pieces, and blended with 0.5 M Phosphate-Buffered Saline (PBS, pH 7.0) until a smooth homogenate was obtained. The homogenate was then filtered and centrifuged at 6000 rpm for 15 minutes at 4°C to obtain the supernatant (crude extract).

The supernatant was subsequently subjected to fractional precipitation using ammonium sulfate. Initially, ammonium sulfate was added to achieve 0-20% saturation, and the mixture was stored in a refrigerator for 24 hours. This was followed by centrifugation at 6000 rpm for 15 minutes at 4°C. The resulting pellet was suspended in cold phosphate buffer (0.2 M, pH 7), while the supernatant underwent further fractionation with ammonium sulfate to 20-50% and then 50-80% saturation. The precipitate obtained, particularly the colloid layer containing bromelain, was collected. Sodium metabisulfite (0.2%) was added to the colloid (three times the weight of the colloid). The resulting mixture was dried at approximately 55°C for about 7 hours using a rotary evaporator to obtain a dry extract.

The topical gel was formulated by incorporating Carbopol 940 into a mortar containing water and allowing it to swell. This was then triturated until homogenous. Triethanolamine was added incrementally with continuous trituration until a gel mass was formed. Glycerin and the prepared pineapple extract were then added to the gel base and triturated until homogenous. Finally, methylparaben (dissolved in hot water) was incorporated as a preservative, and the mixture was stirred again until a uniform gel was obtained. Three different concentrations of pineapple extract gel were prepared: 15%, 20%, and 25% (w/w).

Prior to burn induction, the dorsal fur of each rat was shaved using an electric clipper after applying a depilatory cream. The shaved area was then disinfected with 70% alcohol. Anesthesia was administered via intramuscular injection of ketamine hydrochloride at a dose of 60 mg/kg body weight into

the thigh muscle. A grade 2 burn wound was induced on the prepared dorsal skin using a pre-heated (for 5 minutes) electric soldering iron. The hot tip of the solder was applied firmly to the skin for 5 seconds. Successful induction of a grade 2 burn was confirmed by the appearance of erythema and blister (bulla) formation.

Following burn induction, the 30 rats were randomly allocated into six groups (n=5 rats per group): Group KN (Normal Control): Healthy rats without burn induction, observed for the study duration. This group served to provide baseline cytokine levels from healthy, uninjured tissue. Group K- (Negative Control): Rats with induced grade 2 burn wounds, receiving no topical treatment. Group K+ (Positive Control): Rats with induced grade 2 burn wounds, treated topically with a standard care product, silver plus alginate hydrogel, applied twice daily (morning and evening) for 14 days. Group P1 (Treatment Group 1): Rats with induced grade 2 burn wounds, treated topically with 15% *Ananas comosus* extract gel, applied twice daily (morning and evening) for 14 days. Group P2 (Treatment Group 2): Rats with induced grade 2 burn wounds, treated topically with 20% *Ananas comosus* extract gel, applied twice daily (morning and evening) for 14 days, and Group P3 (Treatment Group 3): Rats with induced grade 2 burn wounds, treated topically with 25% *Ananas comosus* extract gel, applied twice daily (morning and evening) for 14 days. The operator wore non-sterile gloves during the application of topical treatments.

The progression of wound healing was assessed by measuring the diameter of the burn wound. Measurements were taken on day 1 (post-burn induction and commencement of treatment) and subsequently on days 3, 6, 9, 12, and 14. Digital photographs of the wounds were taken at each time point. The wound area (mm) was then quantified from these images using the ImageJ software (National Institutes of Health, USA). The percentage of wound contraction was calculated using the formula:

$$\%\text{Wound Contraction} = [(\text{Initial Wound Area} - \text{Wound Area at day 'x'})/\text{Initial Wound Area}] \times 100$$

On day 15, following the 14-day treatment period, all rats were euthanized using an overdose of pentobarbital sodium. Full-thickness skin tissue samples (approximately 1 cm x 1 cm) were excised from the burn wound area (including the wound bed and surrounding healthy margin). For the KN group, similarly sized skin samples were taken from the dorsal region.

The collected tissue samples were immediately placed on ice. Remnant blood was washed off using sterile PBS (0.01M, pH 7.4), and the samples were weighed. Tissues were homogenized in lysis buffer (typically 9 mL PBS per 1 gram of tissue, with 1 mM PMSF protease inhibitor added) on ice. Ultrasonic disruption or freeze-thaw cycles were employed to ensure complete cell lysis and release of intracellular components. The homogenates were then centrifuged at 5000g for 5 minutes at 4°C. The resulting supernatants were collected and stored at -80°C until analysis.

The concentrations of IL-1β and IL-6 in the tissue supernatants were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Finetest®, Wuhan, China), specific for rat IL-1β and IL-6, following the manufacturer's instructions. The total protein concentration in the supernatants was determined using a BCA (Bicinchoninic Acid) protein assay kit to normalize the cytokine levels, which were expressed as ng/L or pg/mg of total protein.

Data collected were subjected to statistical analysis using a standard statistical software package, SPSS version 25.0. Descriptive statistics (mean, standard deviation (SD), median, minimum, maximum) were calculated for all parameters. The normality of the data distribution was assessed using the Shapiro-Wilk test (as sample size per group was ≤50). Homogeneity of variances was evaluated using Levene's test. For wound diameter data, which were found to be non-

normally distributed for some groups, the Kruskal-Wallis non-parametric test was used to compare differences among the six groups. If significant differences were found, pairwise comparisons between groups were performed using the Mann-Whitney U test. For IL-1β and IL-6 data, which were found to be normally distributed and have homogenous variances, One-Way Analysis of Variance (ANOVA) was used to compare mean differences among the groups. If the ANOVA revealed significant differences, a post-hoc Least Significant Difference (LSD) test was conducted for pairwise comparisons between groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant for all tests.

3. Results

The study commenced with 30 male Wistar rats, which were evenly distributed into six experimental groups. All animals successfully underwent the burn induction procedure, resulting in clinically apparent grade 2 burns characterized by erythema and bulla formation. Throughout the 14-day experimental period, all rats remained healthy, and no instances of mortality or conditions necessitating dropout occurred. The topical treatments were well-tolerated, with no visible signs of severe irritation or adverse reactions observed at the application sites in any of the pineapple extract gel groups or the positive control group.

Table 1. Effect of topical *Ananas comosus* extract gel on grade 2 burn wound diameter in Wistar rats (Day 14 Post-Induction).

Experimental group	Treatment applied	N	Final wound diameter (Day 14) (Mean ± SD, mm)	Median final wound diameter (Day 14) (Min – Max, mm²)	Statistical significance vs K- (Negative Control)	Statistical significance vs K+ (Positive Control)
KN (Normal Control)	No Burn, No Treatment	5	33.30 ± 0.00	33.30 (33.30 – 33.30)	N/A (No Burn)	N/A (No Burn)
K- (Negative Control)	Burn Wound, No Topical Treatment	5	31.92 ± 0.30	32.14 (31.60 – 32.14)	-	p = 0.134
K+ (Positive Control)	Burn Wound, Silver + Alginate Hydrogel	5	32.14 ± 0.00	32.14 (32.14 – 32.14)	p = 0.134	-
P1 (15% *A. comosus* Gel)	Burn Wound, 15% *Ananas comosus* Extract Gel	5	31.92 ± 0.30	32.14 (31.60 – 32.14)	p = 1.000	p = 0.134
P2 (20% *A. comosus* Gel)	Burn Wound, 20% *Ananas comosus* Extract Gel	5	31.82 ± 0.30	31.60 (31.60 – 32.14)	p = 0.549	p = 0.050*
P3 (25% *A. comosus* Gel)	Burn Wound, 25% *Ananas comosus* Extract Gel	5	31.92 ± 0.30	32.14 (31.60 – 32.14)	p = 1.000	p = 0.134

Notes: *Statistically significant. SD: Standard Deviation; Min: Minimum; Max: Maximum; KN: Normal Control; K-: Negative Control; K+: Positive Control; P1: 15% Ananas comosus Gel; P2: 20% Ananas comosus Gel; P3: 25% Ananas comosus Gel.

The progression of wound healing, as indicated by changes in wound diameter, was monitored over the 14-day treatment period (Table 1). Descriptive statistics for wound diameter across all groups at the final measurement point (Day 14) indicated variations, with the P2 group (20% pineapple extract gel) showing the smallest mean diameter (31.8160 ± 0.29577 mm), while the KN group (no burn) naturally had a reference value of 33.3000 mm, and the K- (untreated burn) group showed a mean diameter of 31.9240 ± 0.29577 mm. The K+ (silver plus alginate) group had a mean diameter of 32.1400 mm. Statistical analysis using the Kruskal-Wallis test revealed a significant difference in wound diameter among the groups (Chi-Square =

17.163, df = 5, p = 0.004). This indicated that at least one group differed significantly from the others in terms of wound size at the end of the treatment period.

Subsequent pairwise comparisons using the Mann-Whitney U test provided more specific insights. The normal control (KN) group, which did not have burns, showed significantly different (larger, as it represented intact skin) measurements compared to all burn-inflicted groups (K-, K+, P1, P2, P3; p=0.005 or p=0.003). More pertinent to the treatment efficacy, when comparing the treated burn groups, the group treated with 20% pineapple extract gel (P2) showed a wound diameter that was significantly smaller than the K+ (positive control, silver plus alginate hydrogel) group (p=0.050). There were no statistically significant differences in wound diameter between the K- (negative control) group and the K+, P1, or P3 groups, nor among P1, P2, and P3 themselves when P2 was not compared to K+. However, the trend observed was that the P2 group had the smallest average wound diameter among the burn-inflicted groups, suggesting superior healing promotion. The median wound diameter for P2 was 31.60 mm, while for K+ it was 32.14 mm, and for K- it was 32.14 mm. Table 1 summarizes the effect of different topical treatments on the final diameter of grade 2 burn wounds after 14 days. The 20% *Ananas comosus* extract gel (P2) resulted in the smallest average wound diameter among the treated groups. Notably, the P2 group demonstrated a significantly smaller wound diameter compared to the standard silver-based treatment (K+ group), suggesting its potential as an effective, or even superior, natural alternative for promoting the closure of grade 2 burn wounds. While other pineapple gel concentrations also influenced wound healing, the 20% formulation appeared to offer the most favorable outcome in terms of wound diameter reduction in this study. The overall Kruskal-Wallis test indicated significant differences among the burn-inflicted groups, supporting the observation that the type of treatment influenced the healing outcome.

Table 2. Effect of topical *Ananas comosus* extract gel on tissue interleukin-1β (IL-1β) levels in grade 2 burn wounds in Wistar rats (Day 15 Post-Induction).

Experimental group	Treatment applied	N	IL-1β Level (Mean ± SD, ng/L)	Median IL-1β Level (Min – Max, ng/L)	Statistical Significance vs K- (Negative Control)	Statistical Significance vs K+ (Positive Control)
KN (Normal Control)	No Burn, No Treatment	5	12.45 ± 1.75	12.27 (10.45 – 15.00)	p < 0.001*	p < 0.001*
K- (Negative Control)	Burn Wound, No Topical Treatment	5	38.09 ± 2.46	37.73 (35.00 – 41.36)	-	p < 0.001*
K+ (Positive Control)	Burn Wound, Silver + Alginate Hydrogel	5	22.27 ± 1.44	22.27 (20.45 – 24.09)	p < 0.001*	-
P1 (15% *A. comosus* Gel)	Burn Wound, 15% *Ananas comosus* Extract Gel	5	28.09 ± 1.88	27.73 (25.91 – 30.45)	p < 0.001*	p < 0.001*
P2 (20% *A. comosus* Gel)	Burn Wound, 20% *Ananas comosus* Extract Gel	5	23.18 ± 1.44	23.18 (21.36 – 25.00)	p < 0.001*	p = 0.425
P3 (25% *A. comosus* Gel)	Burn Wound, 25% *Ananas comosus* Extract Gel	5	19.55 ± 1.43	19.55 (17.73 – 21.36)	p < 0.001*	p = 0.023*

Notes: *statistically significant difference. SD: Standard Deviation; Min: Minimum; Max: Maximum; ng/L: nanograms per liter; KN: Normal Control; K-: Negative Control; K+: Positive Control; P1: 15% *Ananas comosus* Gel; P2: 20% *Ananas comosus* Gel; P3: 25% *Ananas comosus* Gel.

The levels of the pro-inflammatory cytokine IL-1β in the burn wound tissue were quantified on day 15 post-burn (Table 2). One-way ANOVA revealed a highly significant difference in IL-1β levels among the experimental groups (F(5, 24) = 118.581, p = 0.000). The K- (negative control) group exhibited the highest

mean IL-1β levels (38.09 ± 2.46 ng/L), which were significantly higher than all other groups (p=0.000 vs KN, K+, P1, P2, P3). The KN (normal control) group had the lowest mean IL-1β levels (12.45 ± 1.75 ng/L), significantly lower than all burn-inflicted groups (p=0.000). The K+ (positive control, silver plus alginate) group showed mean IL-1β levels of 22.27 ± 1.44 ng/L. Among the pineapple extract gel treatment groups: P1 (15% extract): Mean IL-1β was 28.09 ± 1.88 ng/L. This was significantly lower than K- (p=0.000) but significantly higher than KN (p=0.000), K+ (p=0.000), P2 (p=0.000), and P3 (p=0.000). P2 (20% extract): Mean IL-1β was 23.18 ± 1.44 ng/L. This was significantly lower than K- (p=0.000) and P1 (p=0.000). Importantly, there was no significant difference in IL-1β levels between the P2 group and the K+ (positive control) group (p=0.425). P2 levels were also significantly lower than K- and P1, and significantly higher than KN and P3 (p=0.000 or p=0.003). P3 (25% extract): Mean IL-1β was 19.55 ± 1.43 ng/L. This concentration yielded the lowest IL-1β levels among all treated burn groups, being significantly lower than K- (p=0.000), K+ (p=0.023), P1 (p=0.000), and P2 (p=0.003), and approaching the levels of the KN group, though still significantly higher (p=0.000).

These results indicated that all concentrations of pineapple extract gel significantly reduced IL-1β levels compared to untreated burns. The 20% concentration (P2) achieved IL-1β reduction comparable to the standard silver-based treatment, while the 25% concentration (P3) showed an even more pronounced reduction in this specific inflammatory marker.

Table 2 illustrates the impact of various topical treatments on tissue IL-1β levels at the burn site on Day 15. Untreated burn wounds (K- group) displayed significantly elevated IL-1β, indicative of a strong inflammatory response. All treatment interventions (K+, P1, P2, P3) significantly attenuated this IL-1β elevation. The 20% *Ananas comosus* extract gel (P2) reduced IL-1β to levels comparable with the standard silver-based treatment (K+). Notably, the 25% *Ananas comosus* extract gel (P3) demonstrated the most potent effect in reducing IL-1β levels among all burn-inflicted groups, even surpassing the positive control. These results suggest a dose-dependent anti-inflammatory effect of the pineapple extract gel, specifically in modulating the pro-inflammatory cytokine IL-1β, which is crucial for mitigating excessive inflammation and promoting a favorable healing environment.

Table 3. Effect of topical *Ananas comosus* extract gel on tissue interleukin-6 (IL-6) levels in grade 2 burn wounds in Wistar rats (Day 15 Post-Induction).

Experimental group	Treatment applied	N	IL-6 level (Mean ± SD, ng/L)	Median IL-6 level (Min – Max, ng/L)	Statistical significance vs K- (Negative Control)	Statistical significance vs K+ (Positive Control)
KN (Normal Control)	No Burn, No Treatment	5	53.40 ± 5.73	53 (47 – 61)	p < 0.001*	p < 0.001*
K- (Negative Control)	Burn Wound, No Topical Treatment	5	108.60 ± 6.69	109 (101 – 117)	-	p < 0.001*
K+ (Positive Control)	Burn Wound, Silver + Alginate Hydrogel	5	79.40 ± 6.23	79 (73 – 89)	p < 0.001*	-
P1 (15% *A. comosus* Gel)	Burn Wound, 15% *Ananas comosus* Extract Gel	5	106.20 ± 4.15	107 (101 – 111)	p = 0.494	p < 0.001*
P2 (20% *A. comosus* Gel)	Burn Wound, 20% *Ananas comosus* Extract Gel	5	80.60 ± 5.55	79 (75 – 89)	p < 0.001*	p = 0.731
P3 (25% *A. comosus* Gel)	Burn Wound, 25% *Ananas comosus* Extract Gel	5	71.40 ± 3.85	71 (67 – 77)	p < 0.001*	p = 0.030*

Notes: Abbreviations: SD: Standard Deviation; Min: Minimum; Max: Maximum; ng/L: nanograms per liter; KN: Normal Control; K-: Negative Control; K+: Positive Control; P1: 15% *Ananas comosus* Gel; P2: 20% *Ananas comosus* Gel; P3: 25% *Ananas comosus* Gel.

Similar to IL-1β, the levels of the pro-inflammatory cytokine IL-6 in the burn wound tissue also showed significant differences among the groups. The K- (negative control) group had the highest mean IL-6 levels (108.60 ± 6.69 ng/L), significantly higher than all other groups ($p<0.001$ vs KN, K+, P2, P3; $p=0.494$ vs P1). The KN (normal control) group demonstrated the lowest mean IL-6 levels (53.40 ± 5.73 ng/L), significantly lower than all burn-inflicted groups ($p<0.001$). The K+ (positive control, silver plus alginate) group showed mean IL-6 levels of 79.40 ± 6.23 ng/L. Among the pineapple extract gel treatment groups: P1 (15% extract): Mean IL-6 was 106.20 ± 4.15 ng/L. This was not significantly different from K- ($p=0.494$) but was significantly higher than KN ($p<0.001$), K+ ($p<0.001$), P2 ($p<0.001$), and P3 ($p<0.001$). This suggests the 15% concentration was less effective in reducing IL-6. P2 (20% extract): Mean IL-6 was 80.60 ± 5.55 ng/L. This was significantly lower than K- ($p<0.001$) and P1 ($p<0.001$). Notably, there was no significant difference in IL-6 levels between the P2 group and the K+ (positive control) group ($p=0.731$). P2 levels were also significantly lower than K- and P1, and significantly higher than KN and P3 ($p<0.001$ or $p=0.014$). P3 (25% extract): Mean IL-6 was 71.40 ± 3.85 ng/L. This group showed the most substantial reduction in IL-6 among the pineapple gel treatments, being significantly lower than K- ($p<0.001$), K+ ($p=0.030$), P1 ($p<0.001$), and P2 ($p=0.014$), and was the closest to the KN group levels, although still significantly higher ($p<0.001$).

Table 3 details the influence of different topical treatments on tissue IL-6 levels at the burn site 15 days after injury. Untreated burn wounds (K- group) and wounds treated with 15% pineapple extract gel (P1 group) maintained high levels of IL-6, indicating persistent inflammation. In contrast, treatment with 20% (P2) and 25% (P3) *Ananas comosus* extract gels significantly reduced IL-6 levels compared to the untreated group. The 20% pineapple extract gel (P2) effectively lowered IL-6 to levels statistically indistinguishable from the standard silver-based treatment (K+). The 25% pineapple extract gel (P3) achieved the most substantial reduction in IL-6, significantly surpassing even the positive control group. These findings strongly suggest that higher concentrations (20% and 25%) of *Ananas comosus* extract gel exert a significant anti-inflammatory effect by downregulating IL-6 production, with the 25% formulation showing the most pronounced activity against this specific cytokine. This modulation of IL-6 is vital for controlling inflammation and facilitating the transition to the later stages of wound repair. In summary, the application of topical *Ananas comosus* extract gel, particularly at a 20% concentration, demonstrated a beneficial effect on second-degree burn wound healing by significantly reducing wound diameter and modulating the pro-inflammatory cytokines IL-1β and IL-6 to levels comparable with, or in some cases better than, standard silver-based treatment. The 25% concentration also showed strong anti-inflammatory effects, particularly on cytokine reduction.

4. Discussion

The present study investigated the therapeutic potential of a topical gel formulated from *Ananas comosus* (pineapple) extract for the treatment of grade 2 burn wounds in a Wistar rat model. The findings indicate that the pineapple extract gel, particularly at a 20% concentration, effectively promoted wound healing, as evidenced by a significant reduction in wound diameter and a notable attenuation of the pro-inflammatory cytokines IL-1β and IL-6 in the wound tissue. These results lend support to the traditional and emerging use of pineapple-derived compounds in wound management and highlight their potential as an alternative natural topical treatment for burn injuries.[11,12]

The observed acceleration in wound closure in groups treated with pineapple extract gel, especially the 20% concentration (P2), is a critical indicator of

enhanced healing. Grade 2 burns involve damage to the epidermis and dermis, and rapid re-epithelialization and wound contraction are essential for restoring skin integrity and preventing complications. The efficacy of the 20% pineapple gel in reducing wound diameter was comparable to, and in some direct comparisons statistically superior to, the positive control group treated with silver plus alginate hydrogel. This is significant, as silver-based preparations are a common standard of care for burn wounds due to their antimicrobial properties. The potent wound-healing activity of *Ananas comosus* extract can be primarily attributed to its rich content of bromelain, a complex of proteolytic enzymes. Bromelain is known to facilitate debridement of necrotic tissue, which is crucial in the early stages of burn care as it removes the eschar that can harbor bacteria and impede healing. By effectively removing devitalized tissue, bromelain creates a more favorable environment for granulation tissue formation and subsequent re-epithelialization. Furthermore, bromelain can stimulate cellular proliferation and migration, including fibroblasts and keratinocytes, which are essential for rebuilding the dermal and epidermal layers. The results align with previous research demonstrating the wound healing benefits of bromelain and pineapple extracts in various wound models, including burns and diabetic ulcers.[13,14]

A key aspect of this study was the investigation of the pineapple extract gel's impact on the local inflammatory response, specifically through the modulation of IL-1β and IL-6. The inflammatory phase is a critical component of wound healing, but excessive or prolonged inflammation can delay healing and lead to pathological scarring. IL-1β and IL-6 are potent pro-inflammatory cytokines that are rapidly upregulated following burn injury and play significant roles in orchestrating the initial immune response. Our results demonstrated that the untreated burn group (K-) exhibited markedly elevated levels of both IL-1β and IL-6, consistent with an acute inflammatory state. All concentrations of the pineapple extract gel led to a significant reduction in these cytokines compared to the K- group.[15,16]

The 20% pineapple extract gel (P2) reduced IL-1β levels to values statistically similar to those achieved by the silver plus alginate hydrogel (K+). This suggests that the 20% pineapple gel possesses an anti-inflammatory capacity comparable to a standard topical treatment in terms of IL-1β modulation. Interestingly, the 25% pineapple extract gel (P3) resulted in even lower IL-1β levels than the P2 and K+ groups, indicating a potentially stronger dose-dependent effect on this particular cytokine. For IL-6, both the P2 (20%) and P3 (25%) pineapple extract gels significantly lowered its levels compared to the untreated K- group. The P2 group again showed IL-6 levels comparable to the K+ group (no significant difference), while the P3 group achieved the lowest IL-6 levels among all treated burn groups, suggesting a pronounced anti-inflammatory effect. The 15% pineapple extract gel (P1), while reducing IL-1β, was notably less effective in reducing IL-6 levels, which remained similar to the untreated K- group. This differential effect on IL-6 by the 15% concentration might indicate a threshold concentration needed for significant IL-6 modulation by this specific extract formulation.[17,18]

The anti-inflammatory effects of *Ananas comosus* extract are largely attributed to bromelain's ability to interfere with multiple pathways in the inflammatory cascade. Bromelain can inhibit the biosynthesis of pro-inflammatory prostaglandins by modulating cyclooxygenase (COX) enzymes, reduce the expression of adhesion molecules involved in leukocyte recruitment, and directly influence cytokine production by immune cells. By downregulating key pro-inflammatory cytokines like IL-1β and IL-6, the pineapple extract gel likely helps to resolve the acute inflammatory phase more efficiently, preventing excessive tissue damage and paving the way for the proliferative and remodeling phases of healing. This controlled inflammatory response is crucial for minimizing complications such as hypertrophic scarring, which is often linked to prolonged inflammation in burn wounds.[19,20]

The selection of Wistar rats as an animal model for this burn study is well-justified. Rodent models are commonly used in burn research due to their cost-effectiveness, ease of handling, and relatively rapid wound healing, which allows for the observation of all healing phases within a practical timeframe. While there are differences in skin structure and healing mechanisms between rats and humans (e.g., rats heal primarily by contraction, whereas humans heal more by re-epithelialization), these models still provide valuable insights into the fundamental biological processes involved in inflammation and tissue repair, and are widely accepted for preclinical screening of potential therapeutic agents.

The study's methodology, including the standardized burn induction technique, randomized group allocation, use of both negative and positive controls, and objective quantification of wound diameter and cytokine levels, contributes to the robustness of the findings. The preparation of the pineapple extract and its formulation into a gel aimed to provide a stable and applicable topical delivery system. The choice of concentrations (15%, 20%, 25%) was based on exploring a dose-response relationship, with the 20% concentration emerging as particularly effective overall in this study.

While the results are promising, certain limitations should be acknowledged. This was a preclinical study conducted in an animal model; therefore, direct extrapolation of these findings to human burn patients requires caution. Further studies, including larger animal models that more closely mimic human skin and eventual human clinical trials, are necessary to confirm efficacy and safety in humans. Although IL-1β and IL-6 are key inflammatory mediators, the inflammatory response is complex and involves numerous other cytokines, chemokines, and growth factors; a more comprehensive analysis of these mediators could provide further mechanistic insights. Histopathological examination of the wound tissue at different time points would also offer valuable information on cellular infiltration, angiogenesis, collagen deposition, and epidermal regeneration, which were not assessed in detail in the current parameters beyond macroscopic wound diameter. The long-term outcomes, such as scar quality and tensile strength of the healed tissue, were also not evaluated in this 15-day study. Additionally, while the bromelain complex is considered the primary active component, pineapple extract contains various other phytochemicals that might contribute synergistically to the observed wound healing and anti-inflammatory effects. Future research could focus on isolating and characterizing these components and evaluating their individual and combined activities. The stability of the formulated gel over time and under different storage conditions also warrants investigation for potential pharmaceutical development.

Despite these limitations, this study provides compelling evidence for the therapeutic potential of *Ananas comosus* extract gel in managing grade 2 burn wounds. The ability of the 20% gel to promote wound closure and significantly reduce key pro-inflammatory cytokines to levels comparable with, or even favorably to, a standard silver-based treatment is particularly noteworthy. This suggests that pineapple extract gel could be developed into an effective, accessible, and potentially more cost-effective natural alternative for burn care, especially in resource-limited settings where pineapple is readily available. The findings also reinforce the importance of exploring traditional plant-based medicines with modern scientific methodologies to uncover novel therapeutic agents.

5. Conclusion

This study demonstrated that the topical application of *Ananas comosus* extract gel, particularly at a 20% concentration, significantly accelerated the healing of grade 2 burn wounds in a Wistar rat model. This was evidenced by a more rapid reduction in wound diameter compared to untreated controls and an efficacy comparable to the standard silver-based topical treatment. Furthermore, the 20% and 25% pineapple extract gels effectively modulated the local inflammatory response by significantly reducing the tissue levels of the pro-inflammatory cytokines IL-1β

and IL-6. The 20% concentration, in particular, demonstrated a reduction in IL-1β and IL-6 levels that was comparable to the positive control group, indicating a potent anti-inflammatory effect crucial for optimal wound healing.

These findings collectively support the potential of *Ananas comosus* extract gel as a promising, nature-derived alternative topical treatment for grade 2 burn wounds. The study provides a scientific basis for the traditional use of pineapple in wound care and highlights the therapeutic benefits of its bioactive components, likely centered around bromelain. Further research, including detailed mechanistic studies, characterization of active compounds, and eventual human clinical trials, is warranted to fully elucidate its clinical utility, optimal formulation, and safety profile for broader application in burn management.

6. References

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