

Wound healing properties of cow ghee and ethanolic pineapple (*Ananas comosus merr*) leaf extract combination in rabbit (*Oryctolagus cuniculus*)

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ABSTRACT

Medicinal plants and Ayurvedic ghee are natural products with therapeutic properties relevant to wound healing. This study aimed to evaluate the wound-healing effects of different concentrations of cow ghee combined with ethanolic pineapple leaf extract in rabbits. Thirty New Zealand white rabbits were used for this study and divided into five groups. NSS served as the negative control group; MUP received mupirocin ointment as the standard treatment; and CP1, CP2, and CP3 were treated with [75% CG and 25% EPLE (w/w)], [50% CG and 50% EPLE (w/w)], and [25% CG and 75% EPLE (w/w)], respectively and served as the treatment groups. A single 3-cm full-thickness incisional wound was created on each rabbit's back. Approximately 0.5 g of the assigned formulation was applied daily for 21 days. Gross macroscopic and histological wound healing were graded and compared on days 7, 14, and 21. Results showed that CP1, CP2, and CP3 showed better wound repair than NSS and, in several histological parameters, than MUP. These findings suggest that topical application of the formulation at different concentrations on incisional wounds appears to support wound healing and may improve selected histological indices compared with standard mupirocin.

INTRODUCTION

Wounds are a common clinical condition that range from acute injuries to complex chronic lesions. Unlike acute wounds, chronic wounds fail to progress through the normal stages of healing and do not resolve in an orderly and timely manner (Falanga, 2005). This contributes to a global health challenge that burdens the medical and healthcare system, leading to productivity losses, sick leave, and early retirement (Martinengo et al., 2019).

Approximately 1–3% of the total health expenditure is spent on wound treatment in developed countries (Olsson et al., 2019). In the Philippines, the wound care market size reached USD 111.4 million in 2025 and is projected to reach USD 175.8 million by 2034, exhibiting a growth rate (CAGR) of 5.20% during the forecast period of 2026–2034 (IMARC Group, 2026).

Staphylococcus aureus, *Escherichia coli*, and *Pseudomonas aeruginosa* are among the most common bacterial isolates from infected wounds (Sule et al., 2002). These organisms can delay wound healing. Wound healing is a complex, overlapping process of cellular reactions and interactions that restore skin integrity and function (Situm et al., 2013). Although antibiotics remain important in reducing infection and promoting healing, their use is limited by the emergence of resistant microbes and the high cost of commercial topical drugs (Browder et al., 1998), prompting renewed interest in the use of herbal and Ayurvedic medicines that are cost-effective and possess antimicrobial and wound-healing properties.

Cow ghee, a clarified butter obtained from cow's milk, has traditionally been used to treat burns, ulcers, and traumatic wounds for its soothing, rejuvenating, antimicrobial, and anti-inflammatory properties, as well as its ability to penetrate deeper layers of cells and tissues, thereby potentially supporting wound healing (Uppalwar et al., 2014).

Pineapple (*Ananas comosus* Merr.) is a popular plant in the Philippines. In folk medicine, pineapple leaf extract has been used as an antimicrobial, antioxidant, and anti-inflammatory agent. The antimicrobial and anti-inflammatory effects of the plant were attributed to bromelain (Dutta et al., 2013), the protein-digesting enzyme from pineapple; however, its quantities in leaves are lower compared to the fruit (Sriwatanapongse et al., 2000). Flavonoids,

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tannins, and vitamins A and C are major antioxidant constituents of the plant.

A review of the available literature suggests that the combined formulation of cow ghee and ethanolic pineapple leaf extract as an alternative medicine has not been extensively investigated. Hence, the objective of this study was to evaluate macroscopic and histologic wound-healing responses of wounds treated with different cow ghee–EPLA ratios in New Zealand white rabbits.

MATERIALS AND METHODS

Ethical Consideration

This research was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines, Los Baños, with assigned protocol number 2018-0012.

Collection of Milk and Ghee

Cow's milk for ghee preparation was purchased from a local backyard farm in Brgy. McKinley, Catarman, Northern Samar. Cow ghee was prepared by the direct cream method (De, 2000). Soon after collection, two liters of milk were warmed to 40 °C for 10 minutes, cooled, and stored in a refrigerator. The cream was collected daily for 10 days with a spoon, kept in a plastic container, and stored in the refrigerator at 7 °C. Butter was prepared by churning the cream with a hand-operated butter churn, then heated with continuous stirring over a direct flame and clarified into ghee. Afterward, the ghee was filtered through Whatman No. 4 filter paper, poured into a glass bottle, cooled to room temperature, capped, and refrigerated until further use.

Plant Collection and Extract Preparation

Mature pineapple leaves were collected from Lavezares, Northern Samar, where these plants are locally produced. The mature pineapple (*Ananas comosus* Merr.) leaves were cleaned in running tap water, cut into small pieces, and sun-dried for 14 days. This method may have limitations in this study, as it can reduce heat- or light-sensitive compounds. The dried leaves were powdered using an electric blender. A 2-mm-mesh sieve was used to obtain fine powder. For extraction, one hundred (100) grams of powdered material was soaked in a beaker containing 1000 ml of 95% ethanol (1:10 w/v; 1600 g of powder in 16 L solvent for 24 hours). The extract was filtered through muslin cloth, then through Whatman filter paper no. 4. It was then concentrated in a water bath at 40°C until a paste formed. The extract, in paste form, was stored in a sterile amber bottle to minimize light exposure and preserve its active constituents. The extract was filtered through muslin cloth, then through Whatman filter paper no. 4. It was then concentrated in a water bath at 40°C until a paste formed. The extract, in paste form, was stored in a sterile amber bottle to minimize light exposure and preserve its active constituents.

Preparation of Cow Ghee and Ethanolic Pineapple Leaf Extract Combination

Cow ghee and ethanolic pineapple leaf extract were formulated and designated as CP1 [75% CG and 25% EPLA (w/w)], CP2 [50% CG and 50% EPLA (w/w)], and CP3 [25% CG and 75% EPLA (w/w)]. The formulated test material was mixed by weight and stored before use.

Study Animals

All rabbits were acclimatized for 14 days and housed individually in standard cages (18L x 17W x 18H inches) under a 12 h light/12 h dark cycle. They were fed a 160 g pelleted rabbit diet daily and had ad libitum access to water. Physical and hematological assessments were performed to screen for disease, and only rabbits deemed fit for surgery were included in the study.

Study Design

A single-factor completely randomized design (CRD) with balanced replication was utilized. Thirty (30) New Zealand white rabbits were randomly divided into five treatment groups, with six animals per group. A lottery method of randomization was used, in which each rabbit was assigned a number and then randomly drawn to ensure unbiased selection of animals per treatment group. Normal saline solution (NSS) and MUP were treated with mupirocin ointment; CP1, CP2, and CP3 were treated with 75% CG and 25% EPLA (w/w), 50% CG and 50% EPLA (w/w), and 25% CG and 75% EPLA (w/w), respectively.

Surgical Procedure

All rabbits were premedicated with intramuscular (IM) acepromazine (10 mg/kg; Labistress®, Labiana, Pasig City, Philippines) as a pre-anesthetic agent, followed 5 minutes later by general anesthesia with intravenous (IV) propofol (10 mg/kg; IV-PRO™, Macropharma Corporation, Pasig City, Philippines). Perioperative analgesia was not administered in any treatment group, and all procedures were conducted in accordance with protocols approved by the UPLB IACUC. The incisional area was located at the left dorsum of the thoracolumbar area in rabbits. A single incision was made in each of the thirty (30) rabbits. A 3-cm full-thickness incisional wound was created using a number 10 scalpel blade. The day of wound creation was marked as day 0 of the experiment. Treatment application MUP was initiated 24 h after wounding.

Treatment Protocol

All rabbits were randomly assigned to groups and treated once daily with different ratios of cow ghee and EPLA. Before applying the formulated test material, the wound was cleaned with normal saline solution. Sterile cotton-tipped applicators were used to apply approximately 0.5 g of test material once daily to the entire wound surface. The wound was covered with sterile gauze, which was changed daily for 21 days and removed before observation.

Wound Healing Evaluation

Gross wound appearance of the wound was monitored daily. It was assessed on days 7, 14, and 21 for the presence of wound elevation, hyperemia, pus, scab, and scar formation using a standardized data collection form and photographic documentation to compare wound appearance between treatment groups. The length and width of the wound were measured and recorded daily. A 0.5-cm skin biopsy was taken on days 7, 14, and 21 to monitor changes in tissue repair from one representative animal per group in the representative animal per group.

Evaluation of Wound Contraction

All rabbits were monitored for 21 days by measuring wound area contraction with a caliper, and the degree of wound contraction was computed as described by Agarwal et al. (2009). The percentage of wound healing was computed using the following formula:

$$\% \text{ wound contraction} = \frac{[(\text{initial wound area} - \text{wound area on observation day}) / \text{initial wound area}] \times 100}{}$$

$$\begin{aligned} \text{where: Healed area} &= \text{Initial wound area} - \text{present wound area,} \\ \text{Initial wound area} &= \text{Length} \times \text{width of wound at day 0 (3 cm)} \\ \text{Present wound area} &= \text{Length} \times \text{width of the wound on the} \\ &\text{day of observation} \end{aligned}$$

After the surgery was performed, the initial wound dimensions (length and width) on each rabbit were recorded to determine the initial wound area. The percent wound contraction was computed by dividing the healed area by the initial wound area, then multiplying by 100. The healed area was derived by subtracting the present wound area from the day-0. Computation of the present wound area was done by multiplying the wound length by its width (Adao et al., 2017). Representative animals that underwent incisional biopsy were removed.

Macroscopic Grading of Wounds

Gross macroscopic characteristics of wound elevation, hyperemia, pus, scab, and scar formations were scored following the procedure of Chupeco et al., as cited by Adao (2017), from days 0 to 21. Elevation was graded as 0–3: 0 = absent; 1 = slightly raised; 2 = moderately raised; 3 = markedly raised. Hyperemia was graded as either 0–3: 0 = absent; 1 = slight; 2 = moderate pink; 3 = red areas surrounding the wound site. Pus formation was graded as 0–3: 0 = absent; 1 = slight; 2 = moderate; 3 = marked presence of pus in the wounds. Scab formation was graded as 0–5: 0 for 10% coverage; 1 for 20%; 2 for 40%; 3 for 60%; 4 for 80%, and 5 for 100%. Lastly, scar formation was graded 0–3: 0 for absent; 1 for slight; 2 for moderate; and 3 for the marked scarring area surrounding the wound site.

Histologic Grading of Wound Repair

A 0.5 cm biopsy specimen was taken from the wound edges of each representative rabbit in its designated group on days 7, 14, and 21. Soon after collecting the biopsy specimen, representative animals from each group were excluded from the study to minimize stress and any disruption of the normal healing process caused by repeated tissue sampling, thereby avoiding confounding effects on the results. Macroscopic data were analyzed using the remaining animal sample available at each time point, with the corresponding sample size considered in the analysis. At the same time, histopathological results represented independent observations and were analyzed descriptively. The specimens were fixed in 10% buffered formalin and processed. The skin samples were sectioned, stained with hematoxylin and eosin (H&E), and examined microscopically at low to high power. Skin biopsies were evaluated blindly. Levels of collagen tissue, angiogenesis, fibroblast proliferation, and epithelialization were evaluated using Abramov's histological scoring system, modified from Greenhalgh's system. A score of 0–3 was used on each parameter independently. The collagen level was graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant). Angiogenesis was graded as either 0 (none), 1 (up to 5 vessels per high-power field [HPF]), 2 (6–10 vessels per HPF), or 3 (more than 10 vessels per HPF). Fibroblast was graded as 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblasts), or 3 (predominantly fibroblasts). Epithelialization was graded as 0 (none), 1 (partial), 2 (complete but immature or thin), or 3 (complete and mature) (Abramov et al., 2007; Greenhalgh, 1990).

Statistical Analysis

Macroscopic grading of wound healing was analyzed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics, followed by Tukey's HSD test to identify which pairs of treatments were statistically different for the specified parameters since one representative animal from each group was removed at each time point (days 7, 14, and 21), thereby reducing the sample size. The analyses were performed independently using the remaining animals in each treatment group ($n = 5$; $n = 4$; $n = 3$ for days 7, 14, and 21, respectively). The difference was considered significant when p -values < 0.05 . All the data were expressed as mean \pm standard deviation SD.

RESULTS AND DISCUSSION

Macroscopic Data of Samples

The macroscopic wound observations on wound elevation, hyperemia, scab formation, pus, and scar formation are presented in Table 1. Before treatment, all wounds exhibited inflammatory signs, including redness and edge edema, and these signs persisted for 14 days in the control and CP-treated groups, after which they eventually subsided the following week.

On day 7, hyperemia scores did not differ significantly among treatments. CP2 showed the lowest hyperemia score, followed by CP1, CP3, mupirocin ointment (MUP), and normal saline solution (NSS). The slight reduction in hyperemia in the CP-treated groups may reflect previous studies reporting that pineapple contains bioactive compounds, such as bromelain and tannins (Hebbar et al., 2008), and shows anti-inflammatory activity (Sagnia et al., 2014). In addition, bromelain has been postulated to markedly reduce hyperemia in a previous study in some dogs (Boxer breeds) with face, chest, and arm bruises (Blonstein, 1992). Although this study did not screen the plant's phytochemicals, the gradual reduction in hyperemia observed in the CP-treated groups may be attributable to bioactive compounds reported in the previous study.

By day 14, the MUP-treated group showed a significant reduction in hyperemia compared with the CP3 group. Mupirocin has a wide spectrum of activities against Gram-positive bacteria, including *Staphylococci* and *Streptococci*, and against certain Gram-negative bacteria, including *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Roghamann et al., 2021). The reduction of hyperemia observed in MUP-treated groups may be associated with its antibacterial activity, which reduces bacterial colonization at the wound site and consequently minimizes local inflammation.

On day 7, scab formation between treatments did not differ significantly, suggesting comparable scab formation. On day 14, scab ratings in both the MUP and CP1 groups were significantly lower than those in the negative control. The detachment of scab, leaving no raw area behind, was observed on day 15 in both MUP and CP1.

In comparison, scab formation terminated later in the NSS, CP2, and CP3 groups. Eurides et al. (1998) reported that the presence of crust on the wound favors healing, as seen in MUP and CP1, and may support early re-epithelialization. However, other authors have reported that when the formation of a wound scab is prevented, the risk of infection is reduced, and the wound heals faster Goss (1977). Nonetheless, all groups showed no scab remnants at the end of the experimental period, and wound closure was observed on day 21.

Regarding scar formation, the MUP group showed no scarring at initial assessment compared with the other groups. On day 14, results showed no significant differences among groups; however, based on observations, MUP had the highest scar score compared with the CP-treated and NSS groups. By day 21, scar formation was less pronounced in both the CP2 and CP3 groups.

Table 1: Mean \pm SD score of wound elevation, hyperemia, pus, scab, and scar formations on days 7, 14, and 21 in rabbit wounds of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Parameter	Day	NSS	MUP	CP1	CP2	CP3
Wound elevation	7	1.61 \pm 0.71	1.83 \pm 0.59	1.39 \pm 0.39	1.33 \pm 0.60	1.11 \pm 0.17
	14	0.66 \pm 0.34	0.32 \pm 0.32 ^{a,b}	0.87 \pm 0.50	1.06 \pm 0.36 ^a	1.07 \pm 0.15 ^a
	21	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Hyperemia	7	1.55 \pm 0.54	1.50 \pm 0.46	1.11 \pm 0.45	0.94 \pm 0.44	1.44 \pm 0.40
	14	0.60 \pm 0.55	0.13 \pm 0.30 ^a	0.53 \pm 0.51	0.40 \pm 0.36	1.00 \pm 0.24 ^a
	21	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Pus formation	7	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
	14	0.20 \pm 0.45	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

	21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Scab formation	7	2.78 ± 0.78	2.55 ± 1.95	3.22 ± 0.55	3.00 ± 0.56	2.72 ± 0.71
	14	1.26 ± 1.04 ^{a,b}	0.07 ± 0.15 ^{a,c}	0.07 ± 0.15 ^b	0.40 ± 0.72 ^c	0.53 ± 0.38
Scar formation	21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	7	0.06 ± 0.13 ^a	0.00 ± 0.00 ^b	0.28 ± 0.32 ^{a,b,c,d}	0.28 ± 0.32 ^c	0.11 ± 0.17 ^d
	14	1.73 ± 1.01	2.87 ± 0.30	2.60 ± 0.43	2.13 ± 1.26	1.87 ± 1.12
	21	2.75 ± 0.50	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	2.25 ± 1.50

*Different letter superscripts within each row indicate significant differences among treatment groups ($p < 0.05$).

NOTE: (NSS)- negative control group; (MUP)- mupirocin-treated group; CP1 treated group [75% CG and 25% EPLE (w/w)]; CP2 treated group [50% CG and 50% EPLE (w/w)]; CP3 treated group [25% CG and 75% EPLE (w/w)].

When it comes to wound closure, Goss et al., (2012) stated that the greater the reduction in wound size, the greater the medication's efficacy, and it will close more quickly when the medication is more effective. Table 2 shows the wound incision healing percentages on days 7, 14, and 21. Table 2 shows the wound incision healing percentages on days 7, 14, and 21. On the 7th day, the MUP group exhibited smaller wounds than the other groups. By day 14, however, wound contraction from NSS was significantly lower than MUP. By day 21, wound closure was approximately 100% across all groups, except one rabbit in the NSS group.

Despite MUP's advantage in the first week of observation, other studies have reported gradual wound closure using mupirocin ointment. However, the modified formulation of mupirocin has shown enhanced wound healing. Ojo et al. (2022) reported that mupirocin encapsulated in animal bone-derived hydroxyapatite improved wound healing compared to conventional mupirocin ointment. In contrast, Kamlungmak et al. (2021) observed that 2% (w/w) mupirocin ointment resulted in the lowest wound closure in rats.

In contrast, the ethanolic pineapple leaf extract (EPLE) and cow ghee combination at different concentrations showed wound-closure results that differed from those of mupirocin. For instance, a study by Handajani et al. (2025) found that pineapple stem extract aids in the treatment of ulcer wounds in Wistar rats, as evidenced by reduced ulcer wound diameter compared to the control group.

Cow ghee also contains several fatty acids that help in wound repair. The most important are linoleic and linolenic acids, which are referred to as essential fatty acids and play a significant chemotactic role for macrophages and is important in expressing the components of the fibrinolytic system, which regulates collagenase production (Cardoso et al., 2004), and favors autolytic debridement in the wound bed and contributes to the production of matrix metalloproteinases, inducing granulation and accelerating the wound closure (Moch et al., 1990).

In this study, the CP-treated groups showed a higher wound contraction rate than the MUP- and NSS-treated groups. Although their mechanisms were not directly investigated, the enhanced wound contraction may be associated with the bioactive compounds present in EPLE and cow ghee, as reported in the literature.

Table 2: Mean ± SD score of wound contraction on days 7, 14, and 21 in rabbit wounds of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
7	22.78 ± 26.72	19.83 ± 7.67	40.05 ± 15.58	35.83 ± 19.01	50.61 ± 15.58
14	90.13 ± 7.71 ^a	99.53 ± 1.05 ^a	96.26 ± 2.85	95.73 ± 4.05	95.20 ± 1.82
21	97.66 ± 4.67	100.00	100.00	100.00	100.00

*Different letter superscripts within each row indicate significant differences among treatment groups ($p < 0.05$).

Histopathological Indices

Figures 2–5 show histopathological scores among treatment groups on days 7, 14, and 21. Epithelialization, angiogenesis, collagen, and fibroblast proliferation were examined in rabbits treated with NSS, MUP, CP1, CP2, and CP3.

Epithelialization is an essential component of wound healing that defines successful wound closure (Pastar et al., 2014). In this study, immature re-epithelialization was first observed in the CP1-treated group on day 7 and progressed to a mature state by day 14. On day 21, the CP-treated groups demonstrated the highest levels of complete epithelialization, whereas the MUP and NSS groups remained incompletely epithelialized.

The early epithelialization observed in the CP-treated groups may be related to the effects of bromelain, which has been reported to accelerate granulation tissue maturation during burn wound healing (Santi et al., 2017). In addition, cow ghee may facilitate the transport of bioactive compounds into deeper tissue layers due to its lipophilicity and compatibility with cell membranes (Upplawar et al., 2014). Moreover, cow ghee demonstrated over 90% wound healing, with rapid re-epithelialization compared with controls in rat excision-wound models (Prasad et al., 2006). Taken together, these findings suggest that EPLE and cow ghee may have a combined effect that accelerates epithelial repair.

On the other hand, Vaidyanathan et al. (2021) reported that mupirocin stimulates growth factors that promote the resolution of inflammation, cell proliferation, angiogenesis, and extracellular matrix remodeling. However, in a study by Mertz et al. (1985), mupirocin ointment applied to a partial-thickness wound showed little effect on re-epithelialization rate, consistent with the present study.

Angiogenesis plays a vital role in delivering immune cells, nutrients, and oxygen to promote tissue regeneration (Shaw & Martin, 2009). In Figure 3, the formation of new blood vessels was observed in all treatment groups, with similar levels on day 7. By day 21, CP1 and CP3 demonstrated better performance, with a pronounced increase in angiogenesis compared with other groups. The results of the present study may be linked to bromelain's ability to repair damaged extracellular matrix components, such as collagen, laminin, and elastin, through proteolytic activity (Trimarco et al., 2020), as well as cow ghee, which may enhance blood vessel formation during wound excision in rats (Prasad et al., 2006), and improve circulation and re-epithelialization in rodents (Koca et al., 2022).

In fibroblasts, both CP1 and CP3 demonstrated low fibroblast proliferation on day 7 and markedly increased in granulation tissue by day 21. Regarding collagen deposition, a similar trend was

observed: a scant level was initially observed on day 7 in the CP2 group. By day 21, the CP-treated groups showed moderate collagen levels, whereas MUP and NSS showed the least.

According to Rozaini et al. (2005), an increase in fibroblast number is an important indicator of the pharmacological wound-healing effect of an agent. The proliferation of fibroblasts enhances the formation of new extracellular matrix (ECM) and collagen structures that support other cells involved in effective wound healing (Bainbridge, 2013). The presence of bromelain in pineapple further facilitates increased interferon-gamma production, which aids fibroblast mobilization at the wound site (Munzig et al., 2005). Aside from that, cow ghee promotes epithelial growth, collagen synthesis, and epithelialization, and increases dermal deposition due to its vitamin A and E content (Zinder et al., 2019). Based on these studies, the enhanced

proliferation of fibroblasts and collagen deposition in the CP-treated groups may be related to the action of bromelain from pineapple, which helps remodel the extracellular matrix, and to the vitamins A and E in cow ghee, which promote collagen synthesis, thereby enhancing tissue regeneration. However, this notion needs further validation, as no screening for phytochemicals in pineapple leaves and physicochemical parameters in cow ghee is included, which is a limitation of this study.

Finally, the histopathological analysis revealed that the formulations (CP1, CP2, and CP3) promote several key processes in wound repair, including re-epithelialization, angiogenesis, dermal collagen deposition, and fibroblast proliferation. These findings suggest that the formulation may have potential as a natural therapeutic agent to aid wound healing.

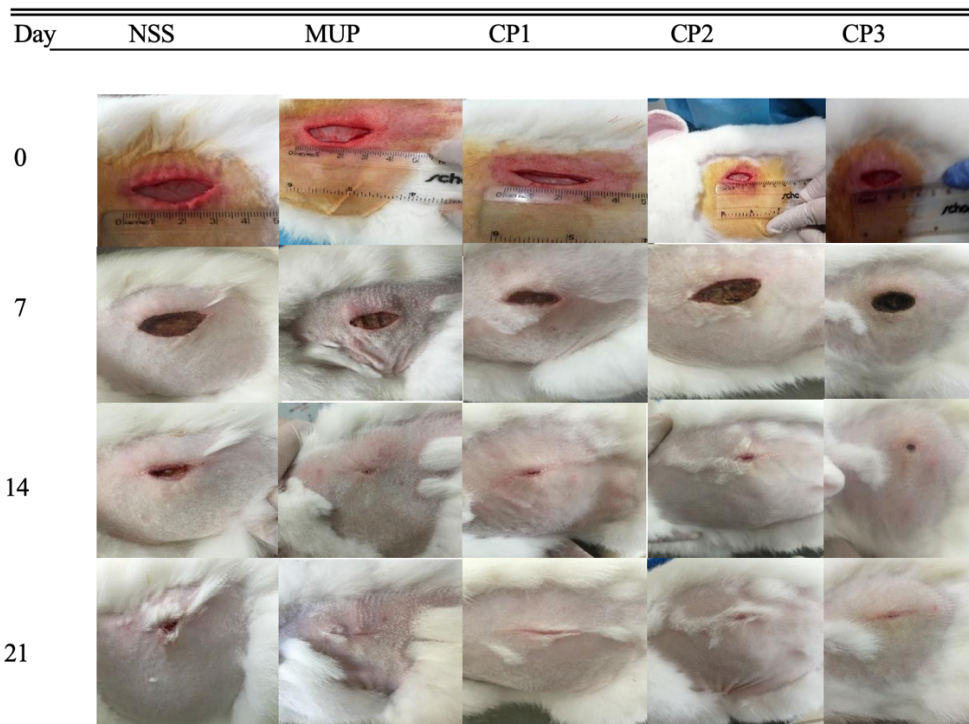


Figure 1: Macroscopic differences in wound size reduction on days 7, 14, and 21 in the wounds of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract. Progressive wound edge apposition was observed among groups during treatment.

NOTE: (NSS)- negative control group; (MUP)- mupirocin treated group; (CP1) treated group [75% CG and 25% EPLE (w/w)]; (CP2) treated group [50% CG and 50% EPLE (w/w)]; (CP3) treated group [25% CG and 75% EPLE (w/w)].

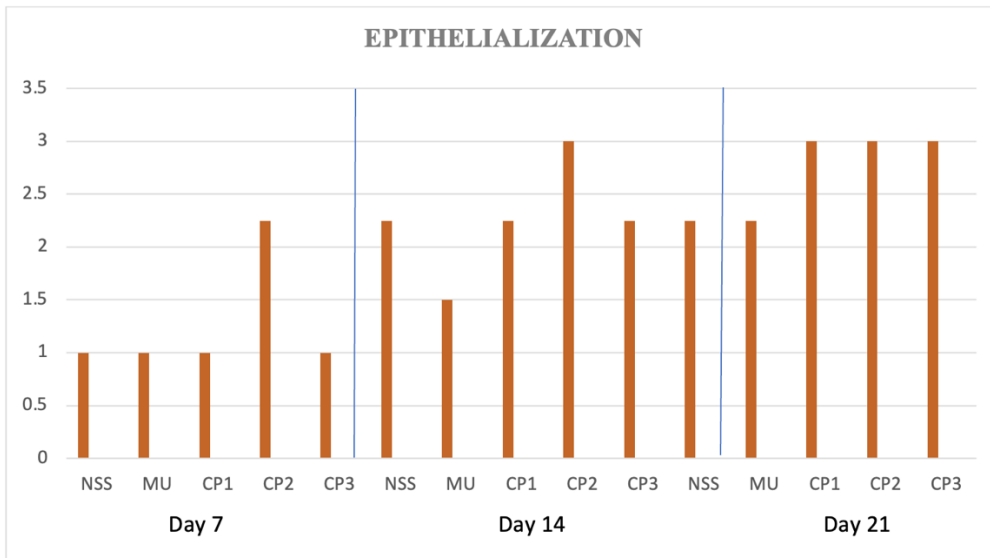


Figure 2: Epithelialization scores on days 7, 14, and 21 in the wound of rabbits treated with NSS, MUP, CP1, CP2, and CP3. Epithelialization was graded as either 0 (none), 1 (partial), 2 (complete but immature or thin), or 3 (complete and mature).

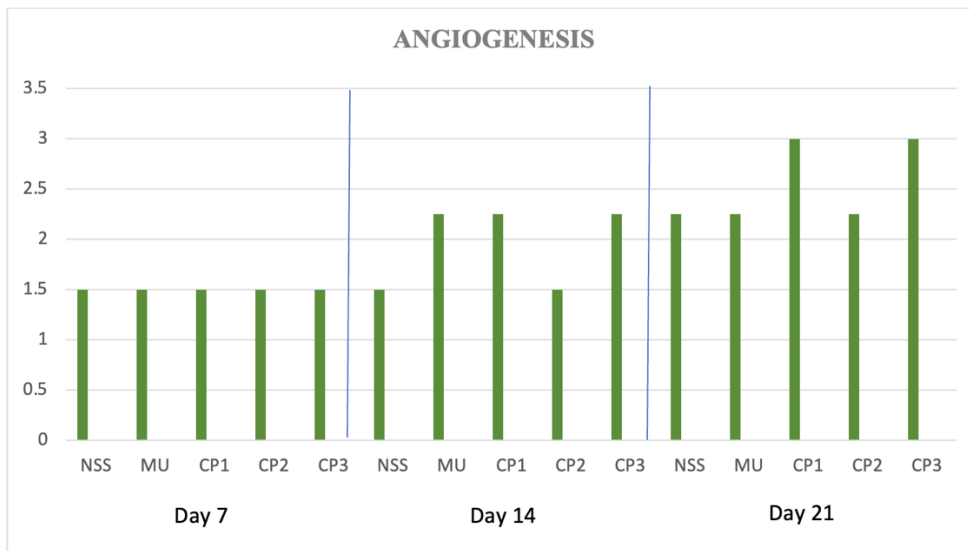


Figure 3: Angiogenesis scores on days 7, 14, and 21 in the wound of rabbits treated with NSS, MUP, CP1, CP2, and CP3. Angiogenesis was graded as either 0 (none), 1 (up to 5 vessels per high-power field [HPF]), 2 (6–10 vessels per HPF), or 3 (more than 10 vessels per HPF).

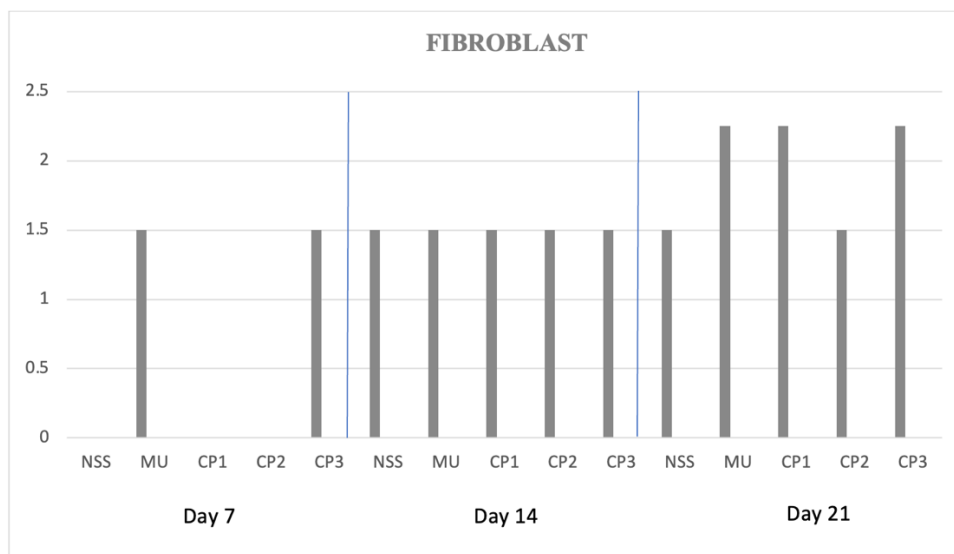


Figure 4: Fibroblast proliferation scores on days 7, 14, and 21 in the wound of rabbits treated with NSS, MUP, CP1, CP2, and CP3. Fibroblast was graded as 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblasts), and 3 (predominantly fibroblasts).

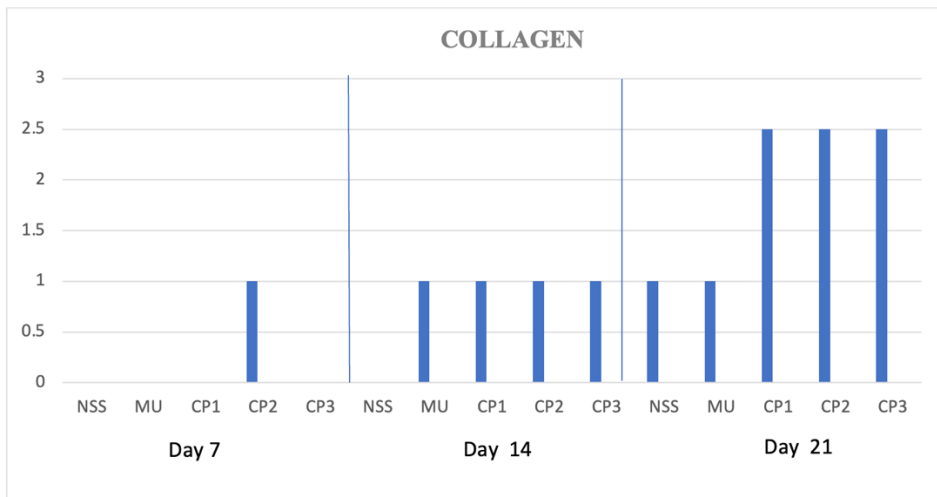


Figure 5: Collagen deposition scores on days 0, 7, 14, and 21 in the wound of rabbits treated with NSS, MUP, CP1, CP2, and CP3. Scoring method: collagen level was graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant).

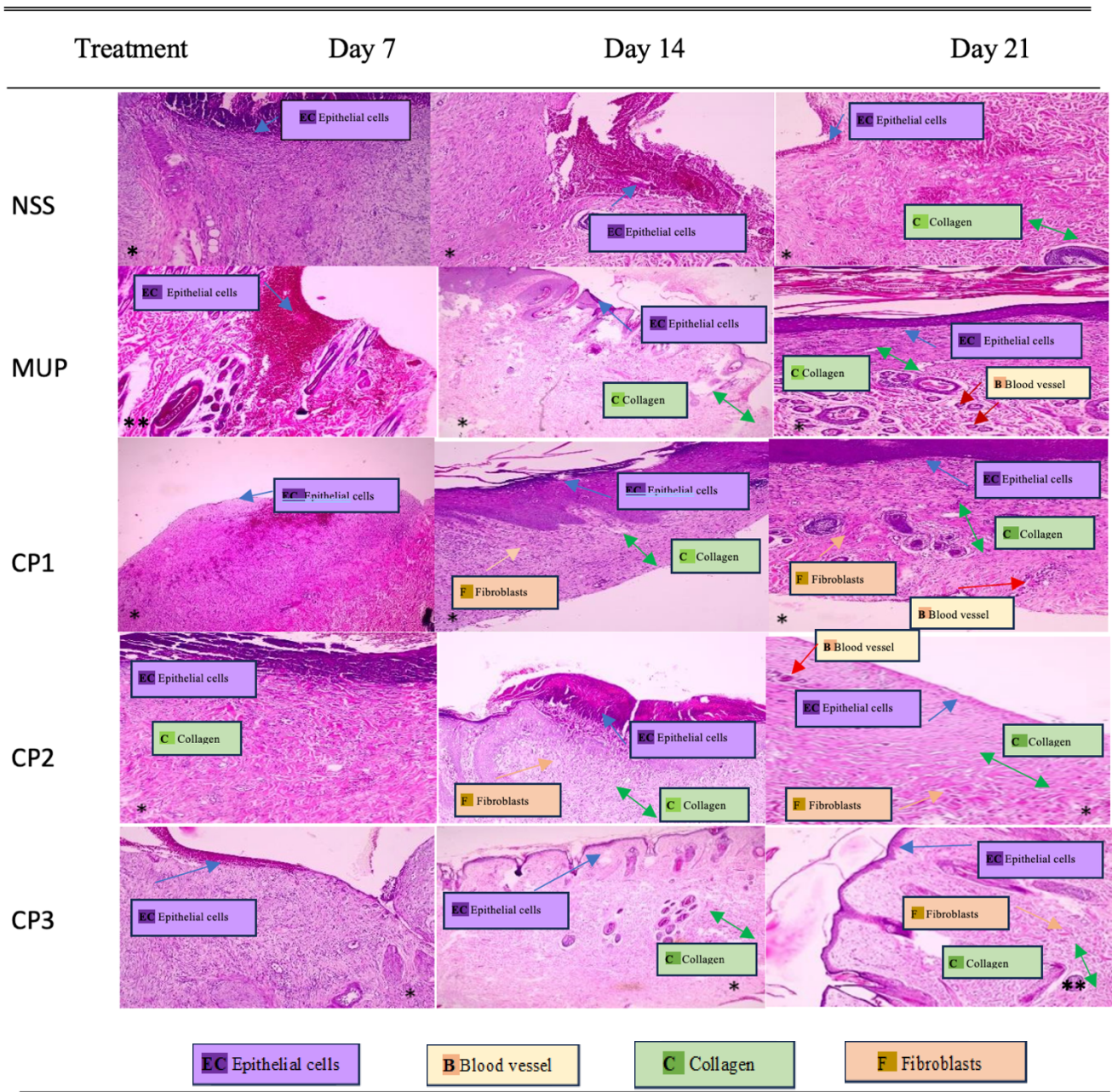


Figure 6: Histopathological features of wound incisions of representative rabbits treated with NSS, MUP, CP1, CP2, and CP3 at days 7, 14, and 21 (Magnifications: "10x" and "40x").

CONCLUSION

This study compared the wound-healing effects of different concentrations of cow ghee and ethanolic pineapple leaf extract with the standard mupirocin and the negative control. It was ascertained that the CP-treated groups had a similar rate of wound contraction to the standard mupirocin. However, they differed in histological wound-healing indices, with CP1, CP2, and CP3 performing well in epithelialization, angiogenesis, collagen levels, and fibroblast proliferation. Thus, it is inferred that topical application of this preparation may accelerate the restoration of wound tissue integrity and support the traditional use of pineapple leaves and cow ghee for wound treatment. The healing components of this topical formulation may be attributed to the bioactive compounds in pineapple leaf and the essential components in cow ghee, as reported in previous studies. Moreover, the present study suggests that this new formulation may support wound healing, but further studies are needed to confirm its efficacy in other animal species and to conduct phytochemical/physicochemical characterization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Conceptualization, JPD, VAM, and JAC; Methodology, JPD, VAM, and JAC; Investigation, JPD, VAM and JAC; Writing—original draft, VAM and JAC- Review and Editing, JPD, VAM, and JAC, Funding, JPD, CHED-K12, and UEP.

REFERENCES

- Abramov Y, Golden B, Sullivan M, Botros SM, Miller JJR, Alshahrour A. Histologic characterization of vaginal vs abdominal surgical wound healing in a rabbit model. *Wound Repair Regen* 2007;15(1):80–86. <https://doi.org/10.1111/j.1524-475X.2006.00188.x>
- Adao J, Matawaran V, Acorda J. Effect of guava (*Psidium guajava*) cream on the gross and microscopic features and healing rate in incisional wounds in short-haired cats. *Philippine Journal of Veterinary and Animal Sciences* [S.l.], v. 43, n. 2, p. 83-91, jun. 2018. ISSN 2619-7731.
- Agarwal P, Singh A, & Gaurav K. (2009). Evaluation of wound healing activity of herbal formulations in experimental animals. *Journal of Ethnopharmacology*, 123(2), 273–279.
- Cardoso CRB, Souza MA, Ferro EAV, Favoreto-Jr S, Pena JDO. Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds. *Wound Repair Regen* 2004; 12:235–243. <https://doi.org/10.1111/j.1067-1927.2004.012216.x>
- Browder W, William D, Lacore P. Effect of enhanced macrophage function on early wound healing. *Immunology* 1998; 104:224–230.
- Chand R, Kumar SS, Srinivasan RA, Batish VK, Chander H. Influence of lactic acid bacteria on oxidative stability of ghee. *Milchwissenschaft* 1986; 41:335–336.
- Chupeco J, Flores, MS, Reyes, MF. Macroscopic and microscopic changes in the wound after intradermal closure, buried knot, and pulley knot-free patterns following ovariectomy in cats. *Philippine Journal of Veterinary and Animal Sciences*, [S.l.], v. 39, n. 2, feb. 2014. ISSN 2619-7731.
- De, S. 2000. *Outlines of Dairy Technology*. Oxford University Press. pp 433–463.
- Dutta S, Bhattacharyya D, Chattopadhyay S. (2013). Bromelain: A potential therapeutic agent for inflammation and oxidative stress. *Food Science and Biotechnology*, 22(2), 1–10.
- Eurides D, Mazzanti A, Gonçalves GF, Belletti ME, Silva LA, Fioravanti S, Ogata AS (1998). Aspectos morfológicos, morfométricos e histológicos da reparação tecidual de feridas cutâneas de camundongos tratadas com óleo de copaíba (*Copaifera langsdorffii*). *Veterinária Notícias*, 4(3), 77-82.
- Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. *The Lancet*, 366(9498), 1736–1743.
- Frykberg R, Banks J, (2015). Challenges in treating chronic wounds. *Advances in Wound Care*, 4(9), 560–582.
- Blonstein, J.L., 1992. Control of swelling in boxing injuries. *Practitioner*, 155: 78.
- Goss, A. Intrauterine healing of feral rat oral mucosal, skin and cartilage wounds. *J oral Pathol Med* 1977; 6:35-43.
- Goss S, Samantha A, Mandall NB, Banerjee S, Chattopadhyay D. Evaluation of the wound healing activity of methanol extract of *Pedilanthus tithymaloides* (L.) Poit leaf and its isolated active constituents in topical formulation. *J Ethnopharmacol*. 2012;142(3):714–722. <https://doi.org/10.1016/j.jep.2012.05.029>.
- Greenhalgh DG, Sprugel KH, Murray MJ, Ross, R. RDGF and FGF stimulate wound healing in diabetic mouse. *American Journal of Pathology*, 136(5), 123–130. *Am J Pathol* 1990;136(6):1235–1246. [https://doi.org/10.1016/S0002-9440\(10\)80367-0](https://doi.org/10.1016/S0002-9440(10)80367-0)
- IMARC Group. (2026). *Philippines wound care market report... 2026–2034* (Report ID: SR112026A23605). IMARC Group. <https://www.imarcgroup.com/philippines-wound-care-market>
- Kamlungmak R, Tansakul C, & Phaechamud T. (2021). Development of mupirocin nanoparticle-loaded hydrogel for topical application and evaluation of wound healing activity. *International Journal of Pharmaceutics*, 599, 120428. <https://doi.org/10.1016/j.ijpharm.2021.120428>
- Martinengo L, Olsson M, Bajpai R, Soljak M, Upton Z, Schmidtchen A, Car J, & Järbrink K. (2019). Prevalence of chronic wounds in the general population: a systematic review and meta-analysis of observational studies. *Annals of Epidemiology*, 29, 8–15. <https://doi.org/10.1016/j.annepidem.2018.10.005>
- Mertz PM, Dunlop BW, Eaglstein WH. The effects of Bactroban ointment on epidermal wound healing in partial-thickness wounds. In Dobson R, Leyden JJ, Noble WC (eds): *Bactroban (Mupirocin)*. Princeton, NJ, Excerpta Medica, 1985, pp 211–215.
- Moch D, Schewe T, Kuhn H, Schmidt D, Buntrock P. The linoleic acid metabolite 9-des-hydroxy-10,12(E,Z)-octadecadienoic acid is a strong proinflammatory mediator in an experimental wound-healing model in rats. *Biomed Biochim Acta* 1990; 4:201–207.
- Munzig E, Eckert K, Harrach T. Bromelain protease F9 reduces the CD44-mediated adhesion of human peripheral blood lymphocytes to human umbilical vein endothelial cells. *FEBS Lett* 2005; 351:215–218.
- Olsson, M, Järbrink, K., Divakar, U., Bajpai, R., Upton, Z., Schmidtchen, Z., & Car, J. (2019). The humanistic and economic burden of chronic wounds: A systematic review. *Wound Repair and Regeneration*, 27(1), 114–125. <https://doi.org/10.1111/wrr.12683>
- Ojo, O, Ilomuanya, M., Sekunowo, O, Gbenedor, O, & Adeosun, S. (2022). Development and characterization of mupirocin encapsulated in animal bone-derived hydroxyapatite for management of chronic wounds. *Beni-Suef University Journal of Basic and Applied Sciences*, 11, Article 262. <https://doi.org/10.1186/s43088-022-00262-8>
- Pastar I, Stojadinovic O, Canic MT. Epithelialization in wound healing: A comprehensive review. *Adv Wound Care (New Rochelle)* 2014; doi:10.1089/wound.2013.0473.
- Prasad V, Dorle AK. (2006). Evaluation of ghee-based formulation for wound healing activity. *J Ethnopharmacol*. 107:38–47. doi: 10.1016/J.JEP.2006.02.006. [DOI] [PubMed] [Google Scholar]
- PFA. *Prevention of Food Adulteration Act, 1955* (as amended). Delhi: Universal Law Publishing Co. Pvt. Ltd., Ansal's Dilskhush of Estate; 2009.
- Quarta S, Santarpino G, Carluccio M, Calabriso N, Scoditti E, Siculella L, Damiano F, Maffia M, Verri T, De Caterina R, et al.

Analysis of the Anti-Inflammatory and Anti-Osteoarthritic Potential of Flonast Fast((R)), a Combination of Harpagophytum Procumbens DC. ex Meisn., Boswellia Serrata Roxb., Curcuma longa L., Bromelain and Escin (Aesculus hippocastanum), Evaluated in In Vitro Models of Inflammation Relevant to Osteoarthritis. *Pharmaceuticals*. 2022;15:1263. doi: 10.3390/ph15101263. [DOI] [PMC free article] [PubMed] [Google Scholar]

Roghmam MC, Lydecker AD, Shardell M. Effect of mupirocin for Staphylococcus aureus decolonization on the microbiome of the nose and throat in community and nursing home dwellings in adults. *PLoS One* 2021;16(6): e0252004. <https://doi.org/10.1371/journal.pone.0252004>

Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V. Antioxidant and anti-inflammatory activities of extracts from *Cassia alata*, *Eleusine indica*, *Eremomastax speciosa*, *Carica papaya*, and *Polyscias fulva* medicinal plants collected in Cameroon. *PLoS One* 2014; 9:e103999. <https://doi.org/10.1371/journal.pone.0103999>

Sritharadol R, Chunhachaichana C, Kumlungmak S, Buatong W, Dechraksa J, Suwandecha T, Sriwiryajan S, Nopparat J, & Srichana, T. (2019). The safety and efficacy of mupirocin topical spray for burn wound healing in a rat model. *International Journal of Pharmaceutical Quality Assurance*, 10(1), 51–59. <http://www.ijpqa.com>

Santi F, Restuhadi F, Ibrahim A. Potensi ekstrak kasar enzim bromelin pada bonggol nanas (*Ananas comosus*) sebagai koagulan alami lateks (*Hevea brasiliensis*) (The potential of crude bromelain enzyme extracted from pineapple fruits (*Ananas comosus* L. Merr) as a natural coagulant of latex (Hevea brasiliensis M) JOM Faperta. 2017;4(1):1–15. [Google Scholar]

Situm M, Kolić M, Aždajić M, & Krolo I. (2013). Wound healing: Physiological mechanisms and clinical implications. *Acta Clinica Croatica*, 52(2), 253–259.

Sule A, Thanni Loa & Sule-odu O. (2002). Bacterial pathogens associated with infected wounds in Ogun State University Teaching Hospital, Sagamu, Nigeria. *Afr J Clin. Exp. Microbiol.*, 3:13-16.

Sriwatanapongse A, Balaban M, Teixeira A. 2000. Thermal inactivation kinetics of Bromelain in pineapple juice. *Trans. Am. Soc. Agric. Eng.* <https://doi.org/10.13031/2013.3071>.

Uppalwar S, Aeri V, Arora A. Evaluation of wound healing properties from five traditional medicinal plants with cow ghee. <https://www.researchgate.net/publication/291338788> *J Med Pharm Innov.* 2014; 1:24–28. [Google Scholar]

Vaidyanathan, L. Growth factors in wound healing: A review. *Biomed. Pharmacol. J.* 2021, 14, 1469–1480. [Google Scholar] [CrossRef] Kotian S R, Padma D, Madhukar R, Bhat KMR. Effect of Natural Medicines on Dermal Fibroblasts in Wound Healing: An In-Vitro Study. *Adv Sci Lett.* 2017;23(3):1949–1956. [Google Scholar]

SUPPLEMENTARY INFORMATION

Table 3: Mean ± SD score of wound elevation in the wounds of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
1	2.61 ± 0.49	2.55 ± 0.59	2.39 ± 0.25	2.33 ± 0.42	1.94 ± 0.33
2	2.39 ± 0.49	2.38 ± 0.32	1.61 ± 0.49	1.94 ± 0.68	1.44 ± 0.34
3	2.11 ± 0.50	2.67 ± 0.42	1.39 ± 0.49	1.83 ± 0.46	0.83 ± 0.41
4	2.00 ± 0.30	2.28 ± 0.32	1.33 ± 0.42	1.33 ± 0.76	0.78 ± 0.27
5	1.78 ± 0.40	1.76 ± 0.55	1.22 ± 0.17	1.61 ± 0.71	0.77 ± 0.35
6	1.72 ± 0.65	1.88 ± 0.29	1.39 ± 0.39	1.61 ± 0.57	0.50 ± 0.41
7	1.61 ± 0.71	1.83 ± 0.59	1.39 ± 0.39	1.33 ± 0.60	1.11 ± 0.17
8	2.00 ± 0.41	1.60 ± 0.72	1.27 ± 0.43	1.33 ± 0.23	1.13 ± 1.02
9	1.80 ± 0.38	1.46 ± 0.45	1.00 ± 0.63	1.20 ± 0.51	1.33 ± 0.47
10	1.26 ± 0.28	1.47 ± 0.51	1.20 ± 0.51	1.13 ± 0.51	1.06 ± 0.43
11	1.53 ± 0.38	1.33 ± 0.47	1.26 ± 0.28	1.26 ± 0.28	1.00 ± 0.75
12	1.60 ± 0.43	1.33 ± 0.41	1.46 ± 0.38	1.06 ± 0.49	0.93 ± 0.28
13	1.20 ± 0.30	1.00 ± 0.34	0.93 ± 0.37	1.20 ± 0.65	1.27 ± 0.43
14	0.66 ± 0.34	0.32 ± 0.32 ^{a,b}	0.87 ± 0.50	1.06 ± 0.36 ^a	1.07 ± 0.15 ^b
15	0.25 ± 0.50	0.08 ± 0.15	0.42 ± 0.42	0.94 ± 0.64	1.08 ± 0.42
16	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.19	0.42 ± 0.50	0.92 ± 0.42
17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.17	1.00 ± 0.54
18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.66 ± 0.54
19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.00
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.17
21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Elevation was graded as 0-3: 0 for absent; 1 for slightly; 2 for moderately, and 3 for markedly raised area surrounding the wound site.

NOTE: (NSS)- negative control group; (MUP)- mupirocin treated group; (CP1)- treated group [75% CG and 25% EPLE (w/w)] (CP2)- treated group [50% CG and 50% EPLE (w/w)]; (CP3)- treated group [25% CG and 75% EPLE (w/w)].

Table 4: Mean±SD score of hyperemia formations in the wound of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
0	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	2.66 ± 0.21	2.66 ± 0.00	2.72 ± 0.14	2.61 ± 0.13	2.38 ± 0.86
2	2.61 ± 0.33	2.39 ± 0.25	2.61 ± 0.13	2.44 ± 0.27	2.38 ± 0.86
3	1.78 ± 0.40	2.00 ± 0.47	2.39 ± 0.32	2.27 ± 0.39	1.89 ± 0.46
4	1.72 ± 0.49	1.89 ± 0.46	1.72 ± 0.14	1.94 ± 0.49	1.66 ± 0.21
5	1.67 ± 0.56	1.44 ± 0.40	1.50 ± 0.28	1.44 ± 0.40	1.61 ± 0.54
6	1.56 ± 0.50	1.61 ± 0.54	1.22 ± 0.34	1.16 ± 0.41	1.44 ± 0.50
7	1.55 ± 0.54	1.50 ± 0.46	1.11 ± 0.45	0.94 ± 0.44	1.44 ± 0.40
8	1.60 ± 0.43	1.46 ± 0.45	1.00 ± 0.24	0.80 ± 0.60	1.33 ± 0.23
9	1.26 ± 0.36	1.13 ± 0.38	0.86 ± 0.19	0.73 ± 0.55	1.07 ± 0.15
10	1.33 ± 0.53	1.13 ± 0.38	0.66 ± 0.41	0.53 ± 0.51	1.00 ± 0.47
11	1.06 ± 0.55	1.06 ± 0.28	0.60 ± 0.36	0.53 ± 0.60	1.40 ± 0.60
12	1.00 ± 0.71	0.86 ± 0.38	0.40 ± 0.55	0.66 ± 0.53	0.99 ± 0.62
13	0.93 ± 0.59	0.80 ± 0.51	0.53 ± 0.60	0.73 ± 0.49	1.26 ± 0.69
14	0.60 ± 0.55	0.13 ± 0.30 ^a	0.53 ± 0.51	0.40 ± 0.36	1.00 ± 0.24 ^a
15	0.25 ± 0.50	0.00 ± 0.00	0.25 ± 0.32	0.33 ± 0.47	0.83 ± 1.04
16	0.25 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.42 ± 0.50	0.50 ± 0.33
17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.33

18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.38
19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Hyperemia was graded as either 0-3: 0 for absent; 1 for slight; 2 for moderate pink and 3 for red areas surrounding the wound site.

NOTE: (NSS)- negative control group; (MUP)- mupirocin treated group; (CP1)- treated group [75% CG and 25% EPLE (w/w)] (CP2)- treated group [50% CG and 50% EPLE (w/w)]; (CP3)- treated group [25% CG and 75% EPLE (w/w)].

Table 5: Mean ± SD score of scab formations in the wound of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	1.78 ± 0.98	1.17 ± 1.07	0.28 ± 0.32	0.61 ± 0.44	1.11 ± 0.78
2	1.94 ± 1.06	1.22 ± 1.07	0.50 ± 0.46	0.78 ± 0.54	1.50 ± 0.62
3	1.83 ± 0.94	1.72 ± 0.91	0.83 ± 0.28	1.11 ± 0.81	1.83 ± 0.69
4	1.50 ± 0.72	2.05 ± 1.59	1.61 ± 0.74	1.38 ± 1.04	2.11 ± 0.83
5	2.05 ± 0.80	1.72 ± 1.18	2.66 ± 0.59	2.72 ± 1.00	2.50 ± 0.96
6	2.17 ± 0.69	1.72 ± 1.18	3.28 ± 0.32	3.00 ± 0.76	2.67 ± 1.11
7	2.78 ± 0.78	2.55 ± 1.95	3.22 ± 0.55	3.00 ± 0.56	2.72 ± 0.71
8	2.53 ± 0.77	3.53 ± 2.80	2.46 ± 0.87	2.73 ± 0.44	3.33 ± 0.63
9	2.20 ± 0.84	3.40 ± 2.73	2.40 ± 0.86	2.06 ± 0.64	3.26 ± 1.46
10	2.00 ± 1.08	3.73 ± 2.98	1.73 ± 0.55	1.60 ± 0.83	2.80 ± 1.40
11	1.93 ± 1.08	2.06 ± 1.26	0.93 ± 0.28	1.40 ± 1.38	1.86 ± 0.93
12	1.66 ± 0.82	1.33 ± 0.97	0.33 ± 0.33	0.86 ± 1.23	1.39 ± 0.72
13	1.33 ± 1.08	0.33 ± 0.33	0.13 ± 0.30	0.46 ± 0.73	0.73 ± 0.28
14	1.26 ± 1.04 ^{a,b}	0.07 ± 0.15 ^a	0.07 ± 0.15 ^b	0.40 ± 0.72	0.53 ± 0.38
15	1.08 ± 0.83	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.50 ± 0.43
16	0.42 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.17	0.42 ± 0.42
17	0.17 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50
18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.33
19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Scab formation was graded as 0-5: 0 for 10%; 1 for 20%; 2 for 40%; 3 for 60%; 4 for 80%; and 5 for 100%.

NOTE: (NSS)- negative control group; (MUP)- mupirocin-treated group; (CP1)- treated group [75% CG and 25% EPLE (w/w)] (CP2)- treated group [50% CG and 50% EPLE (w/w)]; (CP3)- treated group [25% CG and 75% EPLE (w/w)].

Table 6: Mean ± SD score of scar formations in the wound of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
2	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
3	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.17	0.11 ± 0.17	0.06 ± 0.13
4	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.17	0.11 ± 0.17	0.06 ± 0.13
5	0.00 ± 0.00	0.00 ± 0.00	0.28 ± 0.39	0.11 ± 0.17	0.06 ± 0.13
6	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.42	0.17 ± 0.28	0.06 ± 0.13
7	0.06 ± 0.13 ^a	0.00 ± 0.00 ^b	0.78 ± 0.27 ^{a,b,c,d}	0.28 ± 0.32 ^c	0.11 ± 0.17 ^d
8	0.27 ± 0.43	0.07 ± 0.15	0.86 ± 0.19	0.66 ± 0.34	0.60 ± 0.36
9	0.67 ± 0.62	0.93 ± 0.15	1.33 ± 0.23	1.13 ± 0.38	0.66 ± 0.24
10	1.20 ± 0.51	1.40 ± 0.43	1.87 ± 0.30	1.66 ± 0.34	1.20 ± 0.51
11	1.47 ± 0.65	1.80 ± 0.45	2.27 ± 0.43	2.13 ± 0.50	1.53 ± 0.30

12	1.47 ± 0.65	1.87 ± 0.50	2.46 ± 0.45	2.53 ± 0.30	1.86 ± 0.51
13	1.60 ± 0.89	2.33 ± 0.53	2.53 ± 0.51	2.06 ± 1.21	2.33 ± 0.47
14	1.73 ± 1.01	2.87 ± 0.30	2.60 ± 0.43	2.13 ± 1.26	1.87 ± 1.12
15	1.42 ± 0.83	3.00 ± 0.00	3.00 ± 0.00	2.00 ± 1.36	1.67 ± 1.12
16	2.17 ± 0.33	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	1.75 ± 1.20
17	2.50 ± 0.58	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	1.92 ± 1.34
18	2.58 ± 0.50	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	1.92 ± 1.34
19	2.75 ± 0.50	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	2.17 ± 1.45
20	2.75 ± 0.50	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	2.25 ± 1.50
21	2.75 ± 0.50	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 1.50	2.25 ± 1.50

Scar formation was graded 0-3: 0 for absent; 1 for slight; 2 for moderate; and 3 for marked scarred area surrounding the wound site

NOTE: (NSS)- negative control group; (MUP)- mupirocin treated group; (CP1)- treated group [75% CG and 25% EPLE (w/w)] (CP2)- treated group [50% CG and 50% EPLE (w/w)]; (CP3)- treated group [25% CG and 75% EPLE (w/w)].

Table 7: Mean ± SD score of wound contraction at 7, 14, and 21 days in the wound of rabbits treated with different combinations of cow ghee and ethanolic pineapple leaf extract.

Day	NSS	MUP	CP1	CP2	CP3
0	0.00 ± 0.00	0.00 ± 0.00	16.67 ± 40.83	0.00 ± 0.00	0.00 ± 0.00
1	0.04 ± 24.14	-10.39 ± 17.86	09.33 ± 19.50	-06.17 ± 23.89	10.28 ± 29.22
2	10.72 ± 22.05	0.01 ± 09.61	13.94 ± 16.74	-08.61 ± 17.93	10.44 ± 41.10
3	02.72 ± 34.92	06.44 ± 11.67	10.72 ± 16.43	03.06 ± 15.28	13.94 ± 37.45
4	03.83 ± 33.83	06.17 ± 11.85	07.94 ± 17.21	03.22 ± 15.48	18.54 ± 34.79
5	08.56 ± 27.15	11.78 ± 09.34	21.61 ± 22.65	08.28 ± 18.20	25.94 ± 28.69
6	15.39 ± 21.87	15.33 ± 10.45	20.67 ± 22.51	11.50 ± 11.42	41.58 ± 12.1
7	22.78 ± 26.72	19.83 ± 07.67	40.05 ± 15.58	35.83 ± 19.01	50.61 ± 15.31 a
8	34.60 ± 33.09	27.66 ± 10.90	60.73 ± 12.50	54.67 ± 10.23	57.87 ± 14.72
9	49.07 ± 29.42	47.20 ± 13.81	69.20 ± 07.52	69.40 ± 13.93	73.06 ± 08.50
10	71.20 ± 17.54	68.20 ± 10.91	77.80 ± 07.09	79.93 ± 08.07	81.20 ± 07.73
11	71.86 ± 30.73	86.60 ± 04.18	87.60 ± 05.14	87.00 ± 08.99	87.86 ± 03.22
12	82.13 ± 14.69	92.67 ± 01.50	90.53 ± 04.26	90.26 ± 05.58	91.53 ± 02.11
13	88.46 ± 10.54	96.13 ± 02.63	94.53 ± 03.37	92.73 ± 06.39	93.33 ± 02.88
14	90.13 ± 07.71 ^a	99.53 ± 01.05 ^a	96.26 ± 02.86	95.73 ± 04.05	95.20 ± 01.82
15	92.75 ± 10.73	100.00 ± 0.00	99.00 ± 01.30	97.58 ± 03.05	95.83 ± 02.25
16	93.50 ± 11.29	100.00 ± 0.00	100.00 ± 0.00	99.42 ± 01.17	97.83 ± 01.90
17	94.83 ± 09.47	100.00 ± 0.00	100.00 ± 0.00	99.58 ± 00.84	99.25 ± 01.50
18	95.92 ± 07.10	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.33 ± 01.34
19	98.33 ± 03.34	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.58 ± 00.84
20	98.17 ± 03.67	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
21	97.67 ± 04.67	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

NOTE: (NSS)- negative control group; (MUP)- mupirocin-treated group; (CP1)- treated group [75% CG and 25% EPLE (w/w)]; (CP2)- treated group [50% CG and 50% EPLE (w/w)]; (CP3)- treated group [25% CG and 75% EPLE (w/w)].