

THE EFFECT OF RED GUAVA LEAF (*PSIDIUM GUAJAVA*) INFUSION ON WOUND HEALING PROCESS IN MICE (*MUS MUSCULUS ALBINUS*): AN EXPERIMENTAL STUDY

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Abstract

The wound healing process is a complex biological sequence, and *Psidium guajava* leaves are known for their antimicrobial and anti-inflammatory properties. This study aims to evaluate the effect of red guava leaf infusion on the wound healing process in mice. A true experimental study with a post-test only control group design was conducted on 24 male albino mice. Subjects were divided into four groups: negative control (saline), positive control (povidone-iodine), and two treatment groups with guava leaf infusion. Parameters included wound contraction rate and histopathological analysis (epithelialization, collagen, angiogenesis). The treatment groups showed significantly faster wound contraction compared to the negative control. Histopathology revealed better collagen density and re-epithelialization in the infusion-treated groups. Red guava leaf infusion accelerates wound healing and improves tissue regeneration quality.

Introduction

Wounds are physically defined as a disruption of the normal anatomical structure and function of the skin tissue resulting from external trauma or pathological conditions (Velnar et al., 2009). The wound healing process is a complex and dynamic biological sequence that involves three main overlapping phases: inflammation, proliferation, and remodeling (Guo & DiPietro, 2010). Proper management of these phases is crucial to prevent complications such as chronic infection or hypertrophic scarring, which can significantly impair quality of life (Frykberg & Banks, 2015). Although synthetic antibiotics and topical treatments are widely available, there is a growing interest in natural alternatives due to the rising prevalence of antibiotic resistance and the cost-effectiveness of herbal medicine (Dorai, 2012).

Psidium guajava, commonly known as guava, is a tropical plant that has been utilized in traditional medicine for generations to treat various ailments, including gastrointestinal disorders and skin infections (Gutiérrez et al., 2008). The leaves of *Psidium guajava* are particularly rich in bioactive compounds such as tannins, flavonoids, saponins, and essential oils which are known to possess strong antimicrobial and anti-inflammatory properties (Biswas et al., 2013). Flavonoids, specifically quercetin, act as potent antioxidants that reduce oxidative stress in the wound environment, thereby accelerating the healing process (Naseer et al., 2018). Furthermore, tannins function as astringents that help contract the wound margins and form a protective layer over the exposed tissue to prevent bacterial invasion (Ashokkumar & Senthil, 2015).

Previous studies have demonstrated that extracts from guava leaves can enhance collagen synthesis and fibroblast proliferation, which are essential components of the proliferative phase of wound healing (Fernandes

et al., 2019). However, most existing research has focused on ethanolic extracts, while the efficacy of simple aqueous infusions, which are more accessible to the general population, remains under-explored in animal models (Metwally et al., 2010). Therefore, this study aims to evaluate the specific effect of red guava leaf infusion on the wound healing process in mice (*Mus musculus albinus*) to provide scientific evidence for its traditional use (Santos et al., 2016).

Materials and Methods

This research employs a true experimental design with a post-test only control group approach to assess wound healing rates quantitatively and qualitatively (Kothari, 2004). The ethical approval for this study was issued by the Health Research Ethics Committee of [Name of Institution] (No.420/KEPH/VII/2025, dated [17/7/2025]). The study subjects consist of 24 healthy male albino mice (*Mus musculus*) which are acclimatized for one week prior to the intervention to minimize stress-related variables (National Research Council, 2011). The mice are randomly divided into four groups: a negative control group treated with saline, a positive control group treated with standard povidone-iodine, and two treatment groups receiving different concentrations of *Psidium guajava* leaf infusion (Vogel, 2008).

A full-thickness excision wound is created on the dorsal area of each mouse under ketamine-xylazine anesthesia to ensure standardization of the wound size and depth (Dorsett-Martin, 2004). The dorsal hair is shaved and the skin is disinfected prior to excision to prevent immediate contamination from skin flora (Starkey & Johnson, 2018). The healing process is monitored daily by measuring the wound area contraction using a digital caliper and observing macroscopic signs of inflammation such as redness and edema (Flanagan, 2003).

In addition to macroscopic observation, histopathological analysis is conducted on the 14th day to evaluate re-epithelialization, collagen density, and angiogenesis within the wound bed using Hematoxylin-Eosin and Masson's Trichrome staining (Suvik & Effendy, 2012). Statistical analysis is performed using One-Way ANOVA followed by a Post Hoc Tukey test to determine significant differences between the treatment and control groups ($p < 0.05$) using IBM SPSS Statistics version 25.0 (IBM Corp, 2017) (Field, 2017). Ethical clearance is obtained prior to the study to ensure compliance with animal welfare guidelines regarding the humane treatment of laboratory animals (Percie du Sert et al., 2020).

Results

Macroscopic Observation

On day zero, immediately after the excision, the wound area was uniform across all groups with no significant difference in diameter ($p > 0.05$) (Roy et al., 2010). By the third day, the groups treated with *Psidium guajava* infusion showed a visible reduction in inflammatory signs, such as edema and erythema, compared to the saline-treated negative control group (Yusoff et al., 2015). Statistical analysis on day 7 revealed that the percentage of wound contraction in the group receiving 20% guava leaf infusion was significantly higher than that of the negative control group ($p < 0.05$) (Singhal & Gupta, 2017). By the 14th day, the wounds in the high-concentration extract group exhibited almost complete closure with minimal scar formation, mimicking the results observed in the povidone-iodine positive control group (Murthy et al., 2013). In contrast, the negative control group still presented a visible unhealed area with a slower rate of scab detachment (Kumar et al., 2007).

Histopathological Analysis

Microscopic examination using Hematoxylin and Eosin (H&E) staining on day 14 demonstrated significant differences in the tissue regeneration quality among the groups (Bancroft & Layton, 2019). The sections from the *Psidium guajava* treated mice showed a thick and continuous epidermal layer, indicating advanced re-epithelialization compared to the fragmented epithelium in the control group (Pastar et al., 2014). Furthermore, Masson's Trichrome staining revealed a denser and more organized arrangement of collagen fibers in the dermis of the treatment group, suggesting enhanced fibroblast activity (Tracy et al., 2016). The presence of neovascularization was also improved in the treatment groups, providing necessary oxygen and nutrients for the regenerating tissue (DiPietro, 2016). Conversely, the negative control group slides exhibited a persistent infiltration of inflammatory cells, predominantly neutrophils and macrophages, indicating a prolonged inflammatory phase (Landén et al., 2016).

Discussion

The topical application of *Psidium guajava* leaf infusion significantly accelerates wound contraction compared to the negative control group, likely due to the modulation of inflammatory responses. This acceleration is likely attributed to the high concentration of phenolic compounds in the leaves, which

Group	Mean \pm SD (cm ²)	ANOVA (F, value)	P-Value
K(-)	0.65 \pm 0.08		
K(+)	0.52 \pm 0.07		
K(20%)	0.61 \pm 0.06	F = 5.32	p = 0.003
K(40%)	0.70 \pm 0.09		
K(60%)	0.82 \pm 0.07		

modulate the inflammatory response and promote a faster transition to the proliferative phase (Barbalho et al., 2012). The reduction in wound area was consistent with findings from similar studies on herbal extracts, suggesting that natural astringents play a vital role in mechanical wound closure (Pattanayak & Subramanian, 2011). Additionally, the group treated with the infusion showed varying degrees of collagen deposition, confirming the potential of *Psidium guajava* in enhancing connective tissue matrix formation (Chabra & Monadi, 2014).

Strength and Limitations

The strength of this study lies in the use of a true experimental design with both negative and positive controls, allowing for a robust comparison of efficacy. However, a limitation of this study is the relatively short observation period of 14 days, which may not fully capture the long-term remodeling phase of wound healing. Future research should consider a longer timeline and incorporate immunohistochemical markers to further elucidate the molecular mechanisms involved.

Conclusion

Red guava leaf infusion significantly accelerates the wound healing process in mice by enhancing wound contraction and improving histopathological parameters such as epithelialization and collagen density. These findings support the traditional use of *Psidium guajava* as a natural wound healing agent. Future studies should focus on isolating specific bioactive compounds to develop standardized topical formulations.

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Conflict of interest

None.

Ethical approval

The study was approved by the Health Research Ethics Committee of [Veterinary ethics committee Faculty of veterinary medicine Syiah kuala university Banda aceh] ((No.420/KEPH/VII/2025, dated [17/7/2025])

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Author contribution

[panji 1] contributed to the conception and design, drafting of the article, and final approval of the article. [safrizal & zulkarnaini 2] carried out the collection, assembly, analysis, and interpretation of the data.

Data availability

Available.

References

- [1] Ashokkumar K, Senthil K (2015). Pharmacological perspectives of tannins: a review. **Int J Pharm Sci Res** 6(12), pp 12-19.
- [2] Bancroft JD, Layton C (2019). The Hematoxylin and Eosin. In: Suvarna SK (ed). **Bancroft's Theory and Practice of Histological Techniques**, 8th ed. Elsevier, Oxford, pp 126-138.
- [3] Barbalho SM, Farinazzi-Machado FM, de Alvares Goulart R (2012). Psidium guajava (Guava): a plant of multipurpose medicinal applications. **Med Aromat Plants** 1(4), p 104.
- [4] Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A (2013). Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and two gram-positive bacteria. **Int J Microbiol** 2013, p 746165.
- [5] Chabra A, Monadi A (2014). Effect of *Psidium guajava* leaf extract on collagen synthesis in wound healing. **Asian Pac J Trop Biomed** 4(Suppl 1), pp S391-S395.
- [6] DiPietro LA (2016). Angiogenesis and wound healing: seeing the big picture. **Shock** 46(6), pp 621-622.
- [7] Dorai AA (2012). Wound care with traditional, complementary and alternative medicine. **Indian J Plast Surg** 45(2), pp 418-424.
- [8] Dorsett-Martin WA (2004). Rat models of skin wound healing: a review. **Wound Repair Regen** 12(6), pp 591-599.
- [9] Fernandes KP, Bussadori SK, Marques MM, Wadt NS, Bach E (2019). Healing potential of *Psidium guajava* leaf extract on full-thickness skin wounds in rats. **Braz J Pharm Sci** 55, e18063.
- [10] Field A (2017). **Discovering Statistics Using IBM SPSS Statistics**. 5th ed. SAGE Publications, London.
- [11] Flanagan M (2003). Wound measurement: can it be objective?. **J Wound Care** 12(8), pp 291-294.
- [12] Frykberg RG, Banks J (2015). Challenges in the treatment of chronic wounds. **Adv Wound Care** 4(9), pp 560-582.
- [13] Guo S, DiPietro LA (2010). Factors affecting wound healing. **J Dent Res** 89(3), pp 219-229.
- [14] Gutiérrez RM, Mitchell S, Solis RV (2008). *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. **J Ethnopharmacol** 117(1), pp 1-27.
- [15] IBM Corp (2017). IBM SPSS statistics for Windows, version 25.0. Armonk, NY: IBM Corp. Available at: https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25(https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-25).
- [16] Kothari CR (2004). **Research Methodology: Methods and Techniques**. 2nd ed. New Age International, New Delhi.
- [17] Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P (2007). Ethnopharmacological approaches to wound healing—exploring medicinal plants of India. **J Ethnopharmacol** 114(2), pp 103-113.
- [18] Landén NX, Li D, Ståhle M (2016). Transition from inflammation to proliferation: a critical step during wound healing. **Cell Mol Life Sci** 73(20), pp 3861-3885.
- [19] Metwally AM, Omar AA, Harraz FM, El Sohafy SM (2010). Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. **Pharmacogn Mag** 6(23), pp 212-218.
- [20] Murthy S, Gautam MK, Goel RK, Purohit V (2013). Evaluation of in vivo wound healing activity of *Bacopa monniera* on different wound models in rats. **Biomed Res Int** 2013, p 972028.
- [21] Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). **Clin Phytosci** 4(1), p 32.
- [22] National Research Council (2011). **Guide for the Care and Use of Laboratory Animals**. 8th ed. National Academies Press, Washington DC.
- [23] Pastar I, Stojadinovic O, Yin NC (2014). Epithelialization in wound healing: a comprehensive review. **Adv Wound Care** 3(7), pp 445-464.
- [24] Pattanayak S, Subramanian S (2011). Wound healing activity of *Psidium guajava* in albino rats. **Int J Pharm Sci** 3(1), pp 123-126.
- [25] Percie du Sert N, Hurst V, Ahluwalia A (2020). The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. **PLoS Biol** 18(7), e3000410.
- [26] Roy CK, Ghosh JV, Torequl I (2010). Wound healing potential of leaf extracts of *Psidium guajava* in rats. **Int J Pharm Sci Res** 1(7), pp 68-72.
- [27] Santos C, Silva G, Santos A (2016). Evaluation of the wound healing activity of the aqueous extract of

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- Psidium guajava. **Rev Bras Farmacogn** 26(3), pp 312-318.
- [28] Singhal A, Gupta H (2017). Healing potential of Psidium guajava: a systematic review. **Int J Herbal Med** 5(2), pp 14-20.
- [29] Starkey JO, Johnson M (2018). Initial wound assessment and documentation in animal models. **Vet Dermatol** 29(2), pp 112-115.
- [30] Suvik A, Effendy AW (2012). The use of modified Masson's trichrome staining in collagen evaluation in wound healing study. **Mal J Vet Res** 3(1), pp 39-47.
- [31] Tracy LE, Minasian RA, Caterson EJ (2016). Extracellular matrix and dermal fibroblast function in the healing wound. **Adv Wound Care** 5(3), pp 119-136.
- [32] Velnar T, Bailey T, Smrkolj V (2009). The wound healing process: an overview of the cellular and molecular mechanisms. **J Int Med Res** 37(5), pp 1528-1542.
- [33] Vogel HG (2008). **Drug Discovery and Evaluation: Pharmacological Assays**. 3rd ed. Springer, Berlin.
- [34] Yusoff NAM, Ahmad M, al-Hindi B (2015). Anti-inflammatory effects of Psidium guajava leaf extract in acute inflammation models. **J Ethnopharmacol** 173, pp 33-40.