**Borneo Journal of Pharmacy**
Vol 7 Issue 4 November 2024
Pages 404 – 411

*https://journal.umpr.ac.id/index.php/bjop/article/view/5803*
DOI: https://doi.org/10.33084/bjop.v7i4.5803
e-ISSN: 2621-4814

*Research Article*

# Standardized Herbal Extract for Wound Healing: A Comparative Study of *Centella asiatica*, *Curcuma domestica*, and *Heterotrigona itama* Honey Combination in Rabbits

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**Keywords**:
*Centella asiatica*
*Curcuma domestica*
Stingless bee honey
Wound Healing

## Abstract

*Centella asiatica* and *Curcuma domestica* are recognized for their wound-healing properties. This study investigated the synergistic effects of combining these plant extracts with stingless bee (*Heterotrigona itama*) honey on wound healing in rabbits. Ethanolic extracts of *C. asiatica* and *C. domestica* were prepared in a 1 : 10 ratio. Blended formulations containing 20% plant extracts and 80% honey were created in two ratios: 1 : 1 and 2 : 1. The total flavonoid and tannin content of the plant extracts were quantified. Male rabbits were subjected to a wound-healing model, and the formulations were applied topically for seven days. Wound healing was assessed, and the results were compared to a positive control. The ethanolic extracts of *C. asiatica* and *C. domestica* were rich in flavonoids and tannins. The blended honey formulations exhibited promising wound-healing effects, with Formula 2 (2 : 1 ratio of plant extracts) demonstrating slightly superior results compared to Formula 1 and the positive control. These findings suggest the potential of the combined extracts of *C. asiatica*, *C. domestica*, and *H. itama* honey as effective wound-healing agents.

*Received*: September 20th, 2023
*1st Revised*: March 15th, 2024
*2nd Revised*: August 23rd, 2024
*3rd Revised*: September 14th, 2024
*Accepted*: October 14th, 2024
*Published*: November 30th, 2024



## INTRODUCTION

The skin serves as the body's primary barrier, protecting it from environmental insults and pathogens. Skin injuries, such as those caused by sharp objects, disrupt this protective barrier, leading to tissue damage and bleeding, consequently disrupting homeostasis and triggering an inflammatory response[1]. Wound healing is a complex physiological process aimed at restoring the integrity and function of damaged tissues. This multi-stage process typically involves three overlapping phases: inflammation, proliferation, and maturation[2].

The rate and quality of wound healing can vary significantly depending on factors such as the location, severity, and extent of the injury[3]. The healing process typically involves several overlapping phases, including inflammation, proliferation, and remodeling. During the remodeling phase, the initially reddish and thick scar tissue gradually transitions into a paler and thinner scar. This phase also witnesses significant wound contraction. While scar tissue plays a crucial role in wound closure, it is important to note that it rarely achieves the full tensile strength of normal, uninjured skin, typically reaching only 80% of the original strength[4].

Acute wounds, which occur in individuals with otherwise healthy tissues, usually heal predictably within a defined timeframe, restoring normal anatomical and functional integrity[5]. To facilitate the wound healing process, it is crucial to

**How to cite:** Diniatik, Kustiawan PM, Suryadini H. Standardized Herbal Extract for Wound Healing: A Comparative Study of Centella asiatica, Curcuma domestica, and Heterotrigona itama Honey Combination in Rabbits. Borneo J Pharm. 2024;7(4):404-11. doi:10.33084/bjop.v7i4.5803

address the inflammatory response and mitigate the detrimental effects of reactive oxygen species (ROS). Oxidative stress plays a crucial role in various skin aging processes, including collagen degradation and wrinkle formation[6]. Natural products rich in antioxidants and immune-modulating compounds offer promising therapeutic options to support and accelerate wound healing. Antioxidants, by neutralizing free radicals, can help mitigate these effects[7]. Natural sources of antioxidants, such as plant extracts and bee products, have gained significant attention due to their potential safety and efficacy.

*Centella asiatica* (gotu kola), a widely used medicinal herb, is rich in bioactive compounds like saponins, flavonoids, and triterpenoids, exhibiting antioxidant, anti-inflammatory, and wound-healing properties[8]. *Curcuma domestica* (turmeric), another renowned medicinal plant, contains curcuminoids, essential oils, and other bioactive compounds with potent antioxidant and anti-inflammatory activities[9]. The presence of flavonoids and phenols in *C. asiatica* and *C. domestica* is expected to exert antioxidant effects, thereby inhibiting lipid peroxidation. This antioxidant activity may contribute to the prevention of cellular necrosis, improved vascularization, and enhanced collagen fiber viability, ultimately leading to increased strength of the dermal extracellular matrix[10]. Apart from these two natural ingredients, stingless bee honey, produced by various bee species including *Heterotrigona itama*, is also a rich source of polyphenols, flavonoids, and other bioactive compounds with antioxidant and antimicrobial properties[11].

While individual studies have investigated the antioxidant and wound-healing properties of these natural ingredients, limited research has explored their combined effects. This study aims to evaluate the potential synergistic effects of a blended extract containing *C. asiatica*, *C. domestica*, and stingless bee honey on *in vivo* model using male albino rabbits. The findings of this research will contribute to the development of novel and effective natural-based formulations for skin rejuvenation and wound healing.

## MATERIALS AND METHODS

### *Materials*

This study utilized fresh leaves of *C. asiatica* and rhizomes of *C. domestica*, both obtained from Banyumas, Central Java, Indonesia. The plant species were authenticated by the Department of Biology, Universitas Muhammadiyah Purwokerto. Stingless bee honey, specifically from the *H. itama* species, was sourced from East Kalimantan, Indonesia. Other materials used in the study included ethanol, various organic solvents, distilled water, quercetin standards, and a set of *in vivo* wound healing test kits, including male albino rabbits.

### *Methods*

*Total flavonoid and tannin contents of a blended of C. asiatica and C. domestica extracts*

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method. Briefly, 100 mg of the plant extract was subjected to acid hydrolysis. This involved refluxing the extract with 1 mL of 0.5% w/v hexamethylenetetramine, 20 mL of acetone, and 2 mL of 25% HCl in water for 30 minutes. The hydrolyzed mixture was then filtered, and the filtrate was collected in a 50 mL volumetric flask. After cooling, the volume was adjusted to 50 mL with distilled water. Subsequently, 20 mL of the hydrolyzed filtrate was extracted with ethyl acetate. The combined ethyl acetate extracts were collected in a 25 mL volumetric flask and brought to volume with ethyl acetate[12]. The TFC of the extract was determined spectrophotometrically by reacting the ethyl acetate extract with aluminum chloride. The absorbance was measured at the maximum wavelength of 430 nm, and the results were expressed as mg of quercetin equivalents per g of dry extract (mgQE/g). The TFC of each plant extract was determined and standardized according to the methods outlined in the Indonesian Herbal Pharmacopoeia[13]. In addition to TFC, total tannin content (TTC) was also measured using the UV-visible spectrophotometric method at a wavelength of 730 nm. Based on the results of TFC and TTC analysis, blended honey preparations were formulated.

*Preparation of blended honey*

Blended extracts were prepared by combining extracts of *C. asiatica* and *C. domestica* with stingless bee honey. The extracts were initially mixed thoroughly in a mortar and pestle to ensure homogeneity. Honey was then gradually added and mixed continuously until a smooth and uniform blend was achieved (**Figure 1**). Two different concentration ratios of the blended

extracts were utilized for further evaluation, as outlined in **Table I**. These specific ratios were selected based on the outcomes of preliminary experiments aimed at optimizing the combination of plant extracts.

*Animal handling*

Healthy adult male albino rabbits weighing approximately 3 kg were used in this study. The animals were housed individually in clean cages under standard laboratory conditions, including a 12-hour light/dark cycle and controlled temperature and humidity. Prior to the experiment, the rabbits were acclimatized to the laboratory environment for five days. A standard diet of kale was provided to all rabbits throughout the study. To avoid confounding effects on wound healing, carrots were excluded from the diet, as carrot tubers contain saponins, known to possess wound-healing properties[14].

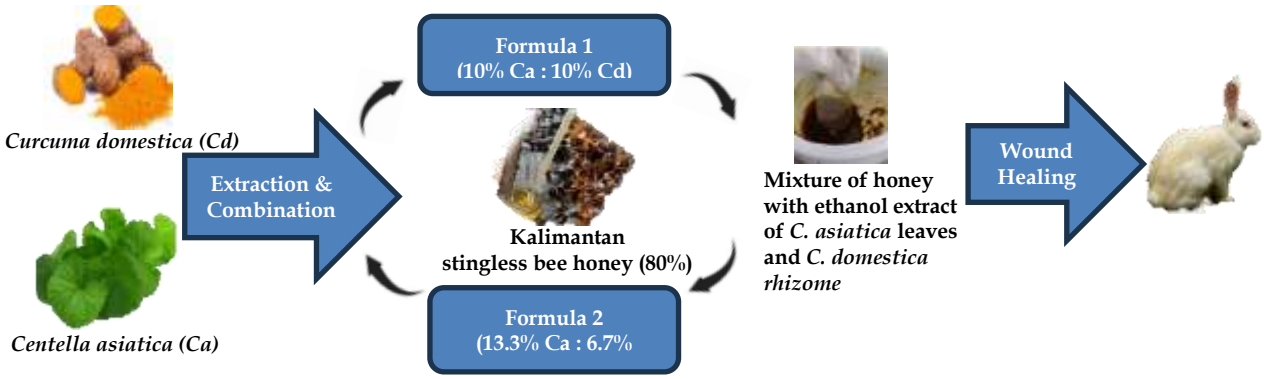


**Figure 1.** Scheme of mixture of stingless bee honey with ethanol extract of *C. asiatica* leaves and *C. domestica* rhizome.

**Table I.** Blended honey formulation.

| Formula | *Centella asiatica* (%) | *Curcuma domestica* (%) | Extract ratio | Stingless bee honey (%) |
|---|---|---|---|---|
| F1 | 10 | 10 | 1:1 | 80 |
| F2 | 13.33 | 6.67 | 2:1 | 80 |

*Determination of wound healing*

This *in vivo* study was conducted with ethical approval from the Health Research Ethics Committee of the Universitas Muhammadiyah Purwokerto, Indonesia (approval number KEPK/UMP/33/III/2022). The study utilized a rabbit model to evaluate wound healing efficacy. Prior to the experiment, the backs of the rabbits were shaved 24 hours beforehand. On the day of the experiment, the shaved area was cleaned with 70% alcohol. A 1.5 cm incision was then made on the subcutaneous layer of the rabbit's skin using sterile surgical instruments. This was achieved by gently lifting the skin with sterile tweezers and making a controlled incision with a sterilized scalpel[15].

***Data analysis***

This study employed a randomized controlled trial design. A total of three rabbits were included in the study. Each rabbit received three wounds, resulting in nine total wounds. Wound I on each rabbit served as the positive control, treated with povidone-iodine. Wound II on each rabbit was treated with varying concentrations of blended honey applied topically three times daily (every eight hours). Wound III on each rabbit served as the negative control, receiving no treatment. The progress of wound healing was monitored and the size of the wound was measured in cm using a ruler every day for eight days.

## RESULTS AND DISCUSSION

***Total flavonoid and tannin contents of a blended of C. asiatica and C. domestica extracts***

A standard curve was generated for quercetin (**Figure 2**), yielding a linear regression equation of $y = 0.0184x + 0.5256944$ with a high coefficient of determination ($R^2 = 0.9444$, $R = 0.9716$), indicating a strong correlation between absorbance and quercetin concentration. **Table II** presents the TFC and TTC of the two ethanol extract mixtures of *C. asiatica* leaves and *C.*

*domestica* rhizome (F1 and F2). Based on these phytochemical analyses, the wound healing activity of both F1 and F2 was subsequently evaluated.

### *Determination of wound healing*

The wound healing process was assessed in male albino rabbits administered with either F1 or F2, alongside negative and positive controls, as shown in **Figure 3**. The negative control group exhibited complete wound closure between days 9 and 14. In contrast, all treatment groups, including the positive control, F1, and F2, demonstrated significantly faster wound healing, with complete closure observed between days 6 and 8. Notably, rabbits treated with F2, containing a higher proportion of *C. asiatica* leaf extract, exhibited a slightly faster wound healing rate compared to those treated with F1. This observation suggests that the increased concentration of *C. asiatica* extract in F2 may contribute to enhanced wound healing activity. These findings align with previous studies that have demonstrated the potent wound healing properties of *C. asiatica*, attributed to its rich content of triterpenoids, flavonoids, and other bioactive compounds[16,17].

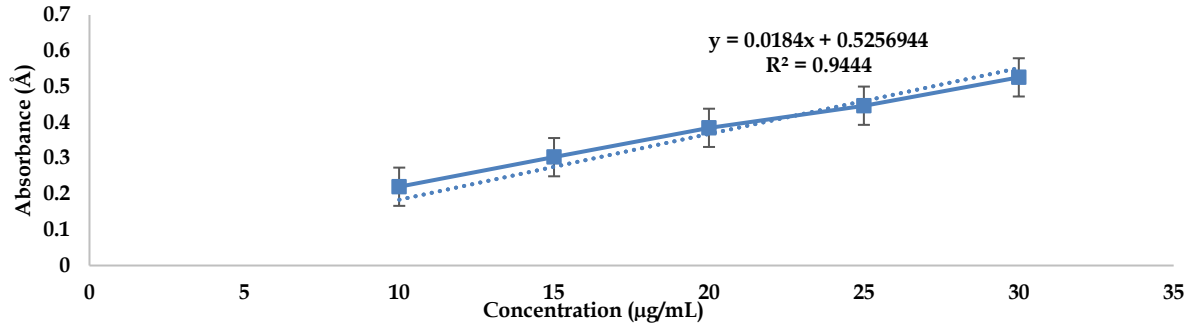


**Figure 2.** Quercetin standard curve.

**Table II.** Total flavonoid content of a blended of *C. asiatica* and *C. domestica* extracts.

| Formula | TFC (mgQE/g) | TTC (mgQE/g) |
|---|---|---|
| F1 | 18.88+1.21 | 4.7+0.3 |
| F2 | 12.57+0.81 | 6.48+0.12 |

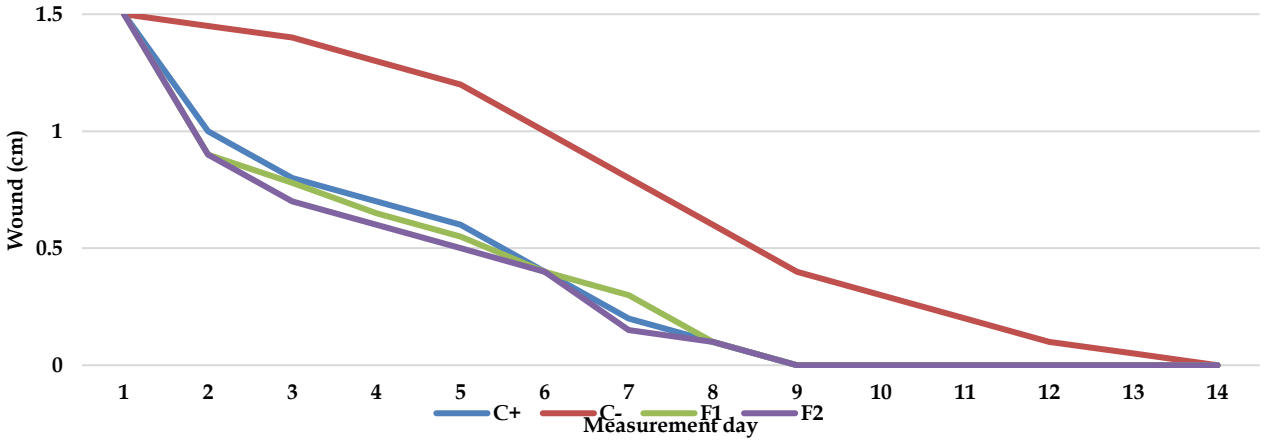


**Figure 3.** Wound healing activity of samples (C+: positive control; C-: negative control).

All groups exhibited an inflammatory phase on the first day, characterized by redness and swelling. The proliferative phase, characterized by granulation tissue formation and re-epithelialization, was observed from the second to the fifth day in the positive control group. Notably, the proliferative phase in the F1 and F2 groups was shorter, lasting from the second to the third day. The maturation phase, marked by collagen deposition and scar formation, commenced on the sixth day in the positive control group and on the fourth day in the F1 and F2 groups. These findings suggest that the combined preparations of *C. asiatica* leaf extract, *C. domestica* rhizome extract, and stingless bee honey demonstrate the potential to accelerate the wound healing process, particularly by shortening the proliferative phase.

The present study demonstrated promising wound healing activity from a topical formulation incorporating extracts of *C. asiatica* leaves, *C. domestica* rhizomes, and stingless bee honey. These findings align with traditional uses within the community where *C. asiatica* has been employed to treat various ailments, including infectious diseases and wounds[18]. Its therapeutic potential is attributed to a diverse array of phytochemicals, including triterpenoid saponins such as asiaticoside, madecassoside, and asiatic acid, which possess anti-inflammatory, antioxidant, and wound healing properties[19,20]. Notably, asiaticoside has been specifically recognized for its anti-leprotic activity[21]. Previous studies have confirmed the antimicrobial activity of *C. asiatica* extracts, with alcoholic extracts demonstrating greater efficacy against *Staphylococcus aureus* and *Escherichia coli* compared to aqueous infusions[22]. This finding aligns with the observed wound healing outcomes in our study. The inclusion of *C. asiatica* extract in the wound healing formulation (F2) likely contributed to its enhanced antimicrobial activity, potentially leading to a slightly faster wound healing rate compared to the formulation with a lower extract concentration (F1). This observation supports the notion that *C. asiatica* extract may play a crucial role in preventing secondary infections and promoting a more rapid healing process.

The potential for anti-inflammatory properties is also observed in *C. domestica*. A study by Fahryl and Carolia (2019) revealed that numerous bioactive compounds within *C. domestica* exhibit diverse pharmacological properties, including antioxidant, antitumor, antidiabetic, and anti-inflammatory activities[23]. These bioactive compounds encompass a range of molecules such as epigallocatechin gallate (EGCG), carnosol, hydroxitirosol, kaempferol, resveratrol, curcumin, and genistein. Notably, curcumin, a prominent phytochemical found in *C. domestica*, has demonstrated anti-inflammatory efficacy in animal models, specifically in reducing tarsal joint edema in mice. This finding further supports the potential of *C. domestica* as a source of natural compounds with anti-inflammatory properties.

Stingless bee honey, a natural product derived from plant nectar and processed by bees, exhibits a wide range of beneficial properties. It serves as a valuable source of nutrients, including vitamins (A, $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, C, D, E, and K), flavonoids, and beta-carotene[24]. Historically, honey has been utilized in traditional medicine for treating respiratory diseases, digestive tract infections, and various other ailments. While sharing some commonalities with other honey types, stingless bee honey possesses distinct characteristics, notably its often-sour taste[25]. These unique properties, combined with its nutritional richness, make it a promising ingredient in wound healing formulations. The current study explored the efficacy of two blended formulations containing stingless bee honey, both of which demonstrated excellent wound healing activity in previous testing.

Povidone-iodine 10% served as the positive control in this study, as it is widely recognized as a gold-standard antiseptic. Povidone-iodine is a complex of polyvinylpyrrolidone and iodine, exerting its antibacterial action through the slow release of iodine upon contact with injured tissue. This released iodine disrupts bacterial metabolism by inhibiting enzymes and damaging bacterial cell structures, ultimately leading to bacterial death[26].

The combination formulations containing *C. asiatica* leaf extract, *C. domestica* rhizome extract, and stingless bee honey were hypothesized to operate through similar mechanisms. Tannins, present in the extracts, possess inherent antibacterial properties. Furthermore, curcuminoids from *C. domestica* exhibit both antibacterial and anti-inflammatory activities[27]. These combined effects within the formulations were expected to synergistically promote wound healing and accelerate the reduction of inflammation. While both formulations (F1 and F2) demonstrated significant wound healing activity, F2 exhibited a slightly faster healing rate. This minor difference might be attributed to subtle variations in the total flavonoid and tannin contents between the two formulations, although these differences were not statistically significant.

## CONCLUSION

The blended F2 combination, comprising *C. asiatica* leaf extract and *C. domestica* rhizome extract with stingless bee honey, demonstrated promising wound healing activity in this study. After seven days of treatment, the wound healing effect of F2 was observed to be slightly superior to that of F1 and the positive control group. This enhanced activity may be attributed to the higher tannin content observed in F2, which is known to possess wound-healing properties. These findings suggest that the F2 combination can serve as a valuable standard blended formula for further in-depth investigations into the potential of these natural ingredients in wound healing applications.

## ACKNOWLEDGMENT

This research was made possible through the generous financial support of the Asosiasi Perguruan Tinggi Farmasi Muhammadiyah Aisyiah (APTFMA) (Grant number: 01/L-APTMFA/I/2022). We are grateful to APTFMA for funding this collaborative research project between Universitas Muhammadiyah Purwokerto, Universitas Muhammadiyah Kalimantan Timur, and Universitas Muhammadiyah Palangkaraya.

## AUTHORS' CONTRIBUTION

**Conceptualization**: Diniatik
**Data curation**: Diniatik, Paula Mariana Kustiawan
**Formal analysis**: Diniatik,
**Funding acquisition**: Diniatik
**Investigation**: Diniatik, Paula Mariana Kustiawan
**Methodology**: Diniatik
**Project administration**: Diniatik
**Resources**: Diniatik, Paula Mariana Kustiawan
**Software**: Halida Suryadini
**Supervision**: Diniatik, Paula Mariana Kustiawan
**Validation**: Diniatik
**Visualization**: Paula Mariana Kustiawan
**Writing - original draft**: Diniatik, Paula Mariana Kustiawan, Halida Suryadini
**Writing - review & editing**: Diniatik, Paula Mariana Kustiawan

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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