Wound healing activities of 
Centella asiatica extracts in incision and burn wound models:
an experimental animal study

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Abstract

**Background:** Efficacy of *Centella asiatica* on incision and burn wounds are not sufficiently known. In this study we evaluated wound healing activities of different extracts of *Centella asiatica*, namely hexane, ethyl acetate, methanol, and water extracts, in incision and partial-thickness burn wound models in rats.

**Results:** On day 7 post-wounding, tensile strength of incision wound in all extract-treated groups was significantly higher than that of the vehicle control (Tween 20®), but comparable to the NSS-treated group. Degree of burn wound healing in ethyl acetate extract-, hexane extract- as well as methanol extract- or water extract-treated groups was significantly higher than that of the control on day 3, 10 and 14, respectively. Similar results were observed in wound lesion and histopathological studies. The wound appearance in the extract-treated groups looked better than those in the control. Analysis by thin layer chromatography demonstrated that the phyto-constituents in hexane extract, ethyl acetate extract and methanol extract included β-sitosterol, asiatic acid, and asiaticoside and madecassocide, respectively.

**Conclusions:** All extracts of *Centella asiatica* could facilitate wound healing process in both incision and burn wounds. Ethyl acetate extract containing asiatic acid seemed to be the most active component for wound healing.

**Keywords**

*Centella asiatica*, wound healing, incision wound, burn wound, asiatic acid, β-sitosterol, asiaticoside, madecassocide
Background

A wound is an injury to a part of the body, especially one in which a break is made in the skin. There are various types of wound such as incised wound, lacerated wound, abrasion, contusion, ulcer and burn wound [1]. Treatment of the wound usually involves infection prevention because the skin, which is the body barrier to infection, is destroyed. Burn wound requires treatment according to the severity of burn. Minor burns are generally treated with topical ointment and dressing while severe burns need immediate medical attention and hospitalization [2]. Inappropriate care of wound may result in delay in the healing process, infection as well as the wound becoming a chronic one. Antimicrobial ointments such as silver sulfadiazine, mafenide, silver nitrate, povidone-iodine, mupirocin and bacitracin, are used to reduce the risk of infection in minor cuts and burns.

Development of drugs or agents to treat wound has been continuously going on. Nowadays, numbers of medicinal plants have been integrated into health care, especially the primary healthcare system. One among them is Centella asiatica (Linn.) Urban commonly found in many parts of the world including Asia and the Middle East [3]. In Asia, Centella asiatica has a long history of use in traditional medicine for the treatment of wounds, to improve mental clarity, and treat skin infectious conditions such as leprosy and psoriasis [4]. The constituents of therapeutic interest in Centella asiatica are saponin-containing triterpene acids and their sugar esters; the most important being asiatic acid, madecassic acid and asiaticosides [5].

Kosalwatna et al. reported that 1% Centella asiatica extract cream improved the wound healing of chronic ulcers in width, length and
depth observed at day 7, 14 and 21. Apparently, the healing rate of the depth was faster than the other two dimensions by about 20% [6]. Later on, Shukla et al. found that asiaticoside isolated from CA[SYG8] increased hydroxyproline content, tensile strength, collagen content and epithelialization in punch wound model [7]. Effect of the extract of Centella asiatica on acute radiation dermatitis in rats has been studied by Chen et al. who concluded that the wounds in treatment group were less severe and repairing processes began earlier than those of the control group [8]. Furthermore, the study of Maquart et al. showed that triterpenes from Centella asiatica were able to increase remodeling of the collagen matrix and stimulate glycosaminoglycan synthesis in rat wound chamber model [9]. A study in incision wound model demonstrated that the ethanolic extract of Centella asiatica significantly increased the wound breaking strength [10]. Oral administration of madecassoside (6, 12, 24 mg/kg) isolated from Centella asiatica herbs facilitated burn wound healing in mice through its antioxidative activity, collagen synthesis and angiogenesis [11]. Coupled with these, clinical efficacy of the oral Centella asiatica extract capsule has been proved to be effective in the promotion of wound healing as well as scar suppression of even diabetic wounds with no demonstrable adverse reactions in the patients [12]. However, there are few studies that investigate the efficacy of Centella asiatica on incision and burn wounds which have peculiar healing mechanisms different from excision wound. Thus, the purpose of this study was to evaluate wound healing activities of hexane-, ethyl acetate-, methanol- and aqueous-extracts of Centella asiatica, in incision and second-degree burn wound models in rats. The test
substances were applied topically once daily. Tensile strength of incision wound was measured on day 7 post-wounding. General appearance and degree of wound healing of burn wound were assessed on day 3, 7, 10 and 14 post-burning and prior to histopathological evaluation. [SYG9].

Methods

Results [SYG10]

Characteristic feature of Centella asiatica extracts
Using thin layer chromatography (TLC) technique, the major active compounds in the hexane-, ethyl acetate and methanol extracted fractions were identified as β-sitosterol, asiatic acid, and asiaticoside and madecassoside, respectively, whereas none of these active components was found in the water extracted fraction.

Effect of Centella asiatica extracts on tensile strength of incision wound
The effect of Centella asiatica extracts on the healing of incision wound is shown in Table 1. On day 7 post-wounding, the tensile strength of the incision wound in untreated group was not significantly different from that of the NSS-treated group or Tween 20-treated group which served as a vehicle control group. In comparison to control, the tensile strength in all animal groups treated with 10% of different fractions of Centella asiatica was significantly increased. However, none of them was significantly different from those of the NSS-treated group.

Effect of Centella asiatica extracts on burn wound lesion
On day 3, the wound in untreated, NSS-treated and Tween 20-treated groups became swollen and bruised. Comparatively, the wound in all extract treated groups showed a mild degree of swelling and wound surface was
rather dry. Most wounds in ethyl acetate-treated group began to reduce in size by slightly contracting from the wound edge.

On day 7, all wounds in untreated, NSS-treated and Tween 20-treated groups showed dark red color, thickening of the skin at the wound site and the wound size remained unchanged from the first day. Most of the wounds treated with the extracts showed wound contraction, smaller in size compared with those in control group. The wound in methanol extract-treated group showed remarkable hair growth. Some wounds in the methanol extract- and water extract-treated groups showed scabs covering the wound surface. On day 10, all wounds in the untreated, NSS-treated and Tween 20-treated groups showed moderate exudation and no hair growth, with scabs covering the wound surface. All wounds in the extract-treated groups showed dry wound surface, progressive wound contraction, reduction of wound size and increased hair growth.

On day 14, which was the end of the experiment, all wounds in the untreated, NSS-treated and Tween 20-treated groups showed moderate exudation and scabs starting to separate from the wound surface. The wound size was slightly decreased from those at the beginning. The wounds in the extract-treated groups showed a remarkable decrease in wound size and continuous growth of hair at the wound site. The wounds in the methanol extract- and water extract-treated groups showed the most remarkable decrease in wound size.

**Effect of Centella asiatica extracts on the degree of burn wound healing**

The effect of *Centella asiatica* extracts on the degree of burn wound healing is shown in Table 2. The degree of wound healing among the
untreated, NSS-treated and Tween 20-treated groups was not significantly
different from each other at every time points. On day 3 post-burning, only in
ethyl acetate-treated group was the degree of wound healing significantly
higher than that of the vehicle control group. On day 7 post-burning, similar
results were observed as of day 3.
On day 10 post-burning, the degree of wound healing in the animals
treated with hexane or ethyl acetate was significantly higher than that of the
vehicle control group. Moreover, the degree of wound healing in hexane
extract-treated group was not significantly different from that of the ethyl
acetate extract-treated group.
On day 14 post-burning, the degree of wound healing in the animals
from all extract-treated groups was significantly higher than that of the vehicle
control group. Furthermore, the degree of wound healing among the extracttreated
groups was not significantly different from each other.

Effect of *Centella asiatica* extracts on histopathology of burn wound

Histological feature of normal skin is illustrated in Figure 1A. At the end
of the experiment on day 14 post-burning, the untreated burn wound showed
prominent fibrinoid necrosis in the subepidermal region, which was
characterized by permeation of collagen with fibrin and additional
degenerative changes. The re-epithelialization was incomplete with less skin
appendages. Wound surface was covered with some exudates (Figure 1B).
The wound in the NSS- and Tween 20-treated groups showed fibrinoid
necrosis in some areas of the subepidermis. There were empty spaces in the
dermal region of the Tween 20-treated group, as evidence of edema. Reepithelialization
was still incomplete but the NSS-treated wounds seemed to
be better than those treated with Tween 20 in this regard (Figures 1C and 1D, respectively). [SYG15] 

The wounds in all extract-treated groups showed fully developed epithelialization and keratinization. Skin appendages can be observed near to normal skin. There were no noticeable necrosis and inflammation (Figures 1E, 1F, 1G and 1H).

Discussion [SYG16]

In this study, four different extracts from *Centella asiatica*, hexane, ethyl acetate, methanol and water extracts, were applied for the treatment of wound on the skin of rats in vivo. The results of this study showed that the extracts from *Centella asiatica* could facilitate the wound healing process during the experimental period of seven and fourteen days in both incision wound and partial thickness burn wound. The results agreed with those which previously claimed that *Centella asiatica* could facilitate wound healing.

Effect of *Centella asiatica* on incision wound

On day 7 post-wounding, the tensile strength of all animals in the extract-treated groups was significantly increased compared to those in Tween 20-treated group. The present finding is in accordance with those of previous studies reporting that crude ethanolic extract from *Centella asiatica* increased the tensile strength in incision wound by increasing collagen concentration and stabilization of the fibers [13, 14]. Asiaticoside isolated from *Centella asiatica* exerted wound healing activity by increasing tensile strength and collagen content [7]. Furthermore, triterpenoids obtained from *Centella asiatica* increased the collagen synthesis both in vivo and in vivo [9, 15].
According to TLC technique, it was shown that ethyl acetate and methanol extracts were composed of triterpenoid, including asiatic acid and asiasioside, respectively. It is well known that collagen is the major protein component of wound connective tissue and the chemical composition of collagen is responsible for its biological function of being the bonding component which holds tissues together and serves as the strength of wound [16]. Thus, the healing activity of the extracts from Centella asiatica characterized by increased tensile strength of incision wound may be due to an increase in collagen synthesis.

Interestingly, the healing activity of each extract from Centella asiatica in incision wound was comparable to the NSS- but not the Tween 20- treated group. This observation could be accounted by the fact that incision wound had minimal cell loss, and tissue injury could be closed within 6-12 hours if they were not contaminated [1]. Additionally, NSS, the most commonly used solution in clinical practice for wound irrigation, preserves the physiological condition and does not contain any surfactants such as Tween 20 which has been postulated to cause skin injury [17].

**Effects of Centella asiatica on burn wound**

Loss of cells and tissues in the burn wound was more extensive than those in incision wound. The reparative process, namely healing by second intention, is more complicated than incision wound [18].

On day 3 post-burning, all wounds in the untreated, NSS- and Tween 20-treated groups became swollen and bruised indicating that all wounds are in the first phase, inflammatory phase, of wound healing process. In contrast, most wounds in the extract-treated groups showed mild swelling and dry
wound surface, suggesting that the extract from *Centella asiatica* could reduce inflammatory process presumably by its anti-inflammatory activity [3, 8]. In addition, most wounds in the extract-treated groups began to slightly contract from the wound edge. Similar results were found with regards to the degree of wound healing which was significantly higher in ethyl acetatetreated group than vehicle control group. Thus, it is suggested that the extract from *Centella asiatica* could facilitate collagen synthesis [9, 15, 19] and subsequently accelerated the wound healing process to the second phase, the proliferative phase.

On day 7 post-burning, all wounds in the untreated, NSS- and Tween 20- treated groups showed dark red color and the wound sizes appeared to be rather similar to those at the beginning indicating that all wounds remained inflamed. In contrast, most of the wounds treated with the extracts showed wound contraction and hair growth. These results indicated that all wounds in extract-treated groups were in the second phase of wound healing process, proliferative phase. In particular, only in the ethyl acetate-treated group was the degree of wound healing higher than that of vehicle control group since day 3 post-burning. TLC technique showed that asiatic acid was the constituent in ethyl acetate extract. Many reports showed that asiatic acid could increase collagen synthesis which is important in healing process [9, 15]. Therefore, it was possible that the ethyl acetate extract promoted the wound healing process by, in addition to inhibiting inflammation as shown on day 3, increasing collagen synthesis. The results supported a previous study which demonstrated that asiatic acid is the most potent triterpenoid components present in *Centella asiatica* in the gene-expression induction of
TNFAIP6, a hyaladherin involved in extracellular matrix remodeling and modulation of inflammation, when applied to human fibroblast [20]. On day 10 post-burning, all wounds in untreated, NSS- and Tween 20-treated groups revealed moderate exudation without hair growth. On the contrary, all wounds in the extract-treated groups showed dry wound surface, progressive wound contraction and reduction in wound sizes as well as increased hair growth, indicating that there was an acceleration in the healing process. The degree of wound healing in hexane and ethyl acetate extract-treated groups were significantly higher than that of Tween 20-treated group. As demonstrated by TLC technique, hexane and ethyl acetate extracts contained β-sitosterol and asiatic acid, respectively. Some studies reported that β-sitosterol showed a potent angiogenic activity, which is essential for wound healing [21, 22]. Thus, healing activity of hexane extract may be partly due to angiogenic activity.

On day 14 post burning, histopathological observation revealed prominent fibrinoid necrosis, incomplete epithelialization, less skin appendages, and exudates covering the wound surface in the untreated group while fully developed epithelialization, keratinization and skin appendages can be observed in all extract-treated groups. The wounds in the extract-treated groups showed a remarkable decrease in wound size and continuous growth of the hair at the wound site. These findings indicated that the extracts could promote wound healing process.

The hexane and ethyl acetate extracts showed wound healing activity since day 3 and day 10 of the experiments, respectively. Thus, it is likely that active components from these fractions, asiatic acid and β-sitosterol might act...
in early stage of wound healing process, inflammatory and proliferative phase, through inhibition of inflammatory process, induction of collagen synthesis and promotion of angiogenesis. In addition, the finding that activity of methanol and water extract were observed on day 14 of the experiment suggested that active components from these fractions might be active in the late stage of wound healing process, the proliferative and remodeling phase. Formation of granulation tissue in the wound was usually initiated in proliferative phase. Collagen and revascularization, which could be facilitated by asiaticoside and β-sitosterol contained in methanol and hexane extracts, respectively, play the major roles in granulation tissue formation [23]. Asiaticoside was previously shown to induce type I collagen synthesis in human dermal fibroblast cells via the activation of the TGFbeta receptor I kinase (TbetaRI kinase)-independent Smad signaling pathway [24]. In addition, asiaticoside isolated from CA induced elevation of antioxidant levels in punch wound model, thereby facilitating the healing [25]. Madecassoside, a major content in methanol extract identified in this study, also facilitated burn wound healing in mice [11]. Neither β-sitosterol, asiatic acid, nor asiaticoside and madecassocide was identified in water extract by TLC technique, the wound healing activity of this extract was also demonstrated on day 14 of the experiment. However, according to the study of Anand et al. (2010), flavonoid content was found to be highest in water extract of Centella asiatica and possesses anti-oxidant property.[26] Antioxidants are postulated to help control wound oxidative stress and thereby accelerate wound healing [27]. Therefore, it is possible that flavonoid in water extract, which could not be determined by TLC technique used, plays an important role as an anti-oxidant agent in the late
stage of wound healing process. Although TLC is the most widely used method to find out any substances because of its simplicity of performance, it still has limited ability to detect some substances which could not be separated. The results of this study offer a support for the notion that *Centella asiatica* could promote the wound healing by inhibiting inflammation, inducing collagen synthesis, promoting angiogenesis, inducing vasodilation, and reducing wound oxidative stress. Besides those findings mentioned, growth factors are interesting to bring into the explanation of wound healing activity of *Centella asiatica* extracts because of their influences on cellular growth and proliferation. In an ideal wound healing situation, new tissue growth would replace damaged tissue resulting from a wound with no functional or cosmetic impairment. The wound healing activity of *Centella asiatica* extracts might be related to growth factors such as endothelial growth factor, fibroblast growth factor and vascular endothelial growth factor. Thus, further study on the effects of *Centella asiatica* extracts on growth factors is needed in order to clarify its mechanism of action on wound healing. Furthermore, microcirculatory studies are also suggested to investigate the anti-inflammatory activity of ethyl acetate extract of CA which was revealed during the early phase of burn wound healing.

**Conclusions**

The present study demonstrates wound healing effect of *Centella asiatica* extracts in both incision and burn wounds.

Ethyl acetate extract containing
asiatic acid, seemed to be the most active in the wound healing models employed. Further mechanistic studies of wound healing activity demonstrated by the active components of *Centella asiatica* as well as an establishment of extract with more well-defined components would be beneficial for the development of wound healing medication from *Centella asiatica*.

**Methods**

**Preparation and extraction of Centella asiatica**

The trunks of *Centella asiatica* were collected, cleaned, dried, powdered, and used for sequential extraction. The extract was prepared into 4 fractions, namely hexane, ethyl acetate, methanol, and water extracts, respectively. First, the powdered plant materials were soaked in hexane 3 times for 5 days each to yield the hexane extract. Next, the rubbish from the first step was soaked in ethyl acetate 3 times for 5 days each to yield the ethyl acetate extract. Then the rubbish from the second step was soaked in methanol 3 times for 5 days each to yield the methanol extract. Finally, the rubbish from the third step was soaked in water 3 times for 5 days each to yield the water extract. Each extract was evaporated under reduced pressure.

**TLC Profiling of Extracts**

Characterization of the extracts was made by thin layer chromatography with silica gel 60 F254 plate. The solvent systems used for analysis of hexane, ethyl acetate, methanol, and water extracts are as follows: chloroform: acetate (9:1), chloroform: methanol (9:1), ethyl acetate: methanol: water (4:0.4:0.2) and ethyl acetate: methanol: water (4:0.4:0.3), respectively. Thin layer chromatography plate of each extract was sprayed with 10%
sulfuric acid in ethanol and then heated by a hot plate the temperature of
which was maintained at 100°C for 15 min.

The concentration of *Centella asiatica* extracts used in this study was
10% (w/v) in 10% Tween 20® (v/v) in freshly prepared solution. First, 1 mL of
Tween 20® was dissolved in 9 mL of distilled water to obtain 10% (v/v) Tween
20®, and then 1 mg of the extract was dissolved in 9 mL of prepared 10%
Tween 20®. The volume was adjusted to obtain 10% (w/v) of the extract for
using topically in the experiment.

**Experimental protocol**

A total of 112 male Sprague-Dawley rats weighing 250-300 g obtained
from the National Laboratory Animal Center, Mahidol University,
Nakornpathom, Thailand were used in this study. The rats were caged in the
room maintained temperature at 25±1°C with free access to commercial pellet
diet and water *ad libitum*. The rats were used after acclimatization to the
laboratory environment for a 7-day period. All animals care and handling were
carried out according to Guide for the Care and Use of Laboratory Animals by
the National Research Council of Thailand and the experimental protocol was
approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences,
Chulalongkorn University.

The animals were divided into two groups of 56 animals for incision
wound and burn wound models. Each group of the animals were further
divided into seven subgroups of eight animals each as follows: 1) untreated
group, 2) NSS-treated group, 3) Tween 20® (polyoxyethylene (20) sorbitan monolaurate)-treated group as a vehicle control group, 4) hexane extract-treated group, 5) ethyl acetate extract-treated group, 6) methanol extract-treated group, and 7) water extract-treated group.

**Effect of *Centella asiatica* extracts on the healing of incision wound**

The effect of *Centella asiatica* extracts on the healing of incision wounds was investigated using the model of Baie and Shiekh (2000) [28]. The animals were anesthetized with intra-peritoneal injection of sodium pentobarbital at 60 mg/kg BW. The right side on the back of each animal was shaved and depilated. Then a 3-cm midline incision was made through the whole skin thickness with a sharp scalpel. Each incised wound was closed with 0.5 cm spaced interrupted sutures with black silk no.3-0 to secure the edges of the incision wound. After wounding, 0.5 mL of the test substance was topically applied to the animals once daily. On day 7 post-wounding, the animals were sacrificed with intra-peritoneal injection of sodium pentobarbital at 100 mg/kg BW. Then the sutures were removed and the tissues were isolated from the healed wound for the measurement of tensile strength as described below.

**Tensile Strength determination of incision wounds**

**Effect of *Centella asiatica* extracts on the healing of burn wound**

The effect of *Centella asiatica* extracts on the healing of burn wounds was investigated using the method described by Somboonwong *et al.* [29] which was modified from Zawacki [30]. The animals were anesthetized with intra-peritoneal injection of sodium pentobarbital at 60 mg/kg BW. The back of the animal between the lower parts of both scapulas were shaved and depilated. Then a partial thickness burn was made by
putting hot plate, 3.5 x 4.6 cm in size, with temperature of 75°C on the prepared area for 10 sec. The burned area was about 10% of total body surface area. After burning, 0.5 ml of the test substances was topically applied to the animals once daily. The burned area of each animal was measured immediately after burning and on day 3, 7, 10 and 14 post-burning using a millimeter scale graph paper and the degree of wound healing was calculated as described below. On day 14 post-burning, the animals were sacrificed with intra-peritoneal injection of sodium pentobarbital at 100 mg/kg BW. Then a specimen sample of the tissue in the healed wound, 0.5 x 0.5 cm in size, was isolated from each animal for histological examination.

**Tensile strength test for incision wounds**

Healing of incision wounds was evaluated by measurement of tensile strength on day 7 post-wounding. The sutures were removed and the skin tissue was cut in the size of 1 cm away from each side of the wound. The isolated wound tissues were used to measure the load (force) to break the tissue with a computerized tensiometer (EZ-TEST I 30804100798, SHUMADZU Corporation, Japan) and tensile strength was calculated using the following formula: tensile strength (N/cm²) = breaking force (N)/ area (cm²); area (cm²) = thickness (cm) x width (cm).

**Gross examination of burn wound lesion**

The wound was grossly examined on day 3, 7, 10 and 14 post-burning. The lesion of wounds was examined using the following criteria: wound bed,
color, exudates, swelling of wound surface and the consistency of the surrounding wound tissues.

**Assessment of the degree of burn wound healing**

On day 3, 7, 10 and 14 post-burning, color photographs of the wounds were taken by digital camera. The areas of the wound were measured by tracing the wound boundaries using a millimeter scale transparent graph paper with permanent marker and the degree of wound healing was calculated using the following formula: the degree of wound healing (%) = 1 - [wound area on corresponding day (cm²) / wound area on zero day (cm²)] x 100 [31].

**Histopathological examination of burn wound**

The specimen of the skin, 0.5 x 0.5 cm in size, was taken from the middle of the burned area. The tissues were preserved in the fresh fixative aqueous 10% neutral buffered solution of formaldehyde for at least 24 hr. Sections were stained with hematoxylin and eosin dyes. The light microscope (Nikon 516609) with x20 and x40 objective lens was used.

**Statistical analysis**

Results are presented as mean ± standard error of mean (SEM). The differences among experimental groups were compared by one-way analysis of variance (ANOVA), followed by the least significant different test (LSD) and were considered statistically significant when $P$ was less than 0.05.

**List of abbreviations**

NSS: normal saline solution; TLC: thin-layer chromatography
Competing interests
All authors declare that they have no competing interests.

Authors' contributions
JS participated in design of the experiments, conducting and analysis of gross and histopathological data and drafting the manuscript. MK was responsible for induction of incision and burn wound as well as statistical analysis. BT prepared the extract and acquisition of animal data. MT conceived of the study and its design, analysis of data and critically revised the manuscript. All authors read and approved the final manuscript.

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**Figures**

Figure 1 Histological features of the partial-thickness burn wounds on day 14 post-burning.
Hematoxylin and eosin stain of the following sections: A) normal skin; B) untreated wound; C) NSS-treated wound; D) Tween 20-treated wound; E) hexane extract-treated wound; F) ethyl acetate extract-treated wound; G) methanol extract-treated wound; H) water extract-treated wound; Ep: epidermis; Kt: keratin; Exd: exudates; FN: fibrinoid necrosis; Ede: edema; F: hair follicle