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Potential Wound Healing Activity of the Different Extract of *Crescentia cujete* in Albino Rats

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Abstract. The aim of the present study was to investigate the wound healing potential of *Crescentia cujete* leaves commonly employed by traditional healers. An excision model was performed to evaluate the wound healing activity. After creating wound healing model on the back of rats, the ethanol and ethyl acetate extracts of plant *Crescentia cujete* was applied. Wound healing rates calculated at 3, 9, and 15 days after wounding. The present study showed that ethanol and ethyl acetate extract were found greater wound healing activity than in negative control. The results indicate that the different extracts of *Crescentia cujete* has significant wound healing activity.

INTRODUCTION

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drug or as models for pharmacologically active compounds. The plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias [1]. Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier [2]. Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue [3]. There are three main phases of wound healing, inflammatory, proliferative and remodeling phase. The inflammatory phase begins immediately after injury with vasoconstriction that favors and releases inflammatory mediators. The proliferative phase is characterized by granulation tissue formation mainly by fibroblasts and angiogenesis. The remodeling phase is characterized by reformulation and improvement in the components of the collagen fiber that increases the tensile strength [4].

Crescentia cujete L (Bignoniaceae) is commonly known as calabash tree. It is widely distributed in the Caribbean region, Mexico, Northern and Southern American and later introduced to tropical Africa from Senegal to Cameroon then to other parts of Africa [5]. In Indonesia, it is known as Maja or Bila. Traditionally, *C. cujete* has been reported to be used among many tribes and different culture around the world to treat different ailments [5]. The fresh leaves are used topically for wound healing while the powdered leaves are used for headaches, and internally as a diuretic and in the treatment of hematomas and tumors [6]. The aim of the present study was to investigate the wound healing potential of *C. cujete* leaves commonly employed by traditional healers.

EXPERIMENT

Preparation Extracts

The leaves of *C. cujete* were collected from West Sulawesi, Indonesia. The plant was identified, confirmed and authenticated by Dr. Syamsiah, M.Si, botanist in the Department of Biology, Faculty of Mathematics and natural sciences, Universitas Negeri Makassar, Indonesia. The leaves of the *C. cujete* was made into coarse powder. Five hundred gram of *C. cujete* was macerated in 75% ethanol for 3 days. The liquid component was filtered through Whitman no.1 filter paper and evaporated to dryness under vacuum at 40°C using a rotary evaporator. All the step will be repeated using ethyl acetate solvent. The sample was stored under refrigeration (-20°C) condition for further analysis. The ethanol extract was labeled (EE) and ethyl acetate extract (EAE).

Evaluation of skin wound healing

Animals

Healthy male wistar albino rats (200-250 g) were selected for all the present in vivo studies. The animals were fed on normal diet and water ad libitum. The animals were used after an acclimatization period of 7 days to the laboratory biology. The study was approved by the ethics committee for animal experimentation, the faculty of Medicine Universitas Hasanuddin.

Excision of Skin Wounds Topical Treatments

Rats were anesthetized with ketamine by subcutaneously with dose of 120 mg / Kg. Next, the dorsocostal area of each rats was shaved and excisional wounds were performed using a sterile sharp dermal biopsy punch of 6 mm [7]. The animals were randomly divided into four groups of 3 animals each. Group 1 was treated 0.5 g ointment base only as the control; group 2 was treated with 5% povidone iodine as positive control; group 3 and 4 were treated topically with EE and EAE ointment. The treatments were applied immediately after the injury and every 24 h during 15 days. All wounds of single animal were subjected to the same treatment throughout the experiment.

Measurement of Wound Contraction

Measurement of the wound area was performed after the injury (day 0) and on days 3, 9, and 15 using a digital caliper before the treatments application. The percentage of wound closure can be calculated using the formula [8] as follows:

$$\% \text{ wound closure} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{initial wound size}} \times 100 \quad (1)$$

Analysis of Data

The results of these experiments are expressed as mean \pm S.E, of three animals in each group. The data were evaluated by one-way ANOVA followed by Tukey's pair-wise comparison test. The values of $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Excision models were used to evaluate the wound healing activity of *C. cujete* leaves. As shown in Figure 1 and 2, the treatments with EE and EAE accelerated the skin wound closure. On day 3, both EE and EAE treatments induced relative wound closure around 15%. On day 9, the animals treated with EE and EAE already presented a relative percentage of wound closure around 50% and 65% respectively, significantly different from negative control showed 35%. On the last day all wounds were almost closed except negative control.

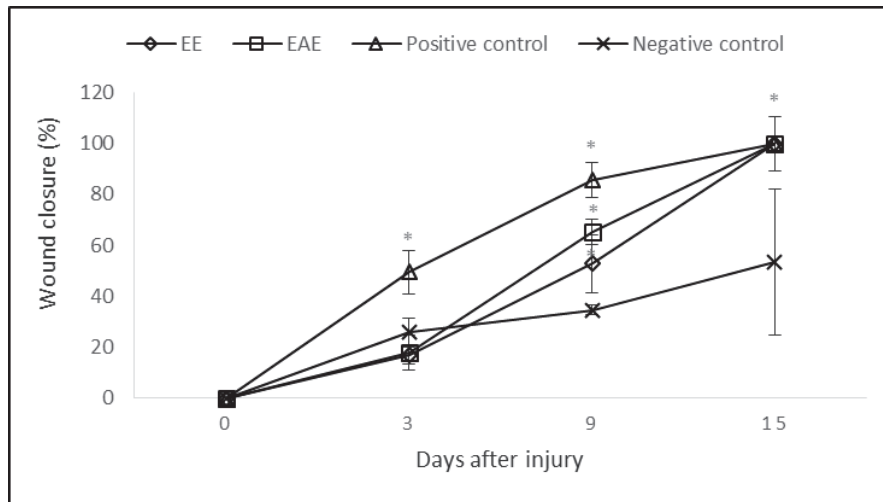
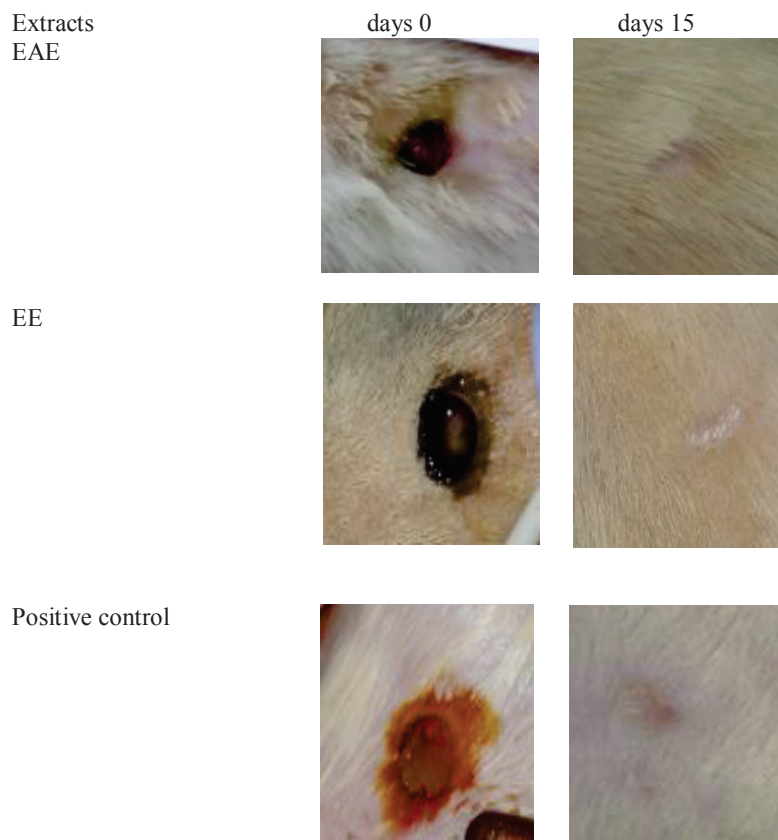


FIGURE 1. Effect of EE and EAE in relative skin wound closure in rats. EE and EAE were topically applied after the injury and every 24 h for 15 days.



Negative control

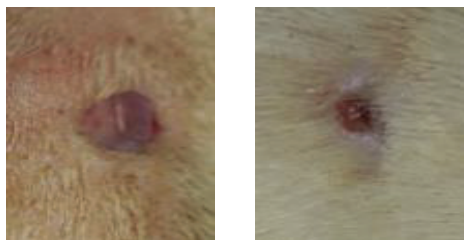


FIGURE 2. Representative images of dorsal excision skin wounds treated with EE, EAE, povidone iodine (positive control) and base gel (negative control) for 15 days.

DISCUSSION

Wound healing is a complicated process. It is a response injury aimed at reconstructing damage tissue and requires precise coordination of connective tissue repair, re-epithelization, and angiogenesis. To generate new tissue and heal the wound, fibroblasts not only proliferate to increase cell number, but also produce several extracellular matrix proteins and growth factors [9,10].

C. cujete has long been used as a traditional medicine for burn and skin wounds in ethnic communities in West Sulawesi. In the present study, topical application of EE and EAE extract significantly enhanced the rate of wound healing. Wound healing effect may be due to upregulation of human collagen I expression [11]. Enhanced healing activity has been attributed to increased collagen formation and angiogenesis [12-14]. Collagen, which is principal component of connective tissue, plays a key role in the healing wounds and provides a structural framework for the regenerating tissue [15,16]. The present study showed that EE and EAE accelerated wound healing in rats, suggesting that EE and EAE exhibited wound healing activities. Probably, flavonoid, tannin, alkaloid were observed in *C. cujete* leaves [17]. Flavonoid found in *C. cujete* can act as antioxidants and protect the cells of the body from radical damage [18]. Alkaloid in *C. cujete* may explain why it has been used as anti-inflammatory agents [17,19].

Ability in rapid wound healing is thought to be caused by leaves (*C. cujete*) has active compounds namely alkaloids, flavonoids, tannins, and saponins. The results of this study are consistent with previous studies showing that *C. cujete* contains alkaloids, saponins, tannins and polyphenols that are potentially antibacterial. Other studies have shown that *C. cujete* contains Flavonoid-quercetin [20], tannins, phenols, saponins, anthraquinones and cardenolides [17]. The process of wound healing is caused by the presence of antibacterial compounds. In line with the study by Mahbub et al., 2011 [21] showed that the ethanol extract of *C. cujete* leaves has an effective antibacterial power inhibiting the growth of *Shigella dysenteriae*, *Bacillus subtilis* and *Escherichia coli*. Other results showed that ethanol extract of *C. cujete* leaves with concentration of 60% and 80% was significantly able to accelerate the cessation of external bleeding of mice [22] and have anti-inflammatory (anti-inflammatory) in mice with in doses of 1680, 3360 and 6720 mg / kg BW [23]. The *C. cujete* plant contains other active compounds such as tartaric acid, cyanohydric, citric acid, cresentia acid, beta-sitosterol, stigmastrol, alpa and beta amyryne, esteric acid, palmitic acid, apigenin, naphthaquinone, iridoid glycosides, 3-hydroxyoktanol glycosides (Marc, 2008). Flavonoid-quercetin found in *C. cujete* has activity as an antioxidant that protects the body cells from free radical damage that contribute to cell damage and various health-related problems [17].

CONCLUSIONS

The study reveals that ethanol and ethyl acetate extracts treated groups possess good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like, flavonoid, alkaloids, saponins and tannins.

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