

Wound Healing Effects of *Piper nigrum* L. and *Coffea canephora* in Rats

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Abstract. The ethanolic and ethyl acetate extracts of *Piper nigrum* L and *Coffea canephora* were evaluated for their wound healing activity in rats. Wound healing activity was studied using excision wound models in rats following topical application. Animals were divided into eight groups with three in each group. Ten Percent w/v extract of each plant was prepared in gel CMC-Na for topical application. *Piper nigrum* L possesses better wound healing activity than *Coffea canephora*.

Introduction

Skin wounds are a disturbance of the epithelial and underlying tissues integrity due to violence or trauma [1]. The skin wound healing is a reparative process, involving the components of the extracellular matrix, resident cells (fibroblasts, keratinocytes, endothelial cells, nerve cells), leukocytes, and chemical mediators, including prostaglandins, cytokines, leukotrienes, and growth factors, which aims for the replacement of the injured tissues by vascularized connective tissues [2, 3]. Several plants are used traditionally in treatment of many skin wounds and burnings in various areas in the world [4]; among them one can refer to plants of jujube, mountain germander, olibanum, Aloe vera and also portulaca, whose effect has been proved through the trend of burning wounds healing in rats [5-8].

Preparations from traditional medicinal plants are often used for wound healing purposes covering a broad area of different diseases. Here we report on 2 plants which are used as wound healing agents in traditional ethnic communities in West Sulawesi medicine. The plant species are *Piper nigrum* L and *Coffea canephora*. Generally, the use of the plants as wound healing agents is merely based on folklore without any scientific evidence of its efficacy. Moreover, the effective compounds during the process of wound healing are still unknown. In order to determine whether their traditional uses are supported by biological effects, the extracts were investigated in several actual biological assays being related to different stages of the wound healing processes.

The leaves of *Piper nigrum* L and *Coffea canephora* were used as wound healing agents in traditional ethnic communities in West Sulawesi medicine. This study investigated the topical wound healing of gels containing the ethanol extract (EE) and ethyl acetate extract (EAE) of leaves of this plant in a model of excision wound healing in rats.

Material and Methods

Preparation extract

The plant materials were collected from West Sulawesi, Indonesia. The plant was identified, confirmed and authenticated by Dr. Syamsiah, M.Si, a botanist in Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Indonesia. The leaves of the *P. nigrum* and *C. canephora* was made into coarse powder. Five hundred gram of *P. nigrum* and *C. canephora* was macerated in 75% ethanol for 3 days. The liquid component was filtered through

Whatman 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 *P. nigrum* was labeled (PEE) and ethyl acetate extract (PEAE), and The etanol extract *C. canephora* was labeled (CEE) and ethyl acetate extract *C. canephora* (CEAE).

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 seven days in the Biology Laboratory. 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 subcutaneously with a single dose of 120 mg / Kg ketamine. Next, the dorsocostal area of each rats was shaved and excisional wounds were performed using a 6 mm sterile sharp dermal biopsy punch [9]. The animals were randomly divided into eight groups of 3 animals each. Group 1 was treated with 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 PEE, PEAE, CEE and CEAE ointment. The treatments were applied immediately after the injury and every 24 h for 15 consecutive days. All wounds of a single rat 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 day 3, 9, and 15 using a digital caliper before the application of the treatments. The percentage of wound closure can be calculated using the formula [10] as follows:

$$\% \text{ Wound Closure} = \frac{\text{Initial Wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \quad (1)$$

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 *P. nigrum* and *C. canephora* leaves. As shown in Figure 1, 2, and 3, the treatments with PEE, PEAE, CEE and CEAE accelerated the skin wound closure. Figure 1 shows that PEE and CEE at concentrations of 10%, 20% and 40% gave the same effect significantly with positive control at the level of α 0.05. Figure 2 shows that PEAE with a concentration of 10% and 20% gave the same effect significantly with positive control and was significantly different from negative control, while CEAE with a concentration of 10%, 20%, and 40% did not show a significant different with negative control.

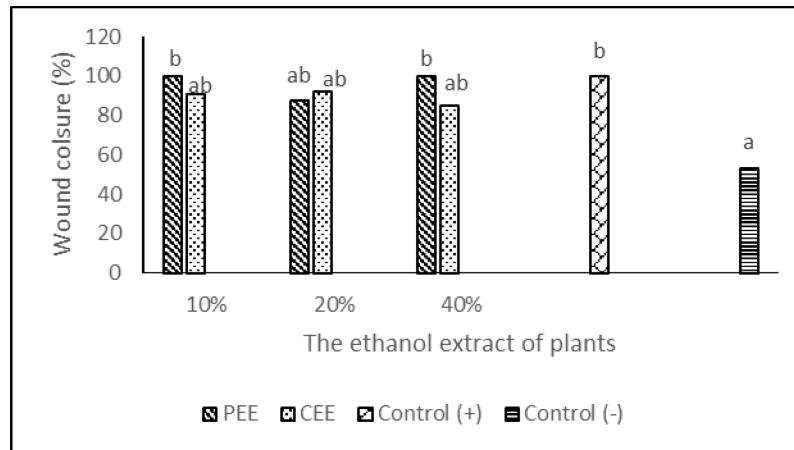


Figure 1. Effect of *P. nigrum* and *C. canephora* ethanol extracts in relative skin wound closure in rats. PEE and CEE were topically applied after the injury and every 24 h for 15 consecutive days. The same letter notation shows no significant difference at the level of α 0.05.

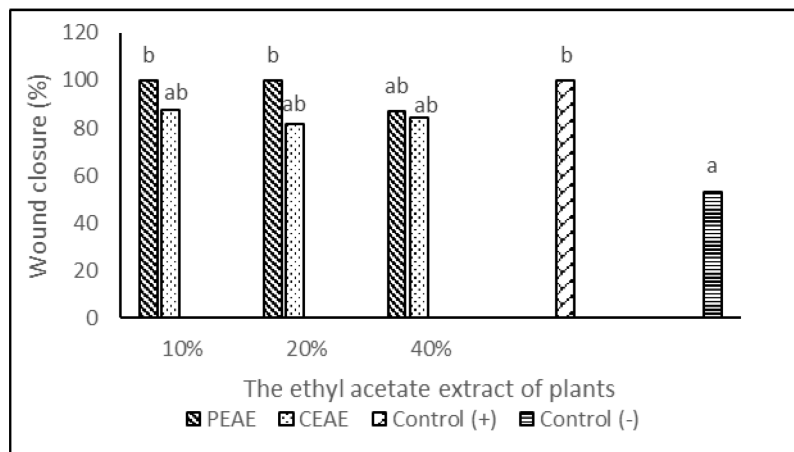


Figure 2. Effect of *P. nigrum* and *C. canephora* ethyl acetate extracts in relative skin wound closure in rats. PEAE and CEAE were topically applied after the injury and every 24 h for 15 consecutive days. The same letter notation shows no significant difference at the level of α 0.05.

P. nigrum and *C. canephora* have a rapid wound healing activity and have the same significant effect with positive control. It is in accordance with the result of previous studies that pepper plants can be used as wound healing agent as it has antibacterial, anti-inflammatory, antioxidant and antifungal activity [11]. The wound healing potential of pepper plant could be attributed to the presence of flavonoids and triterpenes. The former has antibacterial and antioxidant properties, while the latter has their astringent and antimicrobial properties [12]. Besides pepper plant, *C. canephora* also has wound healing potential due to its antibacterial properties. A previous study has showed that the extract of *C. canephora* showed an inhibitory effect against *Streptococcus mutans* [13, 14, 15, 16]. Thus, the extract of *P. nigrum* and *C. canephora* can enhance the rate of epithelization during wound healing process.

Wound healing process consists of several stages, namely (1) acute inflammation, (2) cell proliferation stage, and (3) maturation stage. During acute inflammation, the increasing number of fibroblast in the lesion has a central role to begin the healing process. Increasing number of fibroblast in the dermal showed its healing ability. The healing process of wound area may be hampered by reactive oxygen stress (ROS) produced by microbes or neutrophils in the wound area through a mechanism that lead to DNA damage. Hence, the existence of antimicrobial and antioxidant agents in the wound area is a crucial factor to promote the healing process [17, 18].



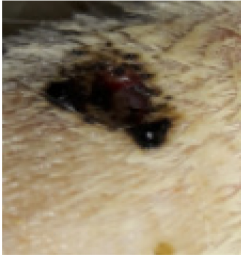








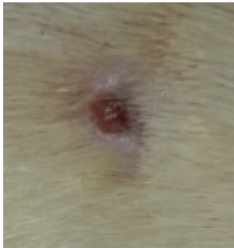
Extracts	Days 0	Days 15
PEAE		
PEE		
CEAE		
CEE		
Positive control		
Negative control		

Figure 3. Skin wound closure with the treatments of PEE, PEAE, CEE and CEAE for 15 days.

Summary

The present study suggests that two plants selected possess wound healing activity and out of two, *Piper nigrum* possesses better activity than *Coffea canephora*.

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