Cutaneous wound healing activity of herbal ointment containing the leaf extract of *Acalypha indica* L. on mice (*Mus musculus*)

M Laut¹*, N A Ndaong¹, T Utami²

¹Division of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Nusa Cendana University, Kupang, Indonesia
²Division of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Nusa Cendana University

*Corresponding author: laut.mm@staf.undana.ac.id

Abstract *Acalypha indica* L. is a wild plant grows in a tropical field which are investigated for medicinal effect to treat and cure illnesses. Previous studies reported the activity of analgesic, anti-inflammatory, antibacterial, antiviral, and antifungal properties of *A. indica*. The present study investigated the potential wound healing activity of ethanol leaf extract of *Acalypha indica* L topical formulation using mice incision wound models, compared to betadine ointment as standard treatment. Ten healthy male mice aged 3-4 months, weighing between 30-40 g were used in this study. Ethanol leaf extract of *A. indica* was formulated as 5%, 10% and 20% ointment. The Vaseline album and betadine 10% ointment served as negative and positive control, respectively. The parameter measured were wound contraction and epithelization period. The mice group treated with 5% and 10% extracts ointment showed better changes in the wound size compared to the placebo and standard groups. However, it was only 10% of the extract ointment promote the formation of granulation tissue allows the reepithelization phase to occur. These results suggest that ointment of ethanol leaf extract of *A. indica* could be an answer to facilitate wound healing, to approve the traditional claims of the plant in wound healing activity.

1. Introduction
Wound is a damage or injury to the body resulted in the loss of tissue integrity. It is well recognized that physical factors, chemical agents, heat or changes in temperatures are the common causes of tissue injuries. To recover the damaged tissues, the body activates a complicated biochemical reaction, intracellularly and extracellularly. Normally, the healing phases consists of inflammation, proliferation and maturation, which are properly arranged in a predictable period. However, many factors may contribute to delay the normal process and result in more serious and complicated wound. To accelerate and prevent complications, wound can be treated with properly drugs or other substances with healing properties. One of the standard wound treatment is povidone iodine ointment. Some medicinal plants have been identified for wound healing properties due to its secondary metabolites such as alkaloids, flavonoids, saponins, tannins, essential oils, terpenoids and phenolic compounds. The World Health Organization (WHO) stated that herbal remedies are preferred by 80% of
populations in Asia and Africa as it is accessible, inexpensive, and had minimal unwanted therapeutic effects[1].

*Acalypha indica* is an uncultivated tropical plant commonly found in Asia, Africa and Central America and has been used since ancient times to treat various diseases and health disorders, including diseases affecting the respiratory system [2]. The local people of Savunese, an ethnic in East Nusa Tenggara (NTT), have had used the extract leaves of *Acalypha indica* to treat screw worm infestation, known as myasis, on their livestock. The leaf is ground to a paste and applied to the wound. Past publications [3][4][5]. The water extract of *A. indica* leaf has effect on wound regeneration and various skin disorders. The phytochemical screening of *A. indica* leaf conducted by Mohideen et al. (2012) showed variations in contents, such as saponins, flavonoids, terpenoids and cardiac glycosides. In addition, Marwah et al. (2005) in their study identified polyphenol compounds, tannins and cyanogenic glycosides which are efficacious as antioxidants. In this study, ethanol extract of *Acalypha indica* leaves were formulated in topical preparation (ointment) and investigated for its pro wound healing activity on incision wound models.

2. Materials and Methods

2.1. Plant material

The leaves of *A. indica* were collected in April, 2018 from areas of Kupang, the capital of NTT province. The leaves were dry sorted to obtain only fresh leaves. The fresh leaves were then washed under running tap water without squeezing, to remove debris and dust particles. The fresh and clean leaves were air dried in shade at room temperature for 14 days.

2.1.1. Extraction of plant material.

The dried leaves were ground into powder using an electrical grinder, passed through a 40-mesh sieve and stored in a closed-glass container for future use. The powdered leaves (200 g) was macerated with 800 ml of 95% ethanol for 48 h and stir intermittently. The ethanol extract was filtered and then subjected to rotary evaporator for 3 hours to evaporate the ethanol and concentrated the extract. A dark-green of 7 g concentrated extract was obtained from the process.

2.2. Ointment formulation

The medicated formulation of unguent was made based on the standard formula as described by Paju et al., (2013). As a standard treatment, 20 g betadine (©Pfizer) 10% ointment were prepared. For preparing medicated ointment, 1 g (5%), 2 g (10%), and 4 g (20%) of the extract was mixed with 19 g, 18 g and 16 g of white Vaseline, respectively. All ingredients of the medicated ointment were mixed in the mortar and stirring constantly until homogenous and form an ointment preparation. In preparing for the control ointment, 20 g of the base was taken and treated in the same manner to formulate the medicated ointment, without the active ingredient. Prior to the mixture, the mortar was heated in the oven at 50°C for 5-6 minutes.

| Table 1. *A. indica* extract leaf ointment formulation |
|------------------------------------------------------|
| Ingredients | Formula |
|             | 5%  | 10% | 20% |
| *A. indica* extract | 1 g | 2 g | 4 g |
| White vaseline | 19 g | 18 g | 16 g |
| m.f. Ungt | 20 g | 20 g | 20 g |

2.3. Experimental animals

Ten healthy male mice (*Mus musculus*) aged 3-4 months old, weighing between 30-40 g, obtained from the Faculty of Veterinary Medicine, Nusa Cendana University, Kupang were used in this study. The animals were placed in plastic cage with wire covered on the top and keep under standard conditions of temperature, 12-hour light and 12-hour dark. The animals were set to adapt to a laboratory situation for one week before the study proceed. The wound models were fed with standard
livestock pellets twice a day and had unrestricted access to clean drinking water 24 h. The animal cages were given sterilized rice husk as bedding and replaced every three days. Ethical clearance was approved and obtained from the Faculty of Veterinary Medicine Animal Ethics Committee, Nusa Cendana University.

2.3.1. Grouping and dosing of animals
The wound models were randomly assigned into five groups (n=2). The first group were treated with simple base ointment only, served as negative control or placebo group. The second group were treated with betadine 10% ointment as standard drugs (positive control). The other 3 groups were treated with 5%, 10% and 20% of the extract ointment, respectively.

2.4. Incision wound model
Prior to the wound creation, all the animals of each group were given EMLA cream contain lidocaine, as local anesthetic, on the depilated area. The hair at particular skin area of the mice was removed with shaver, one day before the wounded. A 1.5 cm incision was made through skin and cutaneous muscles, with a sterile sharp surgical blade. The blood was cleaned with cotton swab and the wound were left undressed (Yunanda and Rinanda, 2016). After hemostatic process, the ointments were applied twice a day, according to the respective grouping, to cover the wounded area for 14 days.

2.5. Wound Healing Activity Test

2.5.1. Wound Contraction Measurement
Wound contraction was assessed by measuring the narrowing of the size of the wound using a ruler

2.5.1.1. Epithelization Time Measurement
The epithelization time was calculated as number of days required for falling off of the dead tissue remnants without any residual raw wound (Kumar et al., 2007).

2.6. Data and Statistical Analysis
All the results were expressed as mean ± S.E.M. Data analysis was performed used Graphpad Prism 7.0 software (GraphPad, San Diego, USA).

3. Result and Discussion
The herbal remedies are preferred and use widely in wound healing process. Apart from inexpensive and availability, the bioactive compounds extracted from plant materials (leaves, roots, bark, fruit, etc) has been clinically proven in wound healing. This study investigated the pro-wound healing activity of topical preparation of A. indica leaves extract on wound incision models. Ethanol 95%, a universal solvent widely known for its characteristic to attract not only polar substances but also the non-polar one. The oily medicated formulation was preferred because it is soft, easy to apply, and used for healing or cosmetic purposes [6]. Moreover, as an oily base, Vaseline album able to maintain skin moisture and supporting the absorption of active ingredient to the target tissue. The advantage of this oily base has an effect on the length of contact time between the active ingredient of the ointment and the target tissue.

| Treatments                        | Wound contraction (cm) |
|-----------------------------------|------------------------|
| Betadine ointment (positive control) | 1.41 ± 0.21           |
| Simple ointment (negative control)    | 1.49 ± 0.12           |
| Extract 5%                          | 1.40 ± 0.24           |
| Extract 10%                         | 1.02 ± 0.46           |
| Extract 20%                         | 1.26 ± 0.32           |

Thus, the healing reaction is accelerated. The parameters measured in this study were the wound contraction and epithelization period. However, the healing process depends on several factors including the ability of tissue to heal, severity of the injury and the health state condition of the origin tissues. Values of wound contraction of all group of treatments are presented in Table 2. The 10%
extract ointment showed significant effect on narrowing the wound area (1.02 cm) compared to the standard drug betadine ointment (1.41cm) (Fig.1). The time required for epithelialization of incision wound of the extract is described in Table 3.

![Figure 1. Wound contraction (cm)](image)

Table 3. Period of epithelization (days)

| Treatments          | Period of Epithelization (days) |
|---------------------|---------------------------------|
| Betadine ointment 10% | 9 ± 0                           |
| Simple ointment     | 8 ± 0                           |
| Extract 5% (w/w)    | 7 ± 0                           |
| Extract 10% (w/w)   | 6 ± 0                           |
| Extract 20% (w/w)   | 10 ± 2.83                       |

Epithelization period was significantly shortened by 10% (w/w) and 5% (w/w) of the extract ointment compared to standard drug and placebo. The 10% medicated concentration shortened the period of epithelization to 6 days compared to the placebo (8 days) and standard group (9 days) (Figure 2.) Wound contraction and epithelization occur on proliferation phase. However, these two stages are not related to each other although the contraction may facilitate the re-epithelization process. The shortening and thickening of the wound resulted in the reduction of wound size and amount of extracellular matrix needed to recover the damaged tissues [7]. Thus, resulted in acceleration of wound closure.

The preliminary phytochemical screening on Acalypha indica leaf indicated flavonoids as bioactive compound served as antimicrobials and antioxidant [9] Flavonoids is known to reduce lipid through inhibition of apoptosis and increased angiogenesis. Therefore, the wound contraction and epithelization are developed faster.

However, wound healing at the higher concentration (20%) as expected to present earlier, didn’t observe in this study. The delayed healing process might be due to inflammation. In addition, other factor that might contribute to the delayed of healing process is limited number and types of wound models used in the present study. It is stated that the impact of drugs on wound degeneration does not depend on a single wound model type only [10].
4. Conclusion
In conclusion, medicated ointment containing extract of *A. indica* leaves exhibited wound healing process in the incision wound models by enhanced wound contraction and shortened epithelization period. Apart from this, other properties such as antioxidant, antibacterial and antifungal activities make it a potential natural product-based ointment. The result of the present study offers pharmacological evidence to support the traditional use of *A. indica* leaves extract for the healing of wounds in East Nusa Tenggara region.

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