

**HEALING EFFECT OF LEAF EXTRACT OF CANDLEBUSH
(CASSIA ALATA L.) ON CUTANEOUS WOUND INFECTED WITH
TRICHOPHYTON RUBRUM**

**Mohammad Kanedi^{1*}, Ahmad Rokiban², Subur Widodo², Nopiyansah², Isbiyantoro²,
Lia Fauziah²**

¹Department of Biology, Faculty of Mathematics and Sciences University of Lampung,
Bandar Lampung, Indonesia.

²Department of Pharmacy, Faculty of Mathematics and Sciences, Tulang Bawang University,
Bandar Lampung, Indonesia.

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***Corresponding Author**

Mohammad Kanedi

Department of Biology,
Faculty of Mathematics
and Sciences University of
Lampung, Bandar
Lampung, Indonesia.

ABSTRACT

Research on antimicrobial activity and healing effect of candlebush (*Cassia alata* L.) extract on wound has been widely done, but its effect on cutaneous wound that were intentionally inoculated with a specific fungus is not known. This study was set to investigate the healing effects of leaf extract of candlebush on rabbit cutaneous wound that intentionally inoculated with *T. rubrum*. Eighteen male rabbits were

divided into six groups: P₀, P₁, P₂, P₃ and P₄, each consisting of three rabbits. P₀ is rabbits that treated with distilled water as the control; P₁, P₂, P₃ and P₄ respectively are the rabbits that received candlebush extract of 15%, 25%, 35%, and 45%; P₅ is rabbits that were given 2% ketoconazole ointment as the positive control. To inflict wound, the withers area on the animal's back were shaved and the shaved skin was then cut of about 2 cm in length using surgical blade. The inflicted wound was inoculated with *T. rubrum* and bandaged with sterile gauze for five days and after which the bandage is opened the treatment is done topically. Five levels of wound score were used as healing parameters namely: 4 (acute stage), 3, 2, 1, and 0 (recover stage). Leaf extracts of candlebush at the concentration of 45% totally heal the wound on day 21 while the positive control, ketoconazole 2%, gave total healing on the day 18. Thus, candlebush extract is potent to heal cutaneous wound infected by *Trichophyton*

rubrum.

KEYWORDS: Candlebush, *Cassia alata*, *Trichophyton rubrum*, Wound Healing, Antifungal Activity.

INTRODUCTION

Candlebush (*Cassia alata* L.) is one of plant species that traditionally having very high medicinal value worldwide. In many developing countries, candle brush is use as a antidote, bactericide, diuretic, fungicide, insecticide, pesticide, purgative, vermifuge, for asthma, bronchitis, constipation, dysentery, eczema, herpes,intestinal parasites, rheumatism, skin disorders, snakebite, stomachache, and venereal diseases. In Indonesia, especially in Java, the plant which is called ketepeng China commonly used to treat herpes, itch, ringworm, scabies, and syphilis.^[1]

The use candlebush to treat various diseases as described above seem to have empirical basis. Scientific researchs on the biological activity of the plant extract supporting the traditional practice of using candlebush in treating human disease caused by bacteria, helminth or fungi. The antibacterial activities of the plant have been reported by numerous researchers, among others faound that leaf extract of *C. alata* highly effective inhibit *Staphylococcus aureus*, *Serratia marcescens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris* and *Neisseria gonorrhoeae*.^[2, 3, 4, 5]

Antihelminth and larvicidal potential of the *C. alata* have also been investigated. The results showed that leaf and stem extracts of the plant exhibit a promising larvicidal activitiy against mosquito larvae^[6], while the leaf extract alone revealed an anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*.^[7]

Concerning its antifungal activities, *Cassia alata* showed high inhibitory effects on numerous types of fungi including *T. rubrum*, *T. mentagrophyte*, *Microsporum canis*, *Microsporum gypseum*, *Candida albicans*, *Rhizopus spp*, *Penicillium oxalicum*, *Aspergillus tamari*, *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium vacitilus*.^[8, 9, 10]

In addition to in vitro test against microbial agents, *C. alata* has also tested in vivo for its healing effects on wound in laboratory animals. By using leaves extract of the plant,^[11] found that the excision wound size in animals were significantly reduced when compared with the negative control group. Previously, it was also reported that the leaves of *Cassia alata*

has been used to cure wounds by the people of Sangli district, Maharashtra, India.^[12]

Although in vitro test of candlebush leaf extract clearly showed antibacterial and antifungal effects, but the effects of extract on specific fungus by in vitro test is not yet known. This study, therefore, was designed and implemented to investigate the healing effects of leaf extract of *C. alata* L. on cutaneous wound that intentionally inoculated with *T. rubrum*.

MATERIALS AND METHODS

Plant Samples and Extraction

Plant samples of *C. alata* L. were collected from the Village Yukum Jaya, Sub-district of Terbanggi Besar, the District of Lampung Tengah, the Province of Lampung, Indonesia. Taxonomic identification of the plant was done by botanist at the Botany Laboratory, University of Lampung, Indonesia. The fresh leaves were washed with water and cut into small pieces and put into a flask for maceration. The fresh leaves were washed with water and cut into small pieces and put into a flask for maceration. The fresh leaves were washed with water and cut into small pieces and put into a flask for maceration.

Maceration was done by soaking 100g of the samples in methanol of 800 ml for 24 hours and stirred three times daily. After 24 hours, the mixture was filtered and the pulp is squeezed and then soaked further in 800 ml of methanol until the pulp is no longer changing the color of the solvent. After being filtered and free from the pulp, macerate then concentrated by rotary vacuum evaporator. The yielded crude extracts diluted with double-distilled water into the appropriate concentration and ready for used.

Fungus Isolates

Trichophyton rubrum isolates used in this study was purchased from Lampung Provincial Health Laboratory. The propagation of the bacteria done by growing the isolates in Sabouraud Dextrose Agar (SDA) slants incubated at 37°C for 48 hours.

Animals and Wound Infliction

Animals used in this study were male rabbits aged 3-4 months weighing 1-1.5 kg obtained from animal house of the Veterinary Centers of Lampung, Indonesia. Before treatment the rabbits were acclimatized for the experimental room for one week. To inflict wound on rabbit skin, the withers area on the animal's back were shaved. Prior to and during wound creation all animals were anesthetized with chloroform using open mask method. On the shaved area a

wound was inflicted by making a cut on skin of about 2 cm in length using a surgical blade. After antiception by alcohol 70% and iodine 10%, the inflicted wound was inoculated with *T. rubrum* and bandaged with sterile gauze for five days to let the fungus infection happened. After five days the bandage is opened and then the treatment is done topically.

Wound Treatment and Healing Parameters

In this study, 18 rabbits were grouped into six consisted of three rabbits each, namely P₀, P₁, P₂, P₃, P₄, and P₅. P₀ is animals treated with distilled water only as the control; P₁ is group treated with candlebush extract of 15%; P₂ is group received extract of 25%; P₃ is group given extract of 35%; P₄ is rabbit group treated with extract of 45%; and P₅ is rabbits that received 2% ketoconazole ointment as the positive control. Treatments are conducted twice daily while wound recovery is observed and measured every three days until the day 21st.

The healing of wound was based on qualitative parameter consists of 5 score levels namely: 4 (acute stage, the wounds show signs of redness, swollen, thick and rough); 3 (redness, rough, less swelling, less thickness); 2 (rough and less redness); 1 (no longer redness but still rough); and 0 (recover stage, skin surface is smooth and hair grows).

Statistical Analysis

Data are presented descriptively and analyzed using one-way analysis of variance (ANOVA) and the differences among samples were determined by Least Significant Difference (LSD) Test at $\alpha < .05$.

RESULTS AND DISCUSSION

The average of recovery scores of wound on rabbit skin after treatment are presented in **Table 1** and the healing pattern according to the day of assessment are visualized in **Figure 1**.

Table 1: Average score of wound healing according to day of assessment, the Anova and LSD test results.

Treatment	Day of assessment						
	3	6	9	12	15	18	21
P0	4 ^a	3.7 ^a	3.3 ^a	2.7 ^a	2 ^a	2 ^a	1.7 ^a
P1	3.7 ^a	3.3 ^a	3 ^a	2.7 ^a	2 ^a	1.3 ^b	0.6 ^a
P2	3.3 ^a	3.3 ^a	3 ^a	2.3 ^a	2 ^a	1 ^b	0.6 ^b
P3	3.3 ^a	3 ^{ab}	2.3 ^b	1.7 ^{ab}	1.3 ^{ab}	0.6 ^c	0.3 ^b
P4	3.3 ^a	3 ^{ab}	2 ^b	1.7 ^{ab}	1 ^b	0.3 ^c	0 ^b
P5	3 ^a	2.3 ^b	1.7 ^b	1 ^b	1 ^b	0 ^{cd}	0 ^b
F-test	1.387 ^{ns}	2.437 ^{ns}	6.802 [*]	4.006 [*]	13.464 [*]	12.754 [*]	4.508 [*]

P₀ is rabbits treated with distilled water only as the control; P₁, P₂, P₃ and P₄ respectively are the

rabbits that treated with candlebush extract of 15%, 25%, 5%, and 45%; P₅ is rabbits that received 2% ketoconazole ointment as the positive control. Values in the same column that share the same superscript are not different at $\alpha=.05$. F-test values labeled 'ns' and '*' consecutively mean 'no significant difference' and 'significantly different' at $\alpha=.05$.

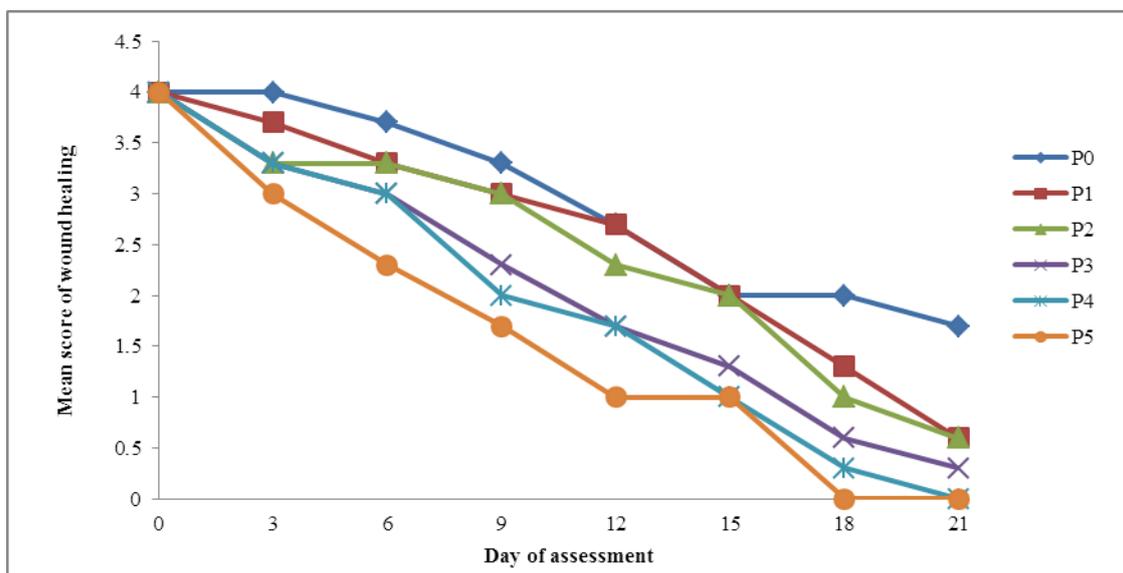


Figure 1: The healing pattern of wound on rabbit skin inoculated with *T. rubrum* after treatment. P₀ is rabbits treated with distilled water only as the control; P₁, P₂, P₃ and P₄ respectively are the rabbits that treated with candlebush extract of 15%, 25%, 35%, and 45%; P₅ is rabbits that received 2% ketoconazole ointment as the positive control.

Based on the data presented in the table and graph mentioned, as shown by P₀ group with a mean score of 1.7, it can be summarized that sores on the skin of rabbits that inoculated with *T. rubrum* can not recovered naturally on day 21. However, leaves extracts of candlebush at the concentration of 45%, on the day 21, are able to totally heal the wound (score of 0). Although candlebush extract of 35% and 25% has not provided a complete recovery on day 21, but given results of the LSD test showed the difference with the control group, it can be said that leaf extracts of candlebush able to recover cutaneous wound that intentionally inoculated with *T. rubrum*. Nevertheless, the healing of wounds by the extract of *C. alata* not as good and fast as healing by standard antifungal drug, ketoconazole 2%, which in this study gave total healing on the day 18 instead of day 21.

The first findings of this study confirmed that leaf extract of candlebush has antifungal effects. There are biochemical compound extracted from plants that are recognized as antifungal namely phenolics, alkaloids, terpenoids, saponins, flavonoids, and peptides.^[13] For

the plant itself, the secondary metabolites are allegedly useful in self-defense mechanism.^[14] Antifungal of flavonoids, terpenoids, and alkaloids against *Trichophyton rubrum* has been shown by organic extract of *Psidium guajava*.^[15] *Cassia alata* is a plant species that is proven to contain alkaloids, tannin, phenols, triterpenes and phytosterols, flavonoids.^[16] The traditional use of candlebush plants in India to cure leprosy, skin diseases, and ringworm infection may be due to the function of these bioactives including flavonoids and phenolic compounds.^[17]

The second findings of the study indicated that methanolic extract of candlebush leaves has a potential effect in wound healing *in vivo*. The wound healing effect of the candlebush extract may be related to antimicrobial activities and/or modulatory activities of the bioactive compounds. The antimicrobial activity of *C.alata* extracts does not only apply to the fungus, as outlined above, but also in bacteria. Leaf extract of candlebush chemically contain 3,4 dihydroxycinnamic acid of which by *in vitro* test revealed antibacterial activity against both Gram-positive and Gram-negative bacteria.^[18] Other phytochemicals of candlebush showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* namely alkaloid, cardioglycoside, fat and oil, flavonoid, gum and mucilage, glycoside, phytosterol, quinone, resin, saponin, tannin and terpenoid.^[19]

Wound healing is a biological process consisting of four phases namely hemostasis, inflammation, proliferation, and remodeling. If there is factor interfere with one of the phases, wound healing by itself will be impaired.^[20] Conversely, factors that can modulate biochemical proces in the phases can be categorized as a wound healing agent. There are plant-derived substances that can function as the modulators of fibroblast and keratinocyte for example alkaloids, terpenoids, polyphenols (flavonoids); modulators of collagen synthesis for example flavonoids, terpenoids, tannins and other phenolic compounds; modulators of cytokines and growth factors for example alkaloids, flavonoids, tannins, terpenoids.^[21] As has been shown by^[19] *C. alata* is contained all of the modulator substances.

Other bioactives showing modulatory activity in wound healing are fatty acids. Unsaturated fatty acids, especially n-9, modulate the inflammation in the wound and enhance reparative response *in vivo*.^[22] Oleic acid is another type of fatty acid that also accelerates the inflammatory response to promote the wound healing.^[23]

One of the conditions that are known to inhibit the healing process of wounds includes

diabetes mellitus. Therefore drugs that can give antidiabetic effects will expectedly promote wound healing process. *Cassia alata* extracts as reported by^[24] dan^[25] effectively reduced blood sugar level in diabetic rats. Interestingly, with regard to diabetes effects, the topically application of insulin on wounds effectively enhance the healing process of cutaneous wounds.^[26]

CONCLUSION

In conclusion, leaf extract of candlebush is potent to heal cutaneous wound infected by *Trichophyton rubrum*.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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