Effects of *Alocasia sp.* Stem Juice On Open Wound Healing In Rats

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**ABSTRACT**

Traditional remedies using herbal plants are fast gaining attention and such plants are increasingly used as products for health care among communities in the world. The local yam, *Alocasia sp.* is a herbal plant used by traditional healers as a wound healing agent. However, there has yet to be a scientific study to prove its potential. In this respect, an experiment was conducted to investigate the effects of *Alocasia sp.* stem juice on open-wound healing in rats. Fifty Wistar rats (250-300 g) were divided into two main groups, *Alocasia sp.* stem juice treated group (15 mg/ml) and pure curcumin treated group (15 mg/ml). Both groups were further subdivided again into five groups of five rats each. Four circular (6 mm in diameter) full-thickness wounds (2 treatments & 2 controls) were induced bilaterally on the dorsal of each rat skin. Each group of rats has their own control wound, which was treated with PBS solution. The study took 14 days to complete with five rats from each group sacrificed on days 1, 3, 6, 10 and 14. The parameters used to monitor the healing effects were the wound macroscopic observation, wound tissue biochemical test (protein) and wound histological observation by Haematoxylin & Eosin (H&E) staining and Masson’s Trichrome (MT) staining. The percentage of healing by the *Alocasia sp.* stem juice treated group showed a higher value with a significant difference (p<0.05) compared to other groups on days 1, 3 and 6. The protein level of the wounded tissue showed that rats treated with *Alocasia sp.* stem juice was of higher value with a significant difference (p<0.001) on day 6 compared to other treated groups on the same day. From the histological findings, rats treated with *Alocasia sp.* stem juice produced a denser collagen deposition and also better distribution at the wound site compared to other groups that also produced the same effect but with slower progression. As a conclusion, the *Alocasia sp.* stem juice showed good healing effects on the open-wound of rats and has the potential to be developed as a wound healing agent.

**Key words:** wound healing, *Alocasia sp.*, skin.

**Introduction**

Wound can be defined as loss or damage of cells and anatomical function of normal tissues. Wound healing or wound repair is a natural process in the body to regenerate a new dermal layer (Kokane et al. 2009) which involves a complex biological process. The success of this process depends on its coordination with various interactions which occur whether at molecular level, cell or tissue level. A wound healing process could be categorized to four phases namely, the coagulation phase, which involves process that prevents blood loss, the inflammation phase, the regeneration phase that involves the proliferation process and also the tissue remodeling and collagen deposition phase [16].

The inflammatory phase is characterized by its signs, which are *rubor* (redness), *calor* (warmth), *tumor* (swelling), *dolor* (pain) and *functio laesa* (loss of function) [24]. As the inflammatory phase nearly ends, proliferation phase will commence. In this phase, fibroblast cells will present, which functions to synthesis extracellular matrix and collagen [19]. As the proliferation phase ends, maturation phase will overtake. In this phase, the fibroblasts will continue to synthesis collagen and the wound tensile strength will improve as the days increase, but will only achieve approximately 80% of its original strength [7].

Traditional medicine as an alternative to modern medicine has proven its effectiveness and has also been admitted with either little or no side effects [9]. According to de Smet [6], some parties also believe that traditional medicine is safer to use and more readily acceptable by our body system compared to modern medicine. Despite modern living, dependence on traditional medicine still prevails all around the world. This is due to the efficacy of traditional medicine which uses various herbal plants and its
advantages in terms of a lack of side effects when compared to modern medicine which uses synthetic material.

*Alocasia sp.* is one of a genus in Araceae family. This herbaceous plant grows wildly at the edges of forests and requires shady and damp conditions. It can be found in any forest in Malaysia and it known to breed easily through its tuber. Traditional medicine practitioners are known to use this yam to heal external wounds and stop bleeding at wound areas immediately. The treatment involves applying the skin of the yam stem onto the wound, before it is bandaged [12]. In spite of its use as a traditional remedy, the biochemical basis of this reaction or its influence on the various phases of wound healing has not been studied in detail. Hence, in the present study, an effort was made to establish the wound healing potential of the plant using rats.

In this experiment, full thickness dermal wounds were made on rats, treated with yam (*Alocasia sp.*) stem juice and its effects on the percentage of wound healing, content of total protein levels and histological observation examined.

**Materials and Methods**

**Samples (yam):**

The yam (*Alocasia sp.*) stems used in this study were collected from an island at Tasik Temenggor, Gerik, Perak, Malaysia. The voucher specimen of the same plant has been deposited at Faculty of Science and Technology Herbarium, Universiti Kebangsaan Malaysia (voucher specimen no. UKMB 29848).

**Animal (rats):**

Fifty male Wistar strain rats weighing between 250-300 g (4 weeks old) were used as experimental animals, purchased from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. The usage of the animals for this project has been approved by Universiti Kebangsaan Malaysia Animal Ethical Committee (approval project code: FSKB/BIOB/2009/MAZLYZAM/20-OCTOBER/281-OCTOBER-2009-JANUARY - 2010). These animal specimens were housed and handled according to the appropriate guidelines laid out by internationally accepted principles for laboratory animal use and care. The rats were then divided randomly into two treated groups and each group was further sub-divided again into five groups of five rats each, one rat per cage. The animals were left for a week to acclimatize to the animal room condition and were maintained on standard pellet diet and tap water ad libitum.

**Preparation Of 15 Mg/Ml Alocasia Sp. Stem Juice:**

The method use is according to Chithra et al., [5] with some modification. The fresh stems of *Alocasia sp.* were cleaned and dried. After drying, they were cut into smaller pieces of 3-4 cm each. Then, distilled water was added at the ratio of 1:1 according to the sample weight. The mixture was then grounded and filtered to remove the debris and fibers, followed by centrifugation at 13 000 rpm for 30 minutes at 4°C. The resulting supernatant was collected in a flask and stored at -40°C. After 24 hours, the sample was lyophilized by a freeze dryer (Stuart Scientific, UK) to form a powder. 15 mg of formed *Alocasia sp.* powder was dissolved in 1 ml of PBS solution. The mixture was then stirred till it become homogen. 1 ml of the mixture was applied to wound area once daily as the test treatment.

**Preparation Of 15 Mg/Ml Pure Curcumin:**

Fifteen mg of pure curcumin powder (Sigma, UK) was dissolved in 1 ml of PBS solution. The mixture was then stirred till it become homogen. 1 ml of the mixture was applied to the wound area once daily as the control positive treatment.

**Experimental Induced Wounds:**

The rats were anaesthetized through the administration of Ketamine (10 mg/kg body weight) intravenally [14]. The dorsal area of each rat was shaved and disinfected with 70% alcohol. By using sterile 6-mm biopsy punches (Stiefel Laboratories, Ireland), four full-thickness wounds (6 mm diameter) were induced on each rat’s dorsal skin bilaterally (two treatment wounds & two control wounds). These animals were then sacrificed on the 1st, 3rd, 6th, 10th and 14th day after wound creation and the entire wound was cut out and stored at -40°C for biochemical test and also stored in formalin for histological preparation.

**Topical Administration:**

Treatment was administered by applying 1 ml of 15 mg/ml *Alocasia sp.* stem juice (treatment), 15 mg/ml pure curcumin (positive control) and PBS solution (negative control) on the wound once daily using a sterile plastic pipette. The period between treatments is at least 24 hours to enable adequate absorption.

**Macroscopic Observation:**

Wounds were measured using a short ruler and photographs were taken on the day of wounding and subsequently on alternate days, until healing was complete. Changes in wound areas were calculated, giving an indication of the rate of wound contraction. The percentage reduction in wound size was calculated using this equation;

\[
\text{Wound size reduction (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100
\]
where \( A_0 \) and \( A_t \) are the initial wound area and wound area after time interval \( t \), respectively. The distance from the right wound margin to the left wound margin was also measured [13].

**Biochemical Estimation:**

The wound area was cut at the size of 1.0 cm x 1.0 cm with a sterile, sharp surgery knife and stored at -20°C. The biochemical test involved is measurement of total protein level [3].

**Histological Observation:**

The wound area was cut at the size of 0.2 cm x 1.0 cm with a sterile, sharp surgery knife. Preparation of histological slides is based on the sequence of processes in the histology tests such as fixation with 10% formaline solution, dehydration, clearing, tissue infiltration, tissue embedding and also staining. The tissues were stained with Haematoxylin and Eosin, and also Masson’s Trichrome to observe collagen deposition [2].

**Statistical Analysis:**

Statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences) 15.0 for Windows software. Results were expressed as mean \( \pm \) S.E.M. The statistical analysis used was one-way ANOVA test for the determination of protein levels to compare median between groups of treatment and to compare median between days of treatment. Kruskal-Wallis test was used to compare median of percentage of wound healing between treatment groups and the median between days of treatment.

**Results and Discussion:**

This pioneering study was carried out to prove that this yam (Alocasia sp.) can heal external wounds and provide a new alternative in modern medicine to accelerate external wound healing process. According To Irvin [8], wound healing process will cause contraction in the wound area because the loss of tissues.

Parameters that have been taken into consideration in this study to determine the effectiveness of treatment in experimental rats were wound macroscopic observation, rate for percentage of wound healing, biochemical test (total protein level) and deposition of collagen in wound tissue through Masson’s Trichrome colouration [15].

From the macroscopic observations (Figure 1), apparent differences can be found when compared between the Alocasia sp. treated, curcumin treated and the PBS treated groups. The difference was the depth of scar groove, which was qualitatively evaluated. The wounds treated with Alocasia sp. showed resulting scars were almost completely closed by day 10\textsuperscript{th}, compared to curcumin and PBS group which showed complete healing only nearing day 14\textsuperscript{th}. Although the wounds were considered healed once the macroscopic scabs had been shed and epidermal layer formed, the actual healing process is still not perfect as proliferation and maturation processes still occur under the newly formed epidermis. This can be supported with studies done by Shakespeare (2001) which states that maturation phase will occur continuously even a few months after the scar covers wound. At this stage, modification of fibrous tissues by fibroblast cells happens to form scars.

For comparison of the percentage of wound healing based on the diameter (Figure 2), the results showed that all treated and control wounds had reached full healing (100%) at day 10\textsuperscript{th}. Full wound healing achieved indicated that the wound healing process occurred properly for all wounds, without interference caused by external factors such as bacterial infections and also internal factors such as dietary habits. As for comparison of the percentage on wound healing of respective groups, Alocasia sp. treated wounds showed the highest percentage of healing and there were significant differences when compared with curcumin and PBS treated wounds on the 1\textsuperscript{st}, 3\textsuperscript{rd} and 6\textsuperscript{th} day. This showed that the healing activities of Alocasia sp. treated wounds were faster compared to both curcumin and PBS treated wounds. This result was supported by macroscopic observation, which the crusts have formed and shed earlier, compared to the other groups.

Based on the biochemical test (total protein level) results obtained, the pattern of changes in total protein level for all types of treatments can be observed. Protein levels were high at the beginning of the study, followed by a decline in the middle of the study, but increased again at the end of the study (Figure 3). Generally, the level of total protein for wound tissue treated with Alocasia sp. juice recorded the highest values on days 1, 3, 6 and 14 compared to curcumin and PBS treated wounds. However, a significance difference was only recorded in day 6 after inducing wounds.

The level of total protein for wound tissue is high at the beginning of the study due to the process of haemostasis and inflammation which begins immediately after injury induced in rats studies [11]. The increase is caused by the chemical mediators such as enzymes, cytokines, hormones, C reactive protein and clotting protein components, debris cells, immunoglobulin and the components of the complement system involved in the inflammatory phase, as well as degradation of defective collagen [18]. This statement is also supported by the report of a study conducted by Walter [23] which states that all components involved in clotting is composed of 13 proteins and one part of calcium ions.
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<th>15 mg/ml pure curcumin</th>
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**Fig. 1:** Representative photographs of wounds treated with *Alocasia sp.* stem juice, pure curcumin (positive control), and PBS (negative control) on different days.
Fig. 2: Graph represent the rate of wound healing of wounds treated with Alocasia sp. stem juice, pure curcumin (positive control), and PBS (negative control) on different days.

The (#) signs showed significant difference (p<0.05) between days of treatment, while (*) signs showed significant difference (p<0.05) between groups within the same day.

Fig. 3: Bar chart represent the level of total protein of wounds treated with Alocasia sp. stem juice, pure curcumin (positive control), and PBS (negative control) on different days.

The (#) signs showed significant difference (p<0.05) between days of treatment, while (*) signs showed significant difference (p<0.001) between groups within the same day.

Decreased levels of total protein of wound tissue suggests that inflammatory processes that occur at the beginning of the study became lighter, and there was a long process of degradation of collagen that has been damaged as a result of the wounds by collagenase, a type of enzyme involved in the process of leucocytic during tissue wound repair. This process along with the synthesis and replacement by new collagen in the wound area and degradation in the form of some proteins by macrophages and neutrophils involved in inflammation [8].

Besides that, more decrease showed on day 6 suggests the end of the inflammatory phase in wound area and the beginning of the proliferation phase characterized by collagen synthesis of fibroblasts cells and macrophages [21,1]. Then on the 10th day, the protein level increase again and suggests an increase in collagen deposition in granulation tissue as a result of synthesis by fibroblasts cells. This statement is supported by Irvin [8] that collagen is the most important structure of protein builder in the granulation tissue and also contributes to 70 percent weight of dry skin.
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**Fig. 4:** Histopathological view of wound healing and epidermal/dermal from day 1 to day 14, treated with *Alocasia sp.* stem aqueous juice (A), curcumin as positive control (B), and PBS as negative control (C). Stained with H&E, at 100x magnification. Arrows pointing events during wound healing; E: epidermis, F: hair follicle, I: inflammatory cells, N: necrotic layer, G: granulation tissue, Fi: fibroblast cells, K: keratin, M: muscle fibre. Bar scale: 50 µm.
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**Fig. 5:** Histopathological view of wound healing and epidermal/dermal from day 1 to day 14, treated with *Alocasia sp.* stem aqueous juice (A), curcumin as positive control (B), and PBS as negative control (C). Stained with Masson’s Trichrome, at 100x magnification. E: epidermis, F: hair follicle, K: keratin, Ko: collagen, K I: type I collagen, K III: type III collagen, Fi: fibroblast cells, I: inflammatory cells, M: muscle fibre, N: necrotic layer. Bar scale: 50 µm.
For histological observations, not much difference could be observed on the 1st and 3rd days when compared to all wounds between groups. On the 1st day, a thick layer of necrotic tissue can be observed, where it is mainly composed of dead tissues, red blood cells and damaged collagen fibers (Figure 4). From the Masson’s Trichrome staining, degradation of damaged collagen fibers can be seen at the wound site. There was also presence of inflammation cells observed from H&E stain, such as neutrophils, which suggests that the inflammation phase has started.

On the 6th day, formation of new epithelium layer can be clearly seen for all groups. Epithelial cells began to enter the proliferation phase which is characterized by the formation of granulation tissue and collagen formation, especially wound tissue of *Alocasia sp.* juice treatment. This situation is clearer if seen on Masson’s Trichrome staining in which the formation of collagen deposition is more clear and concise and quickly dominated the wound. According to the results obtained from previous studies conducted by Panchatcharam *et al.* [16], curcumin treated wounds on the 8th day of microscopic observations showed infiltrating of fibroblast tissues and deposition of collagen together with hyperplasia of the lining epithelium. This proves that the wound treatment with *Alocasia sp.* juice used in this study provide a better healing effect because all changes can be seen beginning on the 6th day induced wound in which fibroblast tissues have dominated the wound area and the formation of collagen deposition can also be seen clearly.

For histological observation of the wound tissue biopsy on the 10th day, the epithelial cells began to divide to form a stratified squamous cell layer which is more structured and dominated by fibroblasts cells that play an important role in the production of collagen. At the end of the study, distribution of collagen deposition is becoming clear and concise in all tissues of the wound, but better results are shown by the treatment of *Alocasia sp.* juice where collagen type III (immature collagen) has been replaced by collagen type I (mature collagen) in the form of unorganized (irregular pattern) from the peripheral area of the wound (Figure 5). According to Chandrasoma and Taylor [4], new fibroblast cells produce collagen type III which is then replaced by collagen type I which are more powerful in terms of its cross-link with collagen fibers.

However, the study found all groups of rats have yet to reach the skin structure and morphology of the collagen sequence that matches the structure of the original skin. This is supported by Tibbs [22] who says that the maturation process of restructuring the collagen into the skin and the appearance of the skin morphology to become as similar to the original, starts at the 3rd week and continues until at least two years.

**Conclusion:**

In conclusion, this study reveals that *Alocasia sp.* stem juice is capable of providing good remedial impact for wound healing on rats through its capacity in improving wound healing percentage, increasing total tissue protein level, and through macroscopic and microscopic observations of wound tissue. Hence, *Alocasia sp.* stem is able to produce significant healing effect on open wounds and prove has the potential to be developed as a new wound healing agent in the modern medicine.

**References**


